NUCLEIC ACID-BINDING PHOTOPROBES AND USES THEREOF

TECHNICAL FIELD OF THE INVENTION

[0001] The present invention relates to photoactivatable compounds and methods of use thereof for identifying RNA transcripts that bind such compounds and are thus druggable, methods of screening drug candidates, and methods of determining drug binding sites and/or reactive site(s) on a target RNA. The invention also provides methods for modulating the biology of RNA transcripts to treat various diseases and conditions.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit under 35 U.S.C. § 119(e) of United States Provisional Patent Application serial number 62/593,175, filed November 30, 2017, the entirety of which is hereby incorporated by reference.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 30, 2018, is named 394457_003WO_163991_SL_ST25.TXT and is 46,324 bytes in size.

BACKGROUND OF THE INVENTION

[0004] Ribonucleic acids (RNAs) have been conventionally considered mere transient intermediaries between genes and proteins, whereby a protein-coding section of deoxyribonucleic acid (DNA) is transcribed into RNA that is then translated into a protein. RNA was thought to lack defined tertiary structure, and even where tertiary structure was present it was believed to be largely irrelevant to the RNA's function as a transient messenger. This understanding has been challenged by the recognition that RNA, including non-coding RNA (ncRNA), plays a multitude of critical regulatory roles in the cell and that RNA can have complex, defined, and functionally-essential tertiary structure.

[0005] All endogenous mammalian diseases are ultimately mediated by the transcriptome. Insofar as messenger mRNA (mRNA) is part of the transcriptome, and all protein expression derives from mRNAs, there is the potential to intervene in protein-mediated diseases by modulating the expression of the relevant protein and by, in turn, modulating the translation of the corresponding upstream mRNA. But mRNA is only a small portion of the transcriptome: other transcribed RNAs also regulate cellular biology either directly by the structure and function of RNA structures (e.g., ribonucleoproteins) as well as via protein expression and action, including (but not limited to) miRNA, lncRNA, lincRNA, snoRNA, snRNA, scaRNA, piRNA, ceRNA, and pseudo-genes. Drugs that intervene at this level have the potential of modulating any and all cellular processes. Existing therapeutic modalities such as antisense RNA or siRNA, in most cases, have yet to overcome significant challenges such as drug delivery, absorption, distribution to target organs, pharmacokinetics, and cell penetration. In contrast, small molecules have a long history of successfully surmounting these barriers and these qualities, which make them suitable as drugs, are readily optimized through a series of analogues to overcome such challeges. In sharp contrast, there are no validated, general methods of screening small molecules for binding to RNA targets in general, much less inside cells. The application of small molecules as ligands for RNA that yield therapeutic benefit has received little to no attention from the drug discovery community.

[0006] Targeting the RNA transcriptome with small molecule modulators represents an untapped therapeutic approach to treat a variety of RNA-mediated diseases. Accordingly, there remains a need to develop small-molecule RNA modulators useful as therapeutic agents.

BRIEF DESCRIPTION OF THE FIGURES

[0007] FIG. 1 shows structures of theophylline ligands with points of attachment for the tethering groups.

[0008] FIG. 2 shows structures of tetracycline ligands with points of attachment for the tethering groups.

[0009] FIG. 3 shows structures of triptycene ligands with points of attachment for the tethering groups.

[0010] FIG. 4 shows structures of triptycene ligands with points of attachment for the tethering groups. X = CH, N, or C-OH; Y = CH or N; R1, R2, R3 = each independently selected from halo,

-OH, -OMe, -NH_2, -NH-(optionally substituted C_{1-10} aliphatic), optionally substituted C_{1-10} aliphatic, or other described tethering groups. The modifier moiety may be attached at any position on R1, R2, or R3, or at the other functional groups on the above structures.

[0011] FIG. 5 shows structures of anthracene-maleimide Diels-Alder adduct ligands with points of attachment for the tethering groups. ***Note:*** The corresponding structures having the succinimido group in the opposite stereochemical orientation may also be prepared. Each R is independently selected from halo, -OH, -OMe, -NH_2, -NH-(optionally substituted C_{1-10} aliphatic), optionally substituted C_{1-10} aliphatic, or other described tethering groups. The modifier moiety may be attached at any position on R, or at the other functional groups on the above structures.

[0012] FIG. 6 shows structures of ribocil ligands with points of attachment for the tethering groups.

[0013] FIG. 7 shows structures of SMN2 ligands with points of attachment for the tethering groups.

[0014] FIG. 8 shows structures of linezolid and tedizolid ligands with points of attachment for the tethering groups.

[0015] FIG. 9 shows structures of exemplary click-ready groups.

[0016] FIG. 10 shows exemplary tethering groups for linking RNA ligands and modifying moieties.

[0017] FIG. 11 shows further examples of tethering groups.

[0018] FIG. 12 shows further examples of tethering groups.

[0019] FIG. 13 shows further examples of tethering groups.

[0020] FIG. 14 shows further examples of tethering groups.

[0021] FIG. 15 shows further examples of tethering groups.

[0022] FIG. 16 shows further examples of tethering groups.

[0023] FIG. 17 shows further examples of tethering groups.

[0024] FIG. 18 shows reaction schemes for accessing several theophylline small molecule ligands that include attachment points for the tethering group.

[0025] FIG. 19 shows reaction schemes for accessing several theophylline small molecule ligands that include attachment points for the tethering group.

[0026] FIG. 20 shows reaction schemes for accessing several theophylline small molecule ligands that include attachment points for the tethering group.

[0027] FIG. 21 shows reaction schemes for accessing several theophylline small molecule ligands that include attachment points for the tethering group.

[0028] FIG. 22 shows reaction schemes for accessing several tetracycline small molecule ligands that include attachment points for the tethering group.

[0029] FIG. 23 shows reaction schemes for accessing several tetracycline small molecule ligands that include attachment points for the tethering group.

[0030] FIG. 24 shows reaction schemes for accessing several tetracycline small molecule ligands that include attachment points for the tethering group.

[0031] FIG. 25 shows reaction schemes for accessing several tetracycline small molecule ligands that include attachment points for the tethering group.

[0032] FIG. 26 shows reaction schemes for accessing several triptycene small molecule ligands that include attachment points for the tethering group.

[0033] FIG. 27 shows reaction schemes for accessing several triptycene small molecule ligands that include attachment points for the tethering group.

[0034] FIG. 28 shows reaction schemes for accessing several triptycene small molecule ligands that include attachment points for the tethering group.

[0035] FIG. 29 shows reaction schemes for accessing several triptycene small molecule ligands that include attachment points for the tethering group.

[0036] FIG. 30 shows reaction schemes for accessing several triptycene small molecule ligands that include attachment points for the tethering group.

[0037] FIG. 31 shows reaction schemes for accessing several triptycene small molecule ligands that include attachment points for the tethering group.

[0038] FIG. 32 shows reaction schemes for accessing several triptycene small molecule ligands that include attachment points for the tethering group.

[0039] FIG. 33 shows reaction schemes for accessing several triptycene small molecule ligands that include attachment points for the tethering group.

[0040] FIG. 34 shows reaction schemes for accessing several tetracycline small molecule ligands that include a tethering group and modifying moiety.

[0041] FIG. 35 shows reaction schemes for accessing several triptycene small molecule ligands that include a tethering group and modifying moiety.

[0042] FIG. 36 shows a synthetic route for compound ARK-132.

[0043] FIG. 37 shows a synthetic route for compound ARK-134.

[0044] FIG. 38 shows a synthetic route for compounds ARK-135 and ARK-136.

[0045] FIG. 39 shows a synthetic route for compound ARK-188.

[0046] FIG. 40 shows a synthetic route for compound ARK-190.

[0047] FIG. 41 shows a synthetic route for compound ARK-191.

[0048] FIG. 42 shows a synthetic route for compound ARK-195.

[0049] FIG. 43 shows a synthetic route for compound ARK-197.

[0050] FIG. 44 shows a synthetic route for compounds based on the ribocil scaffold.

[0051] FIG. 45 shows photochemical reactions of NAz photoprobes which contain a (hetero)aroyl azide, as well as C8 modification reactions of the nitrene intermediate with guanosines.

[0052] FIG. 46 shows several riboswitch/aptamer-ligand pairs useful as positive control model systems for assay development in accordance with the present invention. The $PreQ_1$ ligand and sequence are disclosed in *Nat Struct Mol Biol* **16**, 343-344 (2009), which is hereby incorporated by reference. The TPP ligand and sequence are disclosed in *Nature* **441**, 1167-1171 (2006) and *Structure* **14**, 1459-1468 (2006), each of which is hereby incorporated by reference.

[0053] FIG. 47 shows surface plasmon resonance (SPR) results with the riboswitch/aptamer ligand pairs. While compound 1b (compound **I-1**, ARK-139) binds Aptamer 21, it is known not to bind a mutant sequence, Aptamer 21-E (data not shown).

[0054] FIG. 48 shows ARK-139 binding to Aptamer 21 by SPR; calculated K_D = 568 nM by SPR. Binding was also confirmed by SEC-MS (data not shown)

[0055] FIG. 49 shows SHAPE reactivity results from the use of SHAPE-MaP on Aptamer 21. Higher peak values signify increased solvent exposure and reactivity of individual nucleotides of the aptamer, with and without the presence of the ligand I-1 (ARK-139).

[0056] FIG. 50 shows results from the use of SHAPE-MaP on Aptamer 21-E (bottom). Higher peak values signify increased solvent exposure and reactivity of individual nucleotides of the aptamer, with and without the presence of the ligand I-1 (ARK-139). As can be seen, the presence

of I-1 caused almost no alteration in the SHAPE reactivity, suggesting weak binding of I-1 to Aptamer 21-E.

[0057] FIG. 51 shows the predicted binding mode of photoprobe ARK-547 to Aptamer 21. As the model shows, the predicted binding mode accommodates the linker and photoactivatable group.

[0058] FIG. 52 shows gel results of a PEARL-seq reverse transcriptase pausing assay. The transcriptase pauses at covalently modified nucleotides, leading to accumulation of shortened sequences. ARK-547 treatment leads to production of such shortened sequences of particular lengths, indicating that certain nucleotides are more likely to be covalently modified than others. NAI leads to modification at more accessbile/reactive nucleotides, leading to less selectivity and producing numerous shortened sequences.

[0059] FIG. 53 shows reverse transcriptase (RT) pausing results with Aptamer 21 and PreQ1 RNA. The Aptamer 21 diazirine probe ARK-547 shows specific and UV-dependent cross-linking with Apt21 RNA; PreQ1 probe does not show cross-linking to Apt21 or PreQ1 RNA. Conditions: 1 uM RNA, 10 uM probe, 9 uM PreQ1 probe, 20 mM TrisHCl pH 8, 100 mM KCl, 3 mM $MgCl_2$, 37 °C for 30 min, shielded from light, UV irradiation (~360 nm) for 3 indicated time at room temperature.

[0060] FIG. 54 shows screening results for additional compounds for cross-linking of Aptamer 21. Conditions: 1 μM refolded RNA, 10 μM compound, 20 mM TrisHCl pH 8, 100 mM KCl, 3 mM MgCl2, 2.5% DMSO. Reactions were incubated for 30 min at 37 °C shielded from light, followed by 5 min irradiation with 360 nm light at room temperature in Fisher photo-crosslinker.

[0061] FIG. 55 shows models of Aptamer 21 vs. Aptamer 21-E binding to I-1.

[0062] FIG. 56 shows SPR data for I-1 (ARK-139) binding to Aptamer 21. ARK-139 did not bind to Aptamer 21-E (data not shown). The calculated K_d was 420 nM.

[0063] FIG. 57 shows a gel assay in which Aptamer 21 and Aptamer 21-E were incubated with a biotin-photoaffinity bifunctional probe ARK-670, cross-linked, and then captured on streptavidin beads. Only the Aptamer 21 RNA showed significant pull-down.

shows results of sequencing of cross-linked Aptamer 21 after treatment with ARK-547 measuring MaP signal at positions 43 and 60, and selective drop-off at position 60. Combining with streptavidin capture will identify binding sites from a mixture of RNA.

[0064] FIGS. 58A and 58B show LC-MS results with Aptamer 21. ARK-547 and ARK-581 showed 5% and 10% covalent modification of the RNA, respectively.

[0065] FIG. 59 shows reverse transcriptase (RT) pausing assay results using bifunctional photoactivatable compounds.

[0066] FIG. 60 shows biotin pull-down experiment results. Steps: Cross-linked biotin-diazirine probe (ARK-579) to RNA; Captured on streptavidin magnetic beads; Performed RT on beads; Base-hydrolyzed RNA to elute cDNA; Ran on gel.

[0067] FIG. 61A and 61B show RT pausing and mutation rate results. Photo-crosslinking of Aptamer 21 and photoprobe ARK-547 revealed that ARK-547 does not bind to negative control Aptamer 21-E and yields no photoadduct. Reverse transcriptase (RT) pausing was maximal at nt 59, consistent with predicted binding mode. Sites of normalized mutational rate were also consistent with the ARK-547 binding mode.

[0068] FIG. 62 shows the structures of **I-14** (ARK-729) and **I-15** (ARK-816) and labeling of RNA-photoprobe adducts via a Cu-free click reaction using these compounds. Lanes 1-5 were run with different combinations of denaturant and Cu-free click reaction conditions. Lane 1: no denaturant/10 mM Tris, 1 mM EDTA, pH 8.0, 37 °C click conditions; Lane 2: no denaturant/10 mM Tris, 10 mM EDTA, pH 8.0, 65 °C click conditions; Lane 3: 6 M Urea denaturant/10 mM Tris, 10 mM EDTA, pH 8.0, 65 °C click conditions; Lane 4: 90% formamide denaturant/10 mM Tris, 10 mM EDTA, pH 8.0, 65 °C click conditions; Lane 5: 1 x TBE-Urea buffer, 65 °C. Aptamer 21 was treated with ARK-729 or ARK-816, then was subjected to UV photocrosslinking. The resulting photoadducts were then treated with a Cy7-DBCO conjugate under the indicated conditions. Performing the Cu-free click reaction at 65 °C without any additives enabled detection of the Aptamer 21-probe photoadducts by Cy7 fluorescence.

[0069] FIG. 63 shows results for competition experiments between Aptamer 21 photoprobes and RNA-binding ligands. Aptamer 21 was either incubated with probe alone (ARK-581) or probe plus a 10-fold excess of RNA-binding ligand. Only SPR-active compounds ARK-139 and ARK-852 efficiently inhibited photocrosslinking of ARK-581 to Aptamer 21.

[0070] FIG. 64 shows RT pausing results for photocrosslinking of structurally distinct Aptamer 21 ligands to Aptamer 21.

[0071] FIG. 65 shows RT pausing results for a competition assay. Both ARK-852 and ARK-139 inhibit the photocrosslinking of probes to Aptamer 21.

[0072] FIG. 66 shows RT pausing results relating to photocrosslinking of chemical probes to Aptamer 21.

[0073] FIG. 67A and FIG. 67B shows photocrosslinking of ARK-670 to Aptamer 21, Aptamer 21-E, or a mixture of Aptamer 21 and four other RNAs. The RT pausing signal from probe adducts was specific for Aptamer 21 and increased in strength after bead enrichment of crosslinked RNA.

[0074] FIG. 68 shows selective enrichment of Aptamer 21 by ARK-670 in the presence of other RNA squences. Cross-linking of ARK-670 to a mixture of Aptamer 21 and four other RNAs was followed by avidin bead enrichment of cross-linked RNA and sequencing. Sequencing analysis showed that only Aptamer 21 was enriched by ARK-670, which suggests that ARK-670 binds to Aptamer 21 and cross-links selectively in a proximity-driven manner.

[0075] FIG. 69 shows RT pausing data from click-biotinylated probes after enrichment. Crosslinking of Aptamer 21 or Aptamer 21-E to ARK-729 (phenylazide probe), ARK-2058 (phenylazide warhead-only control), ARK-816 (diazirine probe), ARK-2059 (diazirine warhead-only control) or DMSO was followed by enrichment on avidin beads and sequencing. The probes ARK-729 and ARK-816 showed RT pausing peaks specific to Aptamer 21.

[0076] FIG. 70 shows a cartoon mapping the locations of RT pausing peaks on Aptamer 21's sequence.

[0077] FIG. 71 shows RT pausing on Aptamer 21 spiked into PolyA+ RNA extract. Crosslinking of ARK-816 (diazirine probe) or ARK-2059 (diazirine warhead-only control) to Aptamer 21 spiked into a polyA+ RNA extract and the RT pausing ratio was measured by sequencing. Peaks specific to the ARK-816 probe were observed at the same positions as for isolated Aptamer 21.

[0078] FIG. 72 shows enrichment analysis of Aptamer 21 from a PolyA+ RNA extract. Aptamer 21 was spiked into polyA+ RNA extract and then the mixture was crosslinked to ARK-816 (diazirine probe) and ARK-2059 (warhead-only control) and crosslinked RNA was enriched by avidin capture. Specific enrichment of sequences by the probe as compared to the warhead-only control determined by next-generation sequencing. Enrichment of the sites of probe-specific RT pausing on Aptamer 21 was observed.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

1. General Description of Certain Embodiments of the Invention; Definitions

RNA Targets and Association with Diseases and Disorders

[0079] The vast majority of molecular targets that have been addressed therapeutically are proteins. However, it is now understood that a variety of RNA molecules play important regulatory roles in both healthy and diseased cells. While only 1-2% of the human genome codes for proteins, it is now known that the majority of the genome is transcribed (Carninci *et al., Science* 309:1559-1563; 2005). Thus, the noncoding transcripts (the noncoding transcriptome) represent a large group of new therapeutic targets. Noncoding RNAs such as microRNA (miRNA) and long noncoding RNA (lncRNA) regulate transcription, splicing, mRNA stability/decay, and translation. In addition, the noncoding regions of mRNA such as the 5′ untranslated regions (5′ UTR), the 3′ UTR, and introns can play regulatory roles in affecting mRNA expression levels, alternative splicing, translational efficiency, and mRNA and protein subcellular localization. RNA secondary and tertiary structures are critical for these regulatory activities.

[0080] Remarkably, GWAS studies have shown that there are far more single nucleotide polymorphisms (SNPs) associated with human disease in the noncoding transcriptome relative to the coding transcripts (Maurano *et al*., *Science* 337:1190-1195; 2012). Therefore, the therapeutic targeting of noncoding RNAs and noncoding regions of mRNA can yield novel agents to treat to previously intractable human diseases.

[0081] Current therapeutic approaches to interdict mRNA require methods such as gene therapy (Naldini, *Nature* **2015**, *526*, 351-360), genome editing (Cox et al., Nature Medicine 2015, 21, 121-131), or a wide range of oligonucleotide technologies (antisense, RNAi, etc.) (Bennett & Swayze, *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 259–293). Oligonucleotides modulate the action of RNA via canonical base/base hybridization. The appeal of this approach is that the basic pharmacophore of an oligonucleotide can be defined in a straightforward fashion from the sequence subject to interdiction. Each of these therapeutic modalities suffers from substantial technical, clinical, and regulatory challenges. Some limitations of oligonucleotides as therapeutics (e.g. antisense, RNAi) include unfavorable pharmacokinetics, lack of oral bioavailability, and lack of blood-brain-barrier penetration, with the latter precluding delivery to the brain or spinal cord

after parenteral drug administration for the treatment of neurological diseases. In addition, oligonucleotides are not taken up effectively into solid tumors without a complex delivery system such as lipid nanoparticles. Lastly, a vast majority of the oligonucleotides that are taken up into cells and tissues remain in a non-functional compartment such as endosomes, and only a small fraction of the material escapes to gain access to the cytosol and/or nucleus where the target is located.

[0082] "Traditional" small molecules can be optimized to exhibit excellent absorption from the gut, excellent distribution to target organs, and excellent cell penetration. The use of "traditional" (i.e., "Lipinski-compliant" (Lipinski et al., *Adv. Drug Deliv. Rev.* **2001**, *46*, 3-26) small molecules with favorable drug properties that bind and modulate the activity of a target RNA would solve many of the problems noted above.

[0083] In one aspect, the present invention provides a method of identifying the identity or structure of a binding or active site to which a small molecule binds in a target RNA, comprising the steps of i) contacting the target RNA with a disclosed compound and ii) analyzing the results by an assay disclosed herein, optionally in combination with a computational method. In some embodiments, the target RNA is selected from a mRNA or a noncoding RNA. In some embodiments, the target RNA is an aptamer or riboswitch. In some embodiments, the RNA is the FMN riboswitch, $PreQ_1$, or Aptamer 21. In some embodiments, the assay identifies the location in the primary sequence of the binding site(s) on the target RNA.

Targeting mRNA

[0084] Within mRNAs, noncoding regions can affect the level of mRNA and protein expression. Briefly, these include IRES and upstream open reading frames (uORF) that affect translation efficiency, intronic sequences that affect splicing efficiency and alternative splicing patterns, 3′ UTR sequences that affect mRNA and protein localization, and elements that control mRNA decay and half-life. Therapeutic modulation of these RNA elements can have beneficial effects. Also, mRNAs may contain expansions of simple repeat sequences such as trinucleotide repeats. These repeat expansion containing RNAs can be toxic and have been observed to drive disease pathology, particularly in certain neurological and musculoskeletal diseases (see Gatchel & Zoghbi, *Nature Rev. Gen.* **2005**, *6*, 743-755). In addition, splicing can be modulated to skip

exons having mutations that introduce stop codons in order to relieve premature termination during translation.

[0085] Small molecules can be used to modulate splicing of pre-mRNA for therapeutic benefit in a variety of settings. One example is spinal muscular atrophy (SMA). SMA is a consequence of insufficient amounts of the survival of motor neuron (SMN) protein. Humans have two versions of the SMN gene, SMN1 and SMN2. SMA patients have a mutated SMN1 gene and thus rely solely on SMN2 for their SMN protein. The SMN2 gene has a silent mutation in exon 7 that causes inefficient splicing such that exon 7 is skipped in the majority of SMN2 transcripts, leading to the generation of a defective protein that is rapidly degraded in cells, thus limiting the amount of SMN protein produced from this locus. A small molecule that promotes the efficient inclusion of exon 7 during the splicing of SMN2 transcripts would be an effective treatment for SMA (Palacino *et al., Nature Chem. Biol.*, **2015**, 11, 511-517). Accordingly, in one aspect, the present invention provides a method of identifying a small molecule that modulates the splicing of a target pre-mRNA to treat a disease or disorder, comprising the steps of: screening one or more disclosed compounds for binding to the target pre-mRNA; and analyzing the results by an RNA binding assay disclosed herein. In some embodiments, the pre-mRNA is an SMN2 transcript. In some embodiments, the disease or disorder is spinal muscular atrophy (SMA).

[0086] Even in cases in which defective splicing does not cause the disease, alteration of splicing patterns can be used to correct the disease. Nonsense mutations leading to premature translational termination can be eliminated by exon skipping if the exon sequences are in-frame. This can create a protein that is at least partially functional. One example of the use of exon skipping is the dystrophin gene in Duchenne muscular dystrophy (DMD). A variety of different mutations leading to premature termination codons in DMD patients can be eliminated by exon skipping promoted by oligonucleotides (reviewed in Fairclough *et al.*, *Nature Rev. Gen.*, **2013**, *14*, 373-378). Small molecules that bind RNA structures and affect splicing are expected to have a similar effect. Accordingly, in one aspect, the present invention provides a method of identifying a small molecule that modulates the splicing pattern of a target pre-mRNA to treat a disease or disorder, comprising the steps of: screening one or more disclosed compounds for binding to the target pre-mRNA; and analyzing the results by an RNA binding assay disclosed herein. In some embodiments, the pre-mRNA is a dystrophin gene transcript. In some embodiments, the small

molecule promotes exon skipping to eliminate premature translational termination. In some embodiments, the disease or disorder is Duchenne muscular dystrophy (DMD).

[0087] Lastly, the expression of an mRNA and its translation products could be affected by targeting noncoding sequences and structures in the 5′ and 3′ UTRs. For instance, RNA structures in the 5′ UTR can affect translational efficiency. RNA structures such as hairpins in the 5′ UTR have been shown to affect translation. In general, RNA structures are believed to play a critical role in translation of mRNA. Two examples of these are internal ribosome entry sites (IRES) and upstream open reading frames (uORF) that can affect the level of translation of the main open reading frame (Komar and Hatzoglou, *Frontiers Oncol.* 5:233, 2015; Weingarten-Gabbay *et al., Science* 351:pii:aad4939, 2016; Calvo *et al., Proc. Natl. Acad. Sci. USA* 106:7507-7512; Le Quesne *et al., J. Pathol.* 220:140-151, 2010; Barbosa et al., PLOS Genetics 9:e10035529, 2013). For example, nearly half of all human mRNAs have uORFs, and many of these reduce the translation of the main ORF. Small molecules targeting these RNAs could be used to modulate specific protein levels for therapeutic benefit. Accordingly, in one aspect, the present invention provides a method of producing a small molecule that modulates the expression or translation efficiency of a target pre-mRNA or mRNA to treat a disease or disorder, comprising the steps of: screening one or more disclosed compounds for binding to the target pre-mRNA or mRNA; and analyzing the results by an RNA binding assay disclosed herein. In some embodiments, the small molecule binding site is a 5′ UTR, internal ribosome entry site, or upsteam open reading frame.

Targeting Regulatory RNA

[0088] The largest set of RNA targets is RNA that is transcribed but not translated into protein, termed “non-coding RNA”. Non-coding RNA is highly conserved and the many varieties of non-coding RNA play a wide range of regulatory functions. The term “non-coding RNA,” as used herein, includes but is not limited to micro-RNA (miRNA), long non-coding RNA (lncRNA), long intergenic non-coding RNA (lincRNA), Piwi-interacting RNA (piRNA), competing endogenous RNA (ceRNA), and pseudo-genes. Each of these sub-categories of non-coding RNA offers a large number of RNA targets with significant therapeutic potential. Accordingly, in some embodiments, the present invention provides methods of treating a disease mediated by non-coding RNA. In some embodiments, the disease is caused by a miRNA, lncRNA, lincRNA, piRNA, ceRNA, or pseudo-gene. In another aspect, the present invention provides a method of producing a small

molecule that modulates the activity of a target non-coding RNA to treat a disease or disorder, comprising the steps of: screening one or more disclosed compounds for binding to the target non-coding RNA; and analyzing the results by an RNA binding assay disclosed herein. In some embodiments, the target non-coding RNA is a miRNA, lncRNA, lincRNA, piRNA, ceRNA, or pseudo-gene.

[0089] miRNA are short double-strand RNAs that regulate gene expression (see Elliott & Ladomery, Molecular Biology of RNA, 2nd Ed.). Each miRNA can affect the expression of many human genes. There are nearly 2,000 miRNAs in humans. These RNAs regulate many biological processes, including cell differentiation, cell fate, motility, survival, and function. miRNA expression levels vary between different tissues, cell types, and disease settings. They are frequently aberrantly expressed in tumors versus normal tissue, and their activity may play significant roles in cancer (for reviews, see Croce, *Nature Rev. Genet.* 10:704-714, 2009; Dykxhoorn *Cancer Res.* 70:6401-6406, 2010). miRNAs have been shown to regulate oncogenes and tumor suppressors and themselves can act as oncogenes or tumor suppressors. Some have been shown to promote epithelial-mesenchymal transition (EMT) and cancer cell invasiveness and metastasis. In the case of oncogenic miRNAs, their inhibition could be an effective anti-cancer treatment. Accordingly, in one aspect, the present invention provides a method of producing a small molecule that modulates the activity of a target miRNA to treat a disease or disorder, comprising the steps of: screening one or more disclosed compounds for binding to the target miRNA; and analyzing the results by an RNA binding assay disclosed herein. In some embodiments, the miRNA regulates an oncogene or tumor suppressor, or acts as an oncogene or tumor suppressor. In some embodiments, the disease is cancer. In some embodiments, the cancer is a solid tumor.

[0090] There are multiple oncogenic miRNA that could be therapeutically targeted including miR-155, miR-17~92, miR-19, miR-21, and miR-10b (see Stahlhut & Slack, *Genome Med.* **2013**, *5*, 111). miR-155 plays pathological roles in inflammation, hypertension, heart failure, and cancer. In cancer, miR-155 triggers oncogenic cascades and apoptosis resistance, as well as increasing cancer cell invasiveness. Altered expression of miR-155 has been described in multiple cancers, reflecting staging, progress and treatment outcomes. Cancers in which miR-155 over-expression has been reported are breast cancer, thyroid carcinoma, colon cancer, cervical cancer, and lung

cancer. It is reported to play a role in drug resistance in breast cancer. miR-17~92 (also called Oncomir-1) is a polycistronic 1 kb primary transcript comprising miR-17, 20a, 18a, 19a, 92-1 and 19b-1. It is activated by MYC. miR-19 alters the gene expression and signal transduction pathways in multiple hematopoietic cells, and it triggers leukemogenesis and lymphomagenesis. It is implicated in a wide variety of human solid tumors and hematological cancers. miR-21 is an oncogenic miRNA that reduces the expression of multiple tumor suppressors. It stimulates cancer cell invasion and is associated with a wide variety of human cancers including breast, ovarian, cervix, colon, lung, liver, brain, esophagus, prostate, pancreas, and thyroid cancers. Accordingly, in some embodiments of the methods described above, the target miRNA is selected from miR-155, miR-17~92, miR-19, miR-21, or miR-10b. In some embodiments, the disease or disorder is a cancer selected from breast cancer, ovarian cancer, cervical cancer, thyroid carcinoma, colon cancer, liver cancer, brain cancer, esophageal cancer, prostate cancer, lung cancer, leukemia, or lymph node cancer. In some embodiments, the cancer is a solid tumor.

[0091] Beyond oncology, miRNAs play roles in many other diseases including cardiovascular and metabolic diseases (Quiant and Olson, *J. Clin. Invest.* 123:11-18, 2013; Olson, Science Trans. Med. 6: 239ps3, 2014; Baffy, *J. Clin. Med.* 4:1977-1988, 2015).

[0092] Many mature miRNAs are relatively short in length and thus may lack sufficient folded, thrtee-dimensional structure to be targeted by small molecules. However, it is believed that the levels of such miRNA could be reduced by small molecules that bind the primary transcript or the pre-miRNA to block the biogenesis of the mature miRNA. Accordingly, in some embodiments of the methods described above, the target miRNA is a primary transcript or pre-miRNA.

[0093] lncRNA are RNAs of over 200 nucleotides (nt) that do not encode proteins (see Rinn & Chang, *Ann. Rev. Biochem.* **2012**, *81*, 145-166; (for reviews, see Morris and Mattick, *Nature Reviews Genetics* 15:423-437, 2014; Mattick and Rinn, *Nature Structural & Mol. Biol.* 22:5-7, 2015; Iyer *et al., Nature Genetics* 47(:199-208, 2015)). They can affect the expression of the protein-encoding mRNAs at the level of transcription, splicing and mRNA decay. Considerable research has shown that lncRNA can regulate transcription by recruiting epigenetic regulators that increase or decrease transcription by altering chromatin structure (e.g., Holoch and Moazed, *Nature Reviews Genetics* 16:71-84, 2015). lncRNAs are associated with human diseases including cancer, inflammatory diseases, neurological diseases and cardiovascular disease (for instance,

Presner and Chinnaiyan, *Cancer Discovery* 1:391-407, 2011; Johnson, *Neurobiology of Disease* 46:245-254, 2012; Gutscher and Diederichs, *RNA Biology* 9:703-719, 2012; Kumar *et al., PLOS Genetics* 9:e1003201, 2013; van de Vondervoort *et al., Frontiers in Molecular Neuroscience*, 2013; Li *et al., Int. J. Mol. Sci.* 14:18790-18808, 2013). The targeting of lncRNA could be done to up-regulate or down-regulate the expression of specific genes and proteins for therapeutic benefit (e.g., Wahlestedt, *Nature Reviews Drug Discovery* 12:433-446, 2013; Guil and Esteller, *Nature Structural & Mol. Biol.* 19:1068-1075, 2012). In general, lncRNA are expressed at a lower level relative to mRNAs. Many lncRNAs are physically associated with chromatin (Werner *et al., Cell Reports* 12, 1-10, 2015) and are transcribed in close proximity to protein-encoding genes. They often remain physically associated at their site of transcription and act locally, in *cis*, to regulate the expression of a neighboring mRNA. The mutation and dysregulation of lncRNA is associated with human diseases; therefore, there are a multitude of lncRNAs that could be therapeutic targets. Accordingly, in some embodiments of the methods described above, the target non-coding RNA is a lncRNA. In some embodiments, the lncRNA is associated with a cancer, inflammatory disease, neurological disease, or cardiovascular disease.

[0094] lncRNAs regulate the expression of protein-encoding genes, acting at multiple different levels to affect transcription, alternative splicing and mRNA decay. For example, lncRNA has been shown to bind to the epigenetic regulator PRC2 to promote its recruitment to genes whose transcription is then repressed via chromatin modification. lncRNA may form complex structures that mediate their association with various regulatory proteins. A small molecule that binds to these lncRNA structures could be used to modulate the expression of genes that are normally regulated by an individual lncRNA.

[0095] One examplary target lncRNA is HOTAIR, an lncRNA expressed from the HoxC locus on human chromosome 12. Is expression level is low (~100 RNA copies per cell). Unlike many lncRNAs, HOTAIR can act in *trans* to affect the expression of distant genes. It binds the epigenetic repressor PRC2 as well as the LSD1/CoREST/REST complex, another repressive epigenetic regulator (Tsai *et al., Science* 329, 689-693, 2010). HOTAIR is a highly structured RNA with over 50% of its nucleotides being involved in base pairing. It is frequently dysregulated (often up-regulated) in various types of cancer (Yao *et al., Int. J. Mol. Sci.* 15:18985-18999, 2014; Deng *et al., PLOS One* 9:e110059, 2014). Cancer patients with high expression levels of HOTAIR

have a significantly poorer prognosis, compared with those with low expression levels. HOTAIR has been reported to be involved in the control of apoptosis, proliferation, metastasis, angiogenesis, DNA repair, chemoresistance and tumor cell metabolism. It is highly expressed in metastatic breast cancers. High levels of expression in primary breast tumors are a significant predictor of subsequent metastasis and death. HOTAIR also has been reported to be associated with esophageal squamous cell carcinoma, and it is a prognostic factor in colorectal cancer, cervical cancer, gastric cancer and endometrial carcinoma. Therefore, HOTAIR-binding small molecules are novel anti-cancer drug candidates. Accordingly, in some embodiments of the methods described above, the target non-coding RNA is HOTAIR. In some embodiments, the disease or disorder is breast cancer, esophageal squamous cell carcinoma, colorectal cancer, cervical cancer, gastric cancer, or endometrial carcinoma.

[0096] Another potential cancer target among lncRNA is MALAT-1 (metastasis-associated lung adenocarcinoma transcript 1), also known as NEAT2 (nuclear-enriched abundant transcript 2) (Gutschner *et al., Cancer Res.* 73:1180-1189, 2013; Brown *et al., Nat. Structural & Mol. Biol.* 21:633-640, 2014). It is a highly conserved 7 kb nuclear lncRNA that is localized in nuclear speckles. It is ubiquitously expressed in normal tissues, but is up-regulated in many cancers. MALAT-1 is a predictive marker for metastasis development in multiple cancers including lung cancer. It appears to function as a regulator of gene expression, potentially affecting transcription and/or splicing. MALAT-1 knockout mice have no phenotype, indicating that it has limited normal function. However, MALAT-1-deficient cancer cells are impaired in migration and form fewer tumors in a mouse xenograft tumor models. Antisense oligonucleotides (ASO) blocking MALAT-1 prevent metastasis formation after tumor implantation in mice. Some mouse xenograft tumor model data indicates that MALAT-1 knockdown by ASOs may inhibit both primary tumor growth and metastasis. Thus, a small molecule targeting MALAT-1 is exptected to be effective in inhibiting tumor growth and metastasis. Accordingly, in some embodiments of the methods described above, the target non-coding RNA is MALAT-1. In some embodiments, the disease or disorder is a cancer in which MALAT-1 is upregulated, such as lung cancer.

[0097] In some embodiments, the present invention provides a method of treating a disease or disorder mediated by non-coding RNA (such as HOTAIR or MALAT-1), comprising the step of

administering to a patient in need thereof a compound of the present invention. Such compounds are described in detail herein.

Targeting Toxic RNA (Repeat RNA)

[0098] Simple repeats in mRNA often are associated with human disease. These are often, but not exclusively, repeats of three nucleotides such as CAG ("triplet repeats") (for reviews, see Gatchel and Zoghbi, *Nature Reviews Genetics* 6:743-755, 2005; Krzyzosiak *et al., Nucleic Acids Res.* 40:11-26, 2012; Budworth and McMurray, *Methods Mol. Biol.* 1010:3-17, 2013). Triplet repeats are abundant in the human genome, and they tend to undergo expansion over generations. Approximately 40 human diseases are associated with the expansion of repeat sequences. Diseases caused by triplet expansions are known as Triplet Repeat Expansion Diseases (TRED). Healthy individuals have a variable number of triplet repeats, but there is a threshold beyond which a higher repeat number causes disease. The threshold varies in different disorders. The triplet repeat can be unstable. As the gene is inherited, the number of repeats may increase, and the condition may be more severe or have an earlier onset from generation to generation. When an individual has a number of repeats in the normal range, it is not expected to expand when passed to the next generation. When the repeat number is in the premutation range (a normal, but unstable repeat number), then the repeats may or may not expand upon transmission to the next generation. Normal individuals who carry a premutation do not have the condition, but are at risk of having a child who has inherited a triplet repeat in the full mutation range and who will be affected. TREDs can be autosomal dominant, autosomal recessive or X-linked. The more common triplet repeat disorders are autosomal dominant.

[0099] The repeats can be in the coding or noncoding portions of the mRNA. In the case of repeats within noncoding regions, the repeats may lie in the 5′ UTR, introns, or 3′ UTR sequences. Some examples of diseases caused by repeat sequences within coding regions are shown in Table 1.

Table 1: Repeat Expansion Diseases in Which the Repeat Resides in the Coding Regions of mRNA

Disease	Gene	Repeat	Normal repeat number	Disease repeat number
HD	HTT	CAG	6-35 (SEQ ID NO:1)	36-250 (SEQ ID NO:8)
DRPLA	ATN1	CAG	6-35 (SEQ ID NO:1)	49-88 (SEQ ID NO:9)
SBMA	AR	CAG	9-36 (SEQ ID NO:2)	38-62 (SEQ ID NO:10)
SCA1	ATXN1	CAG	6-35 (SEQ ID NO:1)	49-88 (SEQ ID NO:9)
SCA2	ATXN2	CAG	14-32 (SEQ ID NO:3)	33-77 (SEQ ID NO:11)
SCA3	ATXN3	CAG	12-40 (SEQ ID NO:4)	55-86 (SEQ ID NO:12)
SCA6	CACNA1A	CAG	4-18 (SEQ ID NO:5)	21-30 (SEQ ID NO:13)
SCA7	ATXN7	CAG	7-17 (SEQ ID NO:6)	38-120 (SEQ ID NO:14)
SCA17	TBP	CAG	25-42 (SEQ ID NO:7)	47-63 (SEQ ID NO:15)

[00100] Some examples of diseases caused by repeat sequences within noncoding regions of mRNA are shown in Table 2.

Table 2: Repeat Expansion Diseases in Which the Repeat Resides in the Noncoding Regions of mRNA

Disease	Gene	Repeat	Repeat location	Normal repeat number	Disease repeat number
Fragile X	FMR1	CGG	5′ UTR	6-53 (SEQ ID NO:16)	≥230
DM1	DMPK	CTG	3′ UTR	5-37 (SEQ ID NO:17)	≥50

FRDA	FXN	GAA	Intron	7-34 (SEQ ID NO:18)	≥100
SCA8	ATXN8	CTG	Noncoding antisense	16-37 (SEQ ID NO:19)	110-250 (SEQ ID NO:22)
SCA10	ATXN10	ATTCT	Intron	9-32 (SEQ ID NO:20)	800-4500 (SEQ ID NO:23)
SCA12	PPP2R2B	CAG	5′ UTR	7-28 (SEQ ID NO:21)	66-78 (SEQ ID NO:24)
C9FTD/ALS	C9orf72	GGGGCC	Intron	~30	100s

[00101] The toxicity that results from the repeat sequence can be direct consequence of the action of the toxic RNA itself, or, in cases in which the repeat expansion is in the coding sequence, due to the toxicity of the RNA and/or the aberrant protein. The repeat expansion RNA can act by sequestering critical RNA-binding proteins (RBP) into foci. One example of a sequestered RBP is the Muscleblind family protein MBNL1. Sequestration of RBPs leads to defects in splicing as well as defects in nuclear-cytoplasmic transport of RNA and proteins. Sequestration of RBPs also can affect miRNA biogenesis. These perturbations in RNA biology can profoundly affect neuronal function and survival, leading to a variety of neurological diseases.

[00102] Repeat sequences in RNA form secondary and tertiary structures that bind RBPs and affect normal RNA biology. One specific example disease is myotonic dystrophy (DM1; dystrophia myotonica), a common inherited form of muscle disease characterized by muscle weakness and slow relaxation of the muscles after contraction (Machuca-Tzili *et al., Muscle Nerve* 32:1-18, 2005). It is caused by a CUG expansion in the 3′ UTR of the dystrophia myotonica protein kinase (DMPK) gene. This repeat-containing RNA causes the misregulation of alternative splicing of several developmentally regulated transcripts through effects on the splicing regulators MBNL1 and the CUG repeat binding protein (CELF1) (Wheeler *et al., Science* 325:336-339, 2009). Small molecules that bind the CUG repeat within the DMPK transcript would alter the RNA structure and prevent focus formation and alleviate the effects on these spicing regulators. Fragile X Syndrome (FXS), the most common inherited form of mental retardation, is the consequence of a CGG repeat expansion within the 5′ UTR of the FMR1 gene (Lozano *et al., Intractable Rare Dis. Res.* 3:134-146, 2014). FMRP is critical for the regulation of translation of many mRNAs and for protein trafficking, and it is an essential protein for synaptic development

and neural plasticity. Thus, its deficiency leads to neuropathology. A small molecule targeting this CGG repeat RNA may alleviate the suppression of FMR1 mRNA and FMRP protein expression. Another TRED having a very high unmet medical need is Huntington's disease (HD). HD is a progressive neurological disorder with motor, cognitive, and psychiatric changes (Zuccato *et al., Physiol Rev.* 90:905-981, 2010). It is characterized as a poly-glutamine or polyQ disorder since the CAG repeat within the coding sequence of the HTT gene leads to a protein having a poly-glutamine repeat that appears to have detrimental effects on transcription, vesicle trafficking, mitochondrial function, and proteasome activity. However, the HTT CAG repeat RNA itself also demonstrates toxicity, including the sequestration of MBNL1 protein into nuclear inclusions. One other specific example is the GGGGCC repeat expansion in the C9orf72 (chromosome 9 open reading frame 72) gene that is prevalent in both familial frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) (Ling *et al., Neuron* 79:416-438, 2013; Haeusler *et al., Nature* 507:195-200, 2014). The repeat RNA structures form nuclear foci that sequester critical RNA binding proteins. The GGGGCC repeat RNA also binds and sequesters RanGAP1 to impair nucleocytoplasmic transport of RNA and proteins (Zhang *et al., Nature* 525:56-61, 2015). Selectively targeting any of these repeat expansion RNAs could add therapeutic benefit in these neurological diseases.

[00103] The present invention contemplates a method of treating a disease or disorder wherein aberrant RNAs themselves cause pathogenic effects, rather than acting through the agency of protein expression or regulation of protein expression. In some embodiments, the disease or disorder is mediated by repeat RNA, such as those described above or in Tables 1 and 2. In some embodiments, the disease or disorder is a repeat expansion disease in which the repeat resides in the coding regions of mRNA. In some embodiments, the disease or disorder is a repeat expansion disease in which the repeat resides in the noncoding regions of mRNA. In some embodiments, the disease or disorder is selected from Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), spinal-bulbar muscular atrophy (SBMA), or a spinocerebellar ataxia (SCA) selected from SCA1, SCA2, SCA3, SCA6, SCA7, or SCA17. In some embodiments, the disease or disorder is selected from Fragile X Syndrome, myotonic dystrophy (DM1 or dystrophia myotonica), Friedreich's Ataxia (FRDA), a spinocerebellar ataxia (SCA) selected from SCA8, SCA10, or SCA12, or C9FTD (amyotrophic lateral sclerosis or ALS).

[00104] In some embodiments, the disease is amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), frontotemporal dementia (FTD), myotonic dystrophy (DM1 or dystrophia myotonica), or Fragile X Syndrome.

[00105] In some embodiments, the present invention provides a method of treating a disease or disorder mediated by repeat RNA, comprising the step of administering to a patient in need thereof a compound of the present invention. Such compounds are described in detail herein.

[00106] Also provided is a method of producing a small molecule that modulates the activity of a target repeat expansion RNA to treat a disease or disorder, comprising the steps of: screening one or more disclosed compounds for binding to the target repeat expansion RNA; and analyzing the results by an RNA binding assay disclosed herein. In some embodiments, the repeat expansion RNA causes a disease or disorder selected from HD, DRPLA, SBMA, SCA1, SCA2, SCA3, SCA6, SCA7, or SCA17. In some embodiments, the disease or disorder is selected from Fragile X Syndrome, DM1, FRDA, SCA8, SCA10, SCA12, or C9FTD.

Other Target RNAs and Diseases/Conditions

[00107] An association is known to exist between a large number of additional RNAs and diseases or conditions, some of which are shown below in Table 3. Accordingly, in some embodiments of the methods described above, the target RNA is selected from those in Table 3. In some embodiments, the disease or disorder is selected from those in Table 3.

Table 3: Target RNAs and Associated Diseases/Conditions

GENE	CLASS	UP/ DOWN REGULATED?	TA	INDICATION(S)
MYC	TF	down	Onco	cancer
STAT3	TF	down	Onco	cancer
C9orf72	TRED	down	Neuro	ALS, FTD
FOXP3	TF	down	I&I, I-O	immuno-oncology; I&I
MIR155	miRNA	down	Onco, I&I, Neuro	ALS, fibrosis, cancer
APOC3	apoprotein	down	Cardio	chylomicronemia syndrome
JUN	TF	down	I&I	I&I
RSV	genomic	down	Viral	RSV
KRAS	TF	down	Onco	cancer
BCL2L1	IAP	down	Onco	cancer

GENE	CLASS	UP/ DOWN REGULATED?	TA	INDICATION(S)
HIF1A	TF	down	Onco	cancer
SMARCA2	helicase	down	Onco	cancer
SNCA		down	Neuro	PD
CCNE1	cyclin	down	Onco	cancer
FOXM1	TA	down	Onco	cancer
MYB	TF	down	Onco	cancer
PTPN11	phosphatase	down	Onco, I&I	cancer, SLE
CD40LG	TNF	down	I&I	inflammation
NFE2L2	TF	up	I&I	multiple sclerosis
RORC	NHR	down	I&I	I&I
ZIKV	genomic	down	Viral	ZIKV
DENV	genomic	down	Viral	DENV
AR	NHR	down	Onco	prostate cancer
ASGR1		down	Cardio	CVD
BCL2	IAP	down	Onco	cancer
BDNF	NF	up	Neuro	Huntington's Disease
BRD4	epi	down	Onco	cancer
CD40	TNF	down	I&I	immuno-oncology
CD47	Ig	down	I&I, I-O	immuno-oncology
CTLA4	Ig	down	I&I, I-O	immuno-oncology; I&I
CTNNB1	adhesion	down	Onco	cancer
DMPK	TRED	down	Neuro	Myotonic dystrophy type 1 (DM1)
EIF4E	IF	down	Onco	cancer
FOXA1	TA	down	Onco	cancer
GATA3	TF	down	Onco	cancer
IKZF1	TF	down	Onco	cancer
IKZF3	TF	down	Onco	cancer
IL17A	IL	down	I&I	inflammatory & autoimmune diseases
IL23A	IL	down	I&I	inflammatory & autoimmune diseases
IL6	IL	down	I&I	rheumatoid arthritis
ITGA1	integrin	down	I&I	RA
ITGA5	integrin	down	Onco	solid tumors
ITGAE	integrin	down	I&I	UC, Crohns
ITGB2, ITGAL	integrin	down	I&I	psoriasis

GENE	CLASS	UP/ DOWN REGULATED?	TA	INDICATION(S)
ITGB7	integrin	down	I&I	UC, Crohns
MAPT	cytoskeleton	down	Neuro	Alzheimer's disease
MAX	TF	down	Onco	cancer
MDM2	E3	down	Onco	cancer
MDM4	E3	down	Onco	cancer
MIR21	miRNA	down	Onco	cancer
NR4A2	TF	down	Neuro	PD
PTEN	phosphatase	up	Onco	cancer
PTPN1	phosphatase	down	Metab	Type 2 diabetes
RUNX1	TF	down	Onco	cancer
SIRPA	glycoprotein	down	I&I, I-O	immuno-oncology
SMAD7	TGF	down	I&I	IBD
SOX2	TF	down	Onco	cancer
STAT5A	TF	down	Onco	cancer
TERT	telomerase	down	Onco	cancer
TGFB1	TGF	down	Fibrosis	fibrosis
TNF	TNF	down	I&I	inflammatory disease
TNFRSF11A	TNF	down		osteoporosis
TNFSF11	TNF	down		osteoporosis
TWIST1	TF	down	Onco	cancer
WNT1			Onco	cancer
HepB		down	Viral	HepB
influenza		down	Viral	influenza
DGAT2	transferase	down		NASH
DNMT3	DNMT	down	Onco	cancer
ERBB3	pseudokinase	down	Onco	cancer
FBXW7	F-box (E3)	down	Onco	cancer
FMR1	TRED	down	Neuro	Fragile X Syndrome; FTXAS
FOS	TF	down		
FXN	TRED	down	Neuro	Friedreich's Ataxia
IRAK3	pseudokinase	down	I&I	I&I
MECP2	TF	up/down	Genetic Dz	Rett Syndrome
MIR17HG	miRNA	down	Onco	cancer
NF1		down		neurofibromatosis

GENE	CLASS	UP/ DOWN REGULATED?	TA	INDICATION(S)
ORAI1	ion channel	down	I&I	I&I
PCSK9	convertase	down	Cardio	hypercholesterolemia
PSMB8	protease	down	I&I	I&I
SKP2	F-box (E3)	down	Onco	cancer
USP1	protease	down	Onco	cancer
USP7	protease	down	Onco	cancer
HIF1A	TF	up	I&I	wound repair & regeneration
HOTAIR	lncRNA	down	Onco	cancer
IKBKG		down	I&I	I&I
IKK2	kinase	down	I&I	I&I
MALAT1	lncRNA	down	Onco	cancer
PRMT5	KMT	down	Onco	cancer
BCL6	IAP	down	Onco	cancer
GRN		down	Neuro	neurological diseases
ABCA1	transporter		Cardio	coronary artery disease
ABCB11	transporter			Primary Biliary Sclerosis
ABCB4	transporter			Primary Biliary Sclerosis
ABCG5	transporter		Cardio	coronary artery disease
ABCG8	transporter		Cardio	coronary artery disease
ADIPOQ	hormone	up	Metab	diabetes; obesity; metabolic syndrome
APOA1			Cardio	hypercholesterolemia
APOA5			Cardio	hypercholesterolemia
ATPA2	Ca ATPase	up	Genetic Dz	congestive heart failure
ATXN1	TRED		Neuro	spinocerebellar ataxia 1
ATXN10	TRED	down	Neuro	spinocerebellar ataxia 10
ATXN2	TRED		Neuro	spinocerebellar ataxia 2
ATXN3	TRED		Neuro	spinocerebellar ataxia 3
ATXN7	TRED		Neuro	spinocerebellar ataxia 7
ATXN8	TRED		Neuro	spinocerebellar ataxia 8
BACE1	protease	down	Neuro	Alzheimer's disease
BIRC2	IAP	down	Onco	cancer
BIRC3	IAP	down	Onco	cancer
BIRC5	IAP	down	Onco	cancer
BRCA1	DNA repair	up	Onco	cancer
CACNA1A	ion channel		Neuro	episodic ataxia type 2
CD247	TCR		I&I	I&I

GENE	CLASS	UP/ DOWN REGULATED?	TA	INDICATION(S)
CD274		down	I-O	immuno-oncology
CETP	transfer	down		cardiovascular
CFH	complement			macular degeneration
CFTR	ion channel	up	Genetic Dz	Cystic Fibrosis
CNBP	TRED	down	Neuro	Myotonic dystrophy type 2 (DM2)
CNTF	NF			macular degeneration
DIO2	deiodinase		Metab	dyslipidemia
DMD	cytoskeleton		Neuro	Duchenne Muscular Dystrophy; Becker's MD
F7	protease	up	Hematology	hemophilia
F8	protease	up	Hematology	hemophilia
F9	protease	up	Hematology	hemophilia
FGF3		down	Genetic Dz	achondroplasia
HAMP		down	Genetic Dz	thalassemia; hereditary hemochromatosis
HAVCR2		down	I&I, I-O	inflammatory diseases; immuno-oncology
HBG1, HBG2	hemoglobin	up	Hematology	sickle cell anemia; beta-thalassemia
HIF1AN			Onco	cancer
IDH1	dehydrogenase	down	Onco	cancer
IL1	IL	down	I&I	rheumatoid arthritis
IRAK4	kinase	down	I&I	I&I
IRF5	TF		I-O	immuno-oncology
LAMA1	ECM		Genetic Dz	Merosin-deficient congenital MD (MDCA1)
LARGE1			Genetic Dz	Muscular Dystroglycanopathy Type B,6
LINGO1		down	Neuro	neurodegeneration
MBNL1	splice factor		Neuro	Myotonic Dystrophy
MCL1	IAP	down	Onco	cancer
MERTK	kinase		I&I	Lupus
METAP2	peptidase	down	Onco, I&I	cancer, obesity, autoimmune
MTOR	kinase		Onco	cancer

GENE	CLASS	UP/ DOWN REGULATED?	TA	INDICATION(S)
NANOG	TF		Neuro	neurological diseases
NF2				neurofibromatosis
NSD-3	KMT	down	Onco	cancer
PAH	hydroxylase		Genetic Dz	phenylketonuria
PCSK6	convertase	up	Cardio	hypertension
PDCD1			I-O	immuno-oncology
PDK1, PDK2	kinase			polycystic kidney disease
PDX1	TF		Metab	diabetes
PPARGC1A	PPAR		Neuro	Neurological diseases; obesity
PRKAA1	kinase		Metab	diabetes
PRKAB1	kinase		Metab	diabetes
PRKAG1	kinase		Metab	diabetes
RTN4		down	Neuro	neurodegeneration
RTN4R		down	Neuro	neurodegeneration
SCARB1	HDL receptor		Cardio	coronary artery disease
SIRT6	KDAC	down	Onco	cancer
SMN2		up	Neuro	Spinal Muscular Atrophy
SMURF2		down		
SORT1	glycoprotein		Cardio	coronary artery disease
SSPN	cytoskeleton		Genetic Dz	Duchenne's MD
TBX21			I-O	immuno-oncology
THRB	NHR			dyslipidemia; NASH; NAFLD
TNFAIP3			I&I	inflammatory dz; liver failure; liver transplant
TRIB1	pseudokinase		Cardio	coronary artery disease
TTR		down	Genetic Dz	amyloidosis
UTRN	cytoskeleton		Genetic Dz	Duchenne Muscular Dystrophy
XIAP	IAP	down	Onco	cancer
RAGE				
ANGPTL3				

Table 4: Additional Target RNAs

GENE	COMMON NAME	CLASS	UP/ DOWN REGULATED?	TA	INDICATION(S)
CTSL	cathepsin L	protease	up	neuro	PD
AR	AR-V7	NHR	down	cancer	CRPC
JMJD6	JMJD6	HDM	down	cancer	GBM
DNMT1	DNMT1	Me-transferase	down	cancer	GBM
ASGR1	ASGR1	ASG receptor	down	CVD	CVD
NAMPT	NAMPT	transferase	down	cancer	various
	IRE				
ARID1B	ARID1B				
SOX10	SOX10				
HNF1B	TCF2				
PTPN2	PTPN2				
NLGN3	NLGN3				
	ETS				

2. Compounds and Uses Thereof

[00108] It has now been found that compounds of this invention, and pharmaceutically acceptable compositions thereof, are effective as agents for use in drug discovery and for preparing nucleic acid conjugates that are useful in drug discovery. For example, compounds of the present invention, and pharmaceutical compositions thereof, are useful in determining the location and/or structure of an active site or allosteric sites and/or the tertiary structure of a target RNA.

[00109] In one aspect, disclosed compounds are useful as diagnostic or assay reagents. In some embodiments, the present invention provides a method of determining the three-dimentional structure, binding site of a ligand of interest, or accessibility of a nucleotide in a target nucleic acid, comprising: contacting the target nucleic acid with a disclosed compound; irradiating the compound; determining whether covalent modification of a nucleotide of the nucleic acid has occurred; and optionally deriving the pattern of nucleotide modification, the three-dimentional structure, ligand binding site, or other structural information about the nucleic acid.

[00110] In another aspect, the present invention provides a method of preparing a nucleic acid conjugate, comprising: contacting a target nucleic acid with a disclosed compound; irradiating the compound; and optionally isolating the resulting nucleic acid conjugate by an affinity assay, pull-

down method, or other means known in the art. Such nucleic acid conjugates are useful for determining structural information about the target nucleic acid comprised in the conjugate that allows one of ordinary skill to design small molecule drugs that bind to the target nucleic acid *in vivo* to treat a disease, disorder, or condition, such as those disclosed herein. In some embodiments, the nucleic acid is a RNA, such as a disease-causing RNA as described herein.

[00111] In another aspect, the present invention provides a method of assessing selectivity across a transcriptome for a drug candidate, comprising contacting a biological sample comprising two or more RNA transcripts with a drug candidate comprising a disclosed photoactivatable group tethered to the drug candidate; irradiating the drug candidate; and determining covalent modification of an RNA transcript.

[00112] In another aspect, the present invention provides a method of determining target occupancy in cells of a drug candidate, comprising contacting a biological sample comprising a target RNA with a disclosed compound or drug candidate comprising a disclosed photoactivatable group tethered to the drug candidate; irradiating the compound or drug candidate; and determining covalent modification of the target RNA. In some embodiments, the method confirms target engagement and correlates binding with cellular biology.

[00113] In some embodiments, the method enables assembling a binding site map by identifying subsite binding to explicate the biochemical mode of action.

[00114] In some embodiments, the method further enables relating target engagement to target mutations and cell function. This is useful for understanding the molecular mechanism of drug candidates in cells.

[00115] In another aspect, the present invention provides a method of determining the presence of a RNA binding protein (RBP) that is associated with a target RNA comprising: contacting the target RNA with a disclosed compound; irradiating the compound; and determining whether covalent modification of an amino acid of the RBP has occurred.

[00116] In some embodiments, the present invention provides a compound comprising:

(a) a small molecule ligand that binds selectively to one or more binding sites on a target RNA;

(b) a photoactivatable group (or "warhead") that is covalently conjugated to the small molecule ligand and that forms a covalent bond to the target RNA upon irradiation with visible light or ultraviolet light;

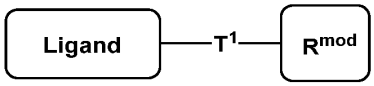
(c) optionally, a click-ready group;

(d) optionally, a pull-down group; and

(e) optionally, one or two tethering groups that covalently link the small molecule ligand and the photoactivatable group and, optionally, the click-ready group.

[00117] Without wishing to be bound by any particular theory, it is believed that compounds of the present invention bind selectively to one or more active or allosteric sites on a target RNA, or other sites determined by binding interactions between the small molecule ligand and the structure of the target RNA; upon irradiation, covalently modify one or more positions of the target RNA, such as a C8 carbon of an adenosine or guanosine nucleotide or a 2′-OH group of the target RNA; and may subsequently be used to identify the active site or other binding sites by sequencing or other analysis of the distribution of modified nucleotides because the pattern of modification will be constrained by the length and conformation of the tether that connects the ligand with the RNA warhead. The target RNA may be inside a cell, in a cell lysate, or in isolated form prior to contacting the compound. Screening of libraries of disclosed compounds will identify highly potent small-molecule modulators of the activity of the target RNA. It is understood that such small molecules identified by such screening may be used as modulators of a target RNA to treat, prevent, or ameliorate a disease or condition in a patient in need thereof.

[00118] In one aspect, the present invention provides a compound of the general Formula **I**:



I

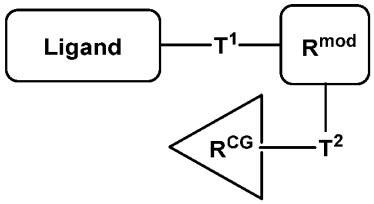
or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

T^1 is a bivalent tethering group; and

R^{mod} is a photoactivatable group; wherein each variable is as defined below.

[00119] In another aspect, the present invention provides a compound of the general Formula **II**:



II

or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

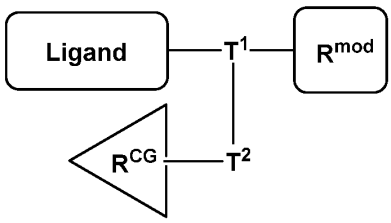
T^1 is a bivalent tethering group;

T^2 is a covalent bond or a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group.

[00120] In another aspect, the present invention provides a compound of the general Formula **III**:



III

or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

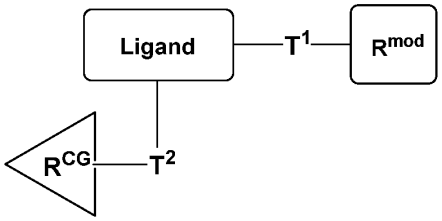
T^1 is a trivalent tethering group;

T^2 is a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group; wherein each variable is as defined below.

[00121] In another aspect, the present invention provides a compound of the general formula **II-a**:



II-a

or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

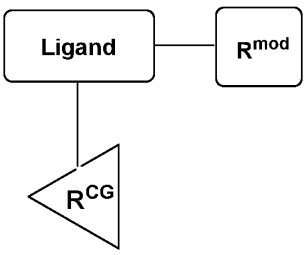
T^1 is a covalent bond or a bivalent tethering group;

T^2 is a covalent bond or a bivalent tethering group;

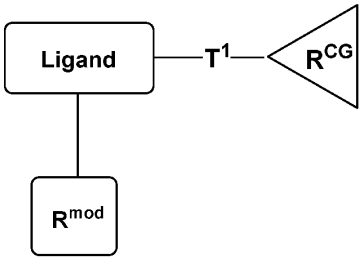
R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group; wherein each variable is as defined below.

[00122] In another aspect, the present invention provides a compound of the general formulae **II-b** or **II-c**:



II-b



II-c

or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

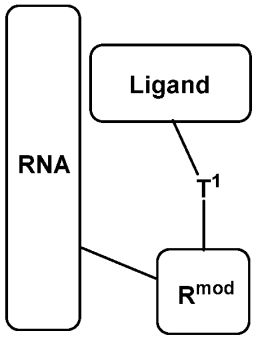
T^1 is a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group; wherein each variable is as defined below.

[00123] In another aspect, the present invention provides a RNA conjugate comprising a target RNA and a compound of any of Formulae **I**, **II**, **II-a**, or **III**, wherein R^{mod} forms a covalent bond to the target RNA after irradiation with visible light or ultraviolet light.

[00124] In some embodiments, the present invention provides a RNA conjugate of Formula **IV**:



IV

wherein **Ligand** is a small molecule that binds to a target RNA;

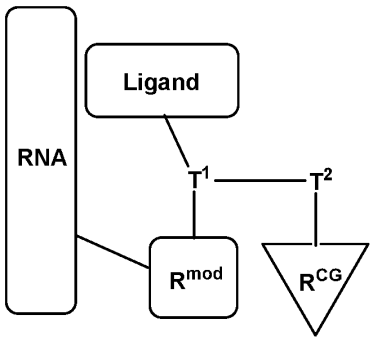
RNA represents the target RNA;

T^1 is a bivalent tethering group; and

R^{mod} is a photoactivatable group;

wherein each variable is as defined below.

[00125] In some embodiments, the present invention provides a RNA conjugate of Formula **V**:



V

wherein **Ligand** is a small molecule that binds to a target RNA;

RNA represents the target RNA;

T^1 is a trivalent tethering group;

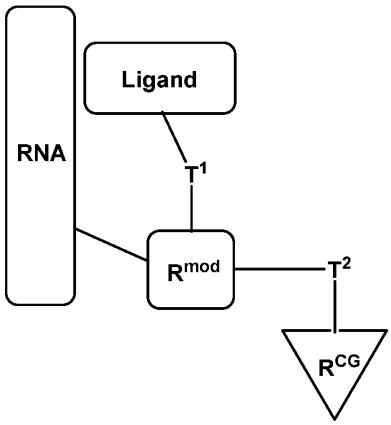
T^2 is a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group;

wherein each variable is as defined below.

[00126] In some embodiments, the present invention provides a RNA conjugate of Formula **VI**:



VI

wherein **Ligand** is a small molecule that binds to a target RNA;

RNA represents the target RNA;

T^1 is a bivalent tethering group;

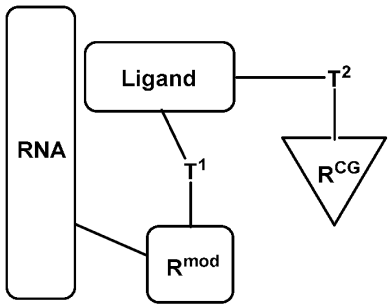
T^2 is a covalent bond or a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group;

wherein each variable is as defined below.

[00127] In some embodiments, the present invention provides a RNA conjugate of Formula **VI-a**:



VI-a

wherein **Ligand** is a small molecule that binds to a target RNA;

RNA represents the target RNA;

T^1 is a bivalent tethering group;

T^2 is a covalent bond or a bivalent tethering group;

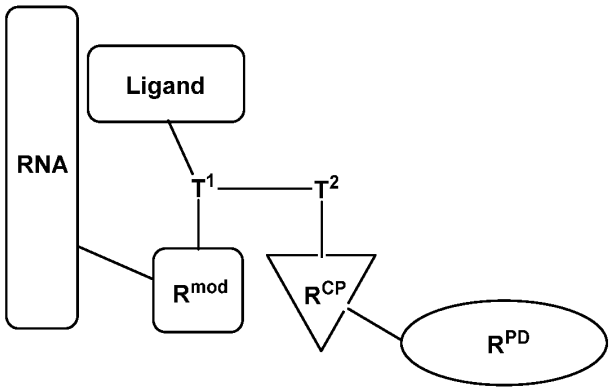
R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group;

wherein each variable is as defined below.

[00128] In another aspect, the present invention provides a conjugate comprising a target RNA, a compound of Formulae **II** or **III**, and a pull-down group, wherein R^{mod} forms a covalent bond to the target RNA.

[00129] In some embodiments, the present invention provides a RNA conjugate of Formula **VII**:



VII

wherein **Ligand** is a small molecule that binds to a target RNA;

RNA represents the target RNA;

T^1 is a trivalent tethering group;

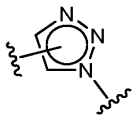
T^2 is a bivalent tethering group;

R^{mod} is a photoactivatable group;

R^{CP} is a reaction product resulting from a click reaction between a click-ready group and an appropriate functional group on R^{PD}; and

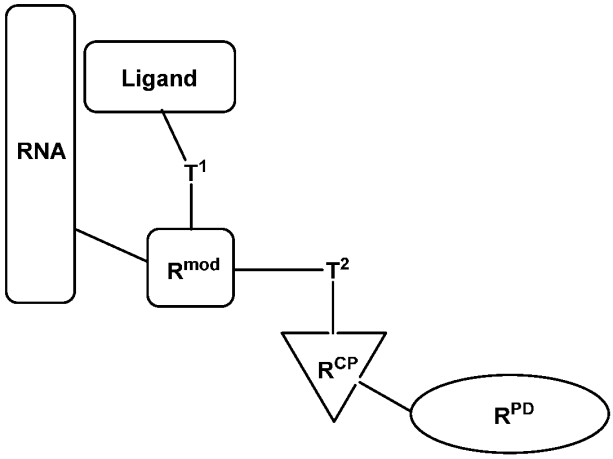
R^{PD} is a pull-down group;

wherein each variable is as defined below. In some embodiments, R^{CP} is



.

[00130] In some embodiments, the present invention provides a RNA conjugate of Formula **VIII**:



VIII

wherein **Ligand** is a small molecule that binds to a target RNA;

RNA represents the target RNA;

T^1 is a bivalent tethering group;

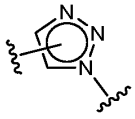
T^2 is a covalent bond or a bivalent tethering group;

R^{mod} is a photoactivatable group;

R^{CP} is a reaction product resulting from a click reaction between a click-ready group and an appropriate functional group on R^{PD}; and

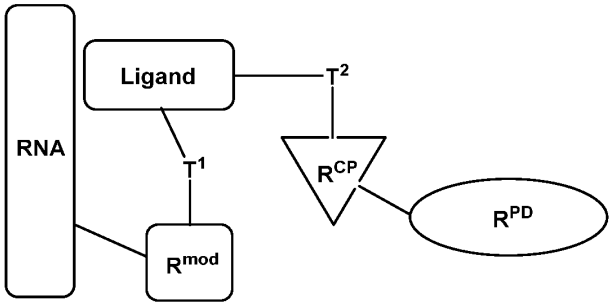
R^{PD} is a pull-down group;

wherein each variable is as defined below. In some embodiments, R^{CP} is



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[00131] In some embodiments, the present invention provides a RNA conjugate of Formula **VIII-a**:



VIII-a

wherein **Ligand** is a small molecule that binds to a target RNA;

RNA represents the target RNA;

T^1 is a bivalent tethering group;

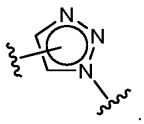
T^2 is a covalent bond or a bivalent tethering group;

R^{mod} is a photoactivatable group;

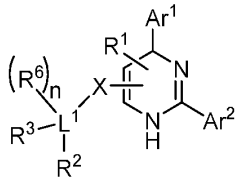
R^{CP} is a reaction product resulting from a click reaction between a click-ready group and an appropriate functional group on R^{PD}; and

R^{PD} is a pull-down group;

wherein each variable is as defined below. In some embodiments, R^{CP} is



[00132] In one aspect, the present invention provides a compound of Formula **X-a**:



X-a

or a tautomer or pharmaceutically acceptable salt thereof, wherein:

Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Ar^2 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6-$, -S-, -C(O)-, $-CO_2-$ -CS-, $-C(NR^6)-$, -S(O)-, or $-S(O)_2-$;

R^1 is selected from $-C(O)R^6$, $-CO_2R$, $-C(O)NR_2$, $-C_{1-6}$ aliphatic, -CN, $-(CH_2)_{1-3}OR$, $-(CH_2)_{1-3}NHR$, $-N(R)C(O)OR^6$, $-N(R^6)C(O)R$, -OC(O)R, -OR, $-NHR^6$, or -N(R)C(O)NHR;

R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent;

R^3 is absent or is a click-ready group or a pull-down group;

each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms;

each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

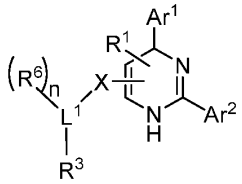
L^1 is a C_{1-20} bivalent, trivalent, or tetravalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, $-SO_2-$, $-SO_2N(R)-$, $-(R)NSO_2-$, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2-$;

each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally

substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

n is 0 or 1.

[00133] In another aspect, the present invention provides a compound of Formula **X-b**:



X-b

or a tautomer or pharmaceutically acceptable salt thereof, wherein:

Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Ar^2 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

wherein one of Ar^1 or Ar^2 is substituted with one R^2;

X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -C(NR^6)-, -S(O)-, or -$S(O)_2$-;

R^1 is selected from -C(O)R^6, -CO_2R, -C(O)NR_2, -C_{1-6} aliphatic, -CN, -$(CH_2)_{1-3}$OR, -$(CH_2)_{1-3}$NHR, -N(R)C(O)OR^6, -N(R^6)C(O)R, -OC(O)R, -OR, -NHR^6, or -N(R)C(O)NHR;

R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent;

R^3 is absent or is a click-ready group or a pull-down group;

each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms;

each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^1 is a C_{1-20} bivalent or trivalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, $-SO_2-$, $-SO_2N(R)-$, $-(R)NSO_2-$, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2-$;

each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

n is 0 or 1.

[00134] As defined generally above, Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00135] In some embodiments, Ar^1 is an optionally substituted phenyl. In some embodiments, Ar^1 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00136] In some embodiments, Ar^1 is phenyl optionally subsituted with 1, 2, 3, or 4 substituents selected from halogen, $-C_{1-6}$ aliphatic, -CN, -OR, $-NR_2$, $-CO_2R$, -C(O)R, -SR, or $-C(O)NR_2$. In some embodiments, the optional substituents are selected from halogen, -CN, $-C_{1-6}$ alkyl, or -OMe. In some embodiments, the optional substituents are halogen. In some embodiments, 1 or 2 substituents are present. In some embodiments, Ar^1 is selected from those depicted in Table 5, below.

[00137] As defined generally above, Ar^2 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00138] In some embodiments, Ar^2 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, Ar^2 is an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00139] In some embodiments, Ar^2 is an optionally substituted pyridinyl, pyrimidinyl, imidazolyl, or pyrrolyl. In some embodiments, Ar^2 is an optionally substituted pyridinyl. In some embodiments, Ar^2 is pyridinyl. In some embodiments, Ar^2 is 3- or 4-pyridinyl. In some embodiments, Ar^2 is selected from those depicted in Table 5, below.

[00140] As defined generally above, X is a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-.

[00141] In some embodiments, X is $-CH_2$-, -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-. In some embodiments, X is a C_2 alkylene chain wherein 1-2 methylene units of

the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -$C(NR^6)$-, -S(O)-, or -$S(O)_2$-. In some embodiments, X is a C_3 alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -$C(NR^6)$-, -S(O)-, or -$S(O)_2$-.

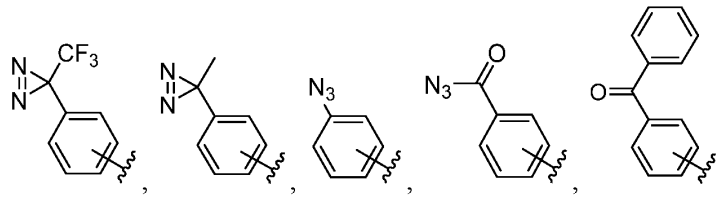
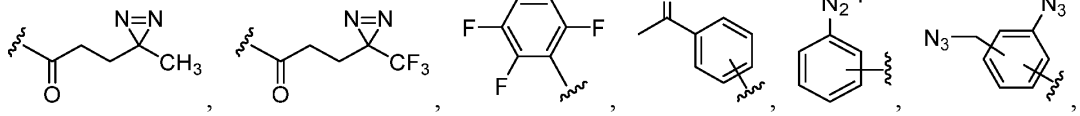
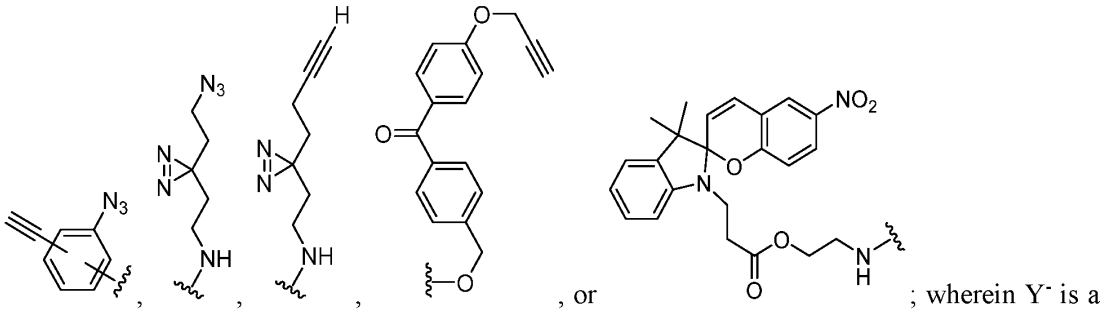
[00142] In some embodiments, X is selected from -CH_2O-, $CH_2C(O)$-, -$C(O)CH_2$-, -$CH_2C(O)O$-, -$C(O)CH_2O$-, -C(O)O-, -$C(O)N(R^6)$-, -$CH_2N(R^6)$-, or -$N(R_6)C(O)$-. In some embodiments, X is -OCH_2- or -CH_2O-. In some embodiments, X is selected from those depicted in Table 5, below.

[00143] As defined generally above, R^1 is selected from -$C(O)R^6$, -CO_2R, -$C(O)NR_2$, -C_{1-6} aliphatic, -CN, -$(CH_2)_{1-3}OR$, -$(CH_2)_{1-3}NHR$, -$N(R)C(O)OR^6$, -$N(R^6)C(O)R$, -OC(O)R, -OR, -NHR^6, or -N(R)C(O)NHR.

[00144] In some embodiments, R^1 is -$C(O)R^6$. In some embodiments, R^1 is -CO_2R. In some embodiments, R^1 is -$C(O)NR_2$. In some embodiments, R^1 is -C_{1-6} aliphatic. In some embodiments, R^1 is –CN. In some embodiments, R^1 is -$(CH_2)_{1-3}OR$. In some embodiments, R^1 is -$(CH_2)_{1-3}NHR$. In some embodiments, R^1 is -$N(R)C(O)OR^6$. In some embodiments, R^1 is -$N(R^6)C(O)R$. In some embodiments, R^1 is -OC(O)R. In some embodiments, R^1 is -OR. In some embodiments, R^1 is -NHR^6. In some embodiments, R^1 is -N(R)C(O)NHR. In some embodiments, R^1 is selected from those depicted in Table 5, below.

[00145] As defined generally above, R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent. In some embodiments, R^2 is a photoactivatable group. In some embodiments, R^2 is a photoactivatable group further substituted with a click-ready group.

[00146] In some embodiments, R^2 is a functional group that generates a radical, an aryl or heteroaryl carbocation, a nitrene, or a carbene intermediate upon irradiation with ultraviolet (UV) radiation, and that is optionally substituted with a click-ready group or pull-down group if R^3 is absent. In some embodiments, R^2 is an optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic azide or 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl azide, optionally substituted benzoyl azide or 5-8 membered heteroaroyl azide or 8-10 membered heteroaroyl azide wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic diazonium salt, optionally substituted 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl

diazonium salt wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted C_{2-6} aliphatic diazo functional group, optionally substituted C_{2-6} aliphatic diazirine, or optionally substituted diphenyl or 8-10-membered diheteroaryl ketone wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted dihydropyrene, optionally substituted spirooxazine, optionally substituted anthracene, optionally substituted fulgide, optionally substituted spiropyran, optionally substituted α-pyrone or optionally substituted pyrimidone; and which is optionally substituted with a click-ready group or pull-down group. In some embodiments, the click-ready group is a C_{1-6} alkyl azide or alkyne. In some embodiments, R^2 is selected from , , ; wherein Y^- is a pharmaceutically acceptable anion.

[00147] In some embodiments, R^2 is selected from those depicted in Table 5, below.

[00148] As defined generally above, R^3 is absent or is a click-ready group or a pull-down group. In some embodiments, R^3 is absent. In some embodiments, R^3 is a click-ready group. In some embodiments, R^3 is a pull-down group. In some embodiments, R^3 is a C_{1-6} alkyl azide, C_{1-6} alkyne, or biotin.

[00149] In some embodiments, R^3 is selected from those depicted in Table 5, below.

[00150] As defined generally above, each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms.

[00151] In some embodiments, R^6 is hydrogen. In some embodiments, R^6 is C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms.

[00152] In some embodiments, R^6 is C_{1-3} alkyl optionally substituted with 1, 2, or 3 halogen atoms.

[00153] In some embodiments, R^6 is selected from those depicted in Table 5, below.

[00154] As defined generally above, L^1 is a C_{1-20} bivalent or trivalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, $-SO_2$-, $-SO_2N(R)$-, $-(R)NSO_2$-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2$-.

[00155] In some embodiments, L^1 is a C_{1-10} bivalent or trivalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, or 5 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, $-SO_2$-, $-SO_2N(R)$-, $-(R)NSO_2$-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-5 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2$-. In some embodiments, L^1 comprises 1-3 natural amino acids. In some embodiments, the amino acids are selected from proline, lysine, glycine, or alanine. In some embodiments, L^1 comprises 1-2 -Cy- groups selected from 1,2,3-triazolylene or 1,2,4-triazolylene. In some embodiments, L^1 comprises 1-10, 1-8, 1-6, 1-4, 1-3, 1-2, 1, 2, or 3 $-OCH_2CH_2$- units.

[00156] In some embodiments, L^1 is selected from those depicted in Table 5, below.

[00157] As defined generally above, each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic

ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00158] In some embodiments, -Cy- is a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring. In some embodiments, -Cy- is an optionally substituted phenylene. In some embodiments, -Cy- is an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, -Cy- is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, -Cy- is an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, -Cy- is an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

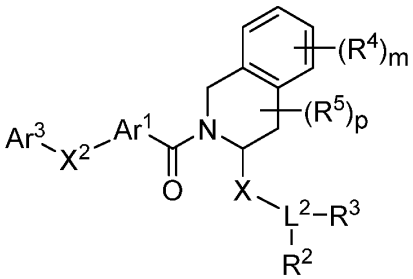
[00159] In some embodiments, -Cy- is phenylene, pyridinylene, pyrimidinylene, 1,2,3-triazolylene, or 1,2,4-triazolylene.

[00160] In some embodiments, -Cy- is selected from those depicted in Table 5, below.

[00161] As defined generally above, n is 0 or 1.

[00162] In some embodiments, n is 0. In some embodiments, n is 1.

[00163] In another aspect, the present invention provides a compound of Formula **XXV**:



XXV

or a pharmaceutically acceptable salt thereof, wherein:

Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Ar^3 is an optionally substituted phenyl, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-12 membered bicyclic aromatic ring, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6-$, -S-, -C(O)-, $-CO_2-$ -CS-, $-C(NR^6)-$, -S(O)-, or $-S(O)_2-$;

X^2 is is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6-$, -S-, -C(O)-, $-CO_2-$ -CS-, $-C(NR^6)-$, -S(O)-, or $-S(O)_2-$;

R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent;

R^3 is absent or is a click-ready group or a pull-down group;

each R^4 is independently R, halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, $-OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, $-N(R)C(O)NR_2$, $-N(R)S(O)_2R$, or $-N(R)S(O)_2NR_2$; or two instances of R^4 may be taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring;

each R^5 is independently R, halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, $-OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, $-N(R)C(O)NR_2$, $-N(R)S(O)_2R$, or $-N(R)S(O)_2NR_2$; or two instances of R^5 may be taken together to form =O or =S;

each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms;

each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

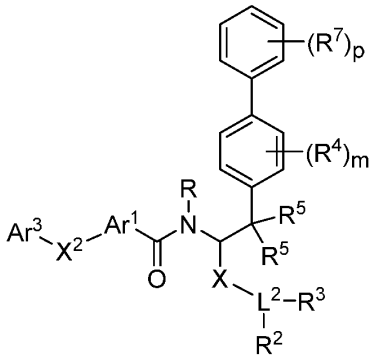
L^2 is a C_{1-20} bivalent or trivalent, straight or branched, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, -SO_2-, -SO_2N(R)-, -(R)NSO_2-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with -OCH_2CH_2-;

each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

m is 0, 1, 2, 3, or 4; and

p is 0, 1, 2, 3, or 4.

[00164] In another aspect, the present invention provides a compound of Formula **XXVI**:



XXVI

or a pharmaceutically acceptable salt thereof, wherein:

Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Ar^3 is an optionally substituted phenyl, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-12 membered bicyclic aromatic ring, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-;

X^2 is is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-;

R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent;

R^3 is absent or is a click-ready group or a pull-down group;

each R^4 is independently R, halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, $-OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, $-N(R)C(O)NR_2$, $-N(R)S(O)_2R$, or $-N(R)S(O)_2NR_2$; or two instances of R^4 may be taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring;

each R^5 is independently R, halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2R$, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, -$OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, -$N(R)C(O)NR_2$, -$N(R)S(O)_2R$, or -$N(R)S(O)_2NR_2$; or two instances of R^5 may be taken together to form =O or =S;

each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms;

each R^7 is independently R, halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2R$, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, -$OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, -$N(R)C(O)NR_2$, -$N(R)S(O)_2R$, or -$N(R)S(O)_2NR_2$; or two instances of R^4 may be taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring;

each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^2 is a C_{1-20} bivalent or trivalent, straight or branched, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, -SO_2-, -$SO_2N(R)$-, -(R)NSO_2-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with -OCH_2CH_2-;

each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring

having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

m is 0, 1, 2, 3, or 4; and

p is 0, 1, 2, 3, or 4.

[00165] As defined generally above, Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00166] In some embodiments, Ar^1 is an optionally substituted phenyl. In some embodiments, Ar^1 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00167] In some embodiments, Ar^1 is phenyl optionally subsituted with 1, 2, 3, or 4 substituents selected from halogen, $-C_{1-6}$ aliphatic, -CN, -OR, $-NR_2$, $-CO_2R$, -C(O)R, -SR, or $-C(O)NR_2$. In some embodiments, the optional substituents are selected from halogen, -CN, $-C_{1-6}$ alkyl, or -OMe. In some embodiments, at least one of the optional substituents is C_{1-6} alkyl. In some embodiments, 1, 2, or 3 substituents are present. In some embodiments, Ar^1 is selected from those depicted in Table 5, below.

[00168] As defined generally above, Ar^3 is an optionally substituted phenyl, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-12 membered bicyclic aromatic ring, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00169] In some embodiments, Ar^3 is an optionally substituted phenyl. In some embodiments, Ar^3 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, Ar^3 is an optionally substituted 8-12 membered bicyclic aromatic ring. In some embodiments, Ar^3 is

an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00170] In some embodiments, Ar^3 is an optionally substituted pyridinyl, pyrimidinyl, imidazolyl, or pyrrolyl. In some embodiments, Ar^3 is an optionally substituted pyridinyl. In some embodiments, Ar^3 is pyridinyl. In some embodiments, the optional substituents are selected from halogen, $-C_{1-6}$ aliphatic, -CN, -OR, $-NR_2$, $-CO_2R$, -C(O)R, -SR, or $-C(O)NR_2$.

[00171] In some embodiments, Ar^3 is phenyl optionally subsituted with 1, 2, 3, or 4 substituents selected from halogen, $-C_{1-6}$ aliphatic, -CN, -OR, $-NR_2$, $-CO_2R$, -C(O)R, -SR, or $-C(O)NR_2$. In some embodiments, the optional substituents are selected from halogen, -CN, $-C_{1-6}$ alkyl, or -OMe. In some embodiments, at least one of the optional substituents is halogen. In some embodiments, 1, 2, or 3 substituents are present. In some embodiments, Ar^3 is phenyl subsituted with 3 substituents selected from halogen, $-C_{1-6}$ aliphatic, and -OR. In some embodiments, Ar^3 is selected from those depicted in Table 5, below.

[00172] As defined generally above, X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-.

[00173] In some embodiments, X is a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-. In some embodiments, X is -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$- (i.e., a C_1 alkylene wherein the methylene unit is replaced with -O-, $-NR^6$-, etc.). In some embodiments, X is a bivalent C_{1-2} alkylene chain wherein one methylene unit of the chain is optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-. In some embodiments, X is -O-, -C(O)-, -C(O)-O-, -O-C(O)-, -NH-C(O)-, or -C(O)-NH-. In some embodiments, X is selected from those depicted in Table 5, below.

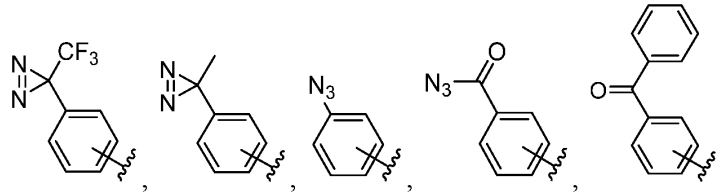
[00174] As defined generally above, X^2 is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-.

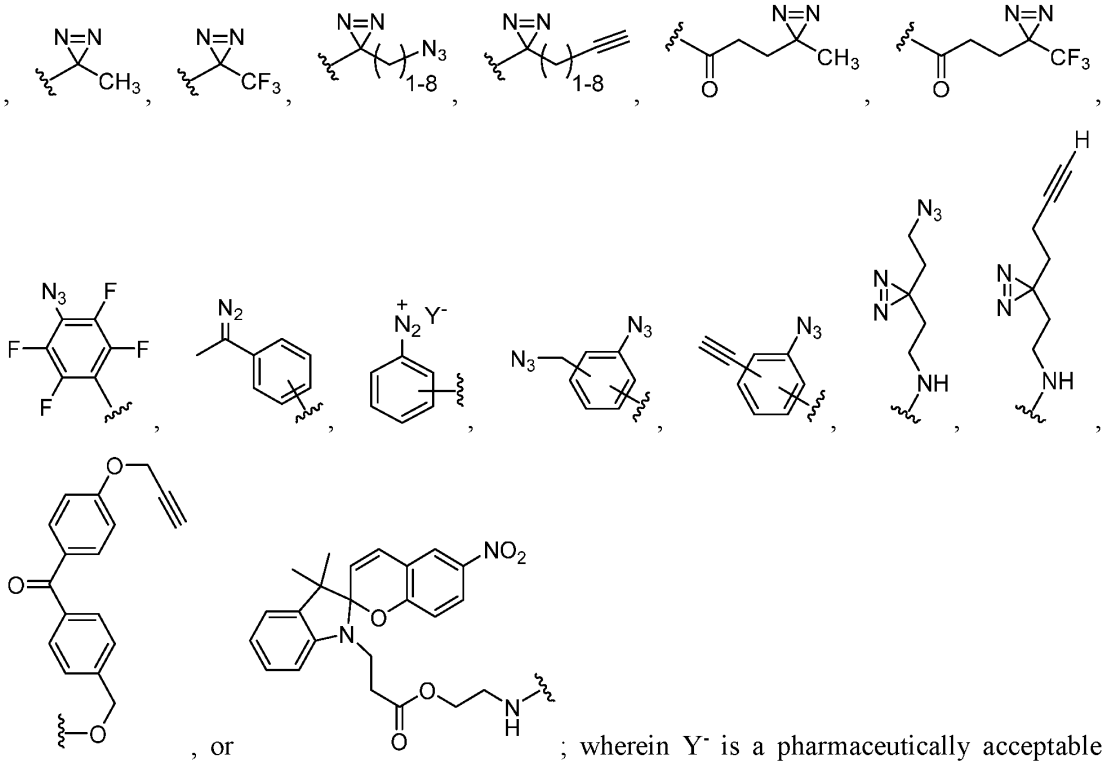
[00175] In some embodiments, X^2 is a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-. In some embodiments, X^2 is -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, -

$C(NR^6)$-, -S(O)-, or $-S(O)_2$- (i.e., a C_1 alkylene wherein the methylene unit is replaced with -O-, $-NR^6$-, etc.). In some embodiments, X^2 is a bivalent C_{1-2} alkylene chain wherein one methylene unit of the chain is optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-. In some embodiments, X^2 is $-CH_2$-NH-, -NH-CH_2-, $-OCH_2$-, $-CH_2O$-, -O-, -C(O)-, -C(O)-O-, -O-C(O)-, -NH-C(O)-, or -C(O)-NH-. In some embodiments, X^2 is selected from those depicted in Table 5, below.

[00176] As defined generally above, R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent. In some embodiments, R^2 is a photoactivatable group. In some embodiments, R^2 is a photoactivatable group further substituted with a click-ready group.

[00177] In some embodiments, R^2 is a functional group that generates a radical, an aryl or heteroaryl carbocation, a nitrene, or a carbene intermediate upon irradiation with ultraviolet (UV) radiation, and that is optionally substituted with a click-ready group or pull-down group if R^3 is absent. In some embodiments, R^2 is an optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic azide or 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl azide, optionally substituted benzoyl azide or 5-8 membered heteroaroyl azide or 8-10 membered heteroaroyl azide wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic diazonium salt, optionally substituted 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl diazonium salt wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted C_{2-6} aliphatic diazo functional group, optionally substituted C_{2-6} aliphatic diazirine, or optionally substituted diphenyl or 8-10-membered diheteroaryl ketone wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted dihydropyrene, optionally substituted spirooxazine, optionally substituted anthracene, optionally substituted fulgide, optionally substituted spiropyran, optionally substituted α-pyrone or optionally substituted pyrimidone; and which is optionally substituted with a click-ready group or pull-down group. In some embodiments, the click-ready group is a C_{1-6} alkyl azide or alkyne. In some embodiments, R^2 is selected from





; wherein Y^- is a pharmaceutically acceptable anion.

[00178] In some embodiments, R^2 is selected from those depicted in Table 5, below.

[00179] As defined generally above, R^3 is absent or is a click-ready group or a pull-down group. In some embodiments, R^3 is absent. In some embodiments, R^3 is a click-ready group. In some embodiments, R^3 is a pull-down group. In some embodiments, R^3 is a C_{1-6} alkyl azide, C_{1-6} alkyne, or a hapten such as biotin.

[00180] In some embodiments, R^3 is selected from those depicted in Table 5, below.

[00181] As defined generally above, each R^4 is independently R, halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, $-OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, $-N(R)C(O)NR_2$, $-N(R)S(O)_2R$, or $-N(R)S(O)_2NR_2$; or two instances of R^4 may be taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring.

[00182] In some embodiments, R^4 is R. In some embodiments, R^4 is halogen. In some embodiments, R^4 is –CN. In some embodiments, R^4 is $-NO_2$. In some embodiments, R^4 is –OR.

In some embodiments, R^4 is –SR. In some embodiments, R^4 is $-NR_2$. In some embodiments, R^4 is $-S(O)_2R$. In some embodiments, R^4 is $-S(O)_2NR_2$. In some embodiments, R^4 is -S(O)R. In some embodiments, R^4 is -C(O)R. In some embodiments, R^4 is -C(O)OR. In some embodiments, R^4 is $-C(O)NR_2$. In some embodiments, R^4 is -C(O)N(R)OR. In some embodiments, R^4 is -OC(O)R. In some embodiments, R^4 is $-OC(O)NR_2$. In some embodiments, R^4 is -N(R)C(O)OR. In some embodiments, R^4 is -N(R)C(O)R. In some embodiments, R^4 is $-N(R)C(O)NR_2$. In some embodiments, R^4 is $-N(R)S(O)_2R$. In some embodiments, R^4 is $-N(R)S(O)_2NR_2$. In some embodiments, two instances of R^4 are taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring.

[00183] In some embodiments, R^4 is hydrogen, C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$, -OC(O)R, or -N(R)C(O)R.

[00184] In some embodiments, R^4 is hydrogen, C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms; a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, -OR, $-NR_2$, $-S(O)_2NR_2$, -C(O)R, -C(O)OR, $-C(O)NR_2$, -OC(O)R, or -N(R)C(O)R. In some embodiments, R^4 is hydrogen, C_{1-6} alkyl, phenyl, halogen, -CN, -OR, or $-NR_2$. In some embodiments, R^4 is selected from those depicted in Table 5, below.

[00185] As defined generally above, each R^5 is independently R, halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$,

-C(O)N(R)OR, -OC(O)R, -OC(O)NR_2, -N(R)C(O)OR, -N(R)C(O)R, -N(R)C(O)NR_2, -N(R)$S(O)_2$R, or -N(R)$S(O)_2NR_2$; or two instances of R^5 may be taken together to form =O or =S.

[00186] In some embodiments, R^5 is R. In some embodiments, R^5 is halogen. In some embodiments, R^5 is –CN. In some embodiments, R^5 is -NO_2. In some embodiments, R^5 is –OR. In some embodiments, R^5 is –SR. In some embodiments, R^5 is -NR_2. In some embodiments, R^5 is -$S(O)_2$R. In some embodiments, R^5 is -$S(O)_2NR_2$. In some embodiments, R^5 is -S(O)R. In some embodiments, R^5 is -C(O)R. In some embodiments, R^5 is -C(O)OR. In some embodiments, R^5 is -C(O)NR_2. In some embodiments, R^5 is -C(O)N(R)OR. In some embodiments, R^5 is -OC(O)R. In some embodiments, R^5 is -OC(O)NR_2. In some embodiments, R^5 is -N(R)C(O)OR. In some embodiments, R^5 is -N(R)C(O)R. In some embodiments, R^5 is -N(R)C(O)NR_2. In some embodiments, R^5 is -N(R)$S(O)_2$R. In some embodiments, R^5 is -N(R)$S(O)_2NR_2$. In some embodiments, two instances of R^5 are taken together to form =O or =S.

[00187] In some embodiments, R^5 is hydrogen, C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2$R, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -C(O)NR_2, -OC(O)R, or -N(R)C(O)R.

[00188] In some embodiments, R^5 is hydrogen, C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms; a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, -OR, -NR_2, -$S(O)_2NR_2$, -C(O)R, -C(O)OR, -C(O)NR_2, -OC(O)R, or -N(R)C(O)R. In some embodiments, R^5 is hydrogen, C_{1-6} alkyl, phenyl, halogen, -CN, -OR, or -NR_2.

[00189] As defined generally above, each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms.

[00190] In some embodiments, R^6 is hydrogen. In some embodiments, R^6 is C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms.

[00191] In some embodiments, R^6 is C_{1-3} alkyl optionally substituted with 1, 2, or 3 halogen atoms.

[00192] In some embodiments, R^6 is selected from those depicted in Table 5, below.

[00193] As defined generally above, each R^7 is independently R, halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, $-OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, $-N(R)C(O)NR_2$, $-N(R)S(O)_2R$, or $-N(R)S(O)_2NR_2$; or two instances of R^7 may be taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring.

[00194] In some embodiments, R^7 is R. In some embodiments, R^7 is halogen. In some embodiments, R^7 is –CN. In some embodiments, R^7 is $-NO_2$. In some embodiments, R^7 is –OR. In some embodiments, R^7 is –SR. In some embodiments, R^7 is $-NR_2$. In some embodiments, R^7 is $-S(O)_2R$. In some embodiments, R^7 is $-S(O)_2NR_2$. In some embodiments, R^7 is -S(O)R. In some embodiments, R^7 is -C(O)R. In some embodiments, R^7 is -C(O)OR. In some embodiments, R^7 is $-C(O)NR_2$. In some embodiments, R^7 is -C(O)N(R)OR. In some embodiments, R^7 is -OC(O)R. In some embodiments, R^7 is $-OC(O)NR_2$. In some embodiments, R^7 is -N(R)C(O)OR. In some embodiments, R^7 is -N(R)C(O)R. In some embodiments, R^7 is $-N(R)C(O)NR_2$. In some embodiments, R^7 is $-N(R)S(O)_2R$. In some embodiments, R^7 is $-N(R)S(O)_2NR_2$. In some embodiments, two instances of R^7 are taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring.

[00195] In some embodiments, R^7 is hydrogen, C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms

independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2R$, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -OC(O)R, or -N(R)C(O)R.

[00196] In some embodiments, R^7 is hydrogen, C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms; a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, -OR, -NR_2, -$S(O)_2NR_2$, -C(O)R, -C(O)OR, -$C(O)NR_2$, -OC(O)R, or -N(R)C(O)R. In some embodiments, R^7 is hydrogen, C_{1-6} alkyl, phenyl, halogen, -CN, -OR, or -NR_2. In some embodiments, R^7 is selected from those depicted in Table 5, below.

[00197] As defined generally above, L^2 is a C_{1-20} bivalent or trivalent, straight or branched, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, -SO_2-, -$SO_2N(R)$-, -$(R)NSO_2$-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with -OCH_2CH_2-.

[00198] In some embodiments, L^2 is a C_{1-10} bivalent or trivalent, straight or branched, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, or 5 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, -SO_2-, -$SO_2N(R)$-, -$(R)NSO_2$-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-5 of the methylene units of the chain are independently and optionally replaced with -OCH_2CH_2-. In some embodiments, L^2 comprises 1-3 natural amino acids. In some embodiments, the amino acids are selected from proline, lysine, glycine, or alanine. In some embodiments, L^2 comprises 1-2 -Cy- groups selected from 1,2,3-triazolylene or 1,2,4-triazolylene. In some embodiments, L^2 comprises 1-10, 1-8, 1-6, 1-4, 1-3, 1-2, 1, 2, or 3 -OCH_2CH_2- units.

[00199] In some embodiments, L^2 is a C_{1-20} bivalent or trivalent, straight or branched, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, or 5 methylene units of the chain are independently and optionally replaced with -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -S-, -SO-, $-SO_2-$, -C(S)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2-$.

[00200] In some embodiments, L^2 is a C_{1-20} bivalent or trivalent, straight, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, or 5 methylene units of the chain are independently and optionally replaced with -O-, -C(O)-, -N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2-$.

[00201] In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 methylene units of the chain are replaced with $-OCH_2CH_2-$. In some embodiments, 1, 2, 3, 4, 5, 6, 7, or 8; or 1, 2, 3, 4, or 5 methylene units of the chain are replaced with $-OCH_2CH_2-$.

[00202] In some embodiments, L^2 is selected from those depicted in Table 5, below.

[00203] As defined generally above, each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00204] In some embodiments, -Cy- is a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring. In some embodiments, -Cy- is an optionally substituted phenylene. In some embodiments, -Cy- is an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, -Cy- is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, -Cy- is an

optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, -Cy- is an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

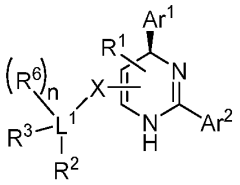
[00205] In some embodiments, -Cy- is phenylene, pyridinylene, pyrimidinylene, 1,2,3-triazolylene, or 1,2,4-triazolylene.

[00206] In some embodiments, -Cy- is selected from those depicted in Table 5, below.

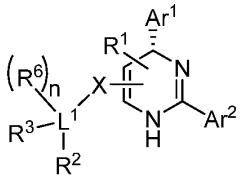
[00207] As defined generally above, m is 0, 1, 2, 3, or 4. In some embodiments, m is 0. In some embodiments, m is 1. In some embodiments, m is 2. In some embodiments, m is 3. In some embodiments, m is 4. In some embodiments, m is 0, 1, 2, or 3. In some embodiments, m is 0, 1, or 2. In some embodiments, m is 1 or 2.

[00208] As defined generally above, p is 0, 1, 2, 3, or 4. In some embodiments, p is 0. In some embodiments, p is 1. In some embodiments, p is 2. In some embodiments, p is 3. In some embodiments, p is 4. In some embodiments, p is 0, 1, 2, or 3. In some embodiments, p is 0, 1, or 2. In some embodiments, p is 1 or 2.

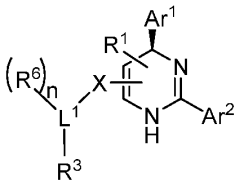
[00209] In some embodiments, the present invention provies a compound of Formulae **X-c**, **X-d**, **X-e**, or **X-f**:



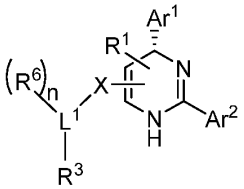
X-c



X-d



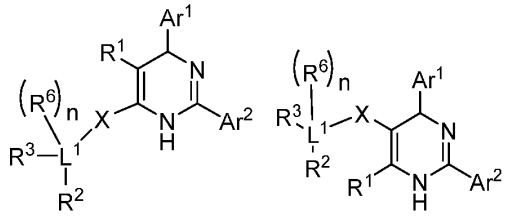
X-e



X-f

or a tautomer or pharmaceutically acceptable salt thereof, wherein each of Ar^1, Ar^2, X, L^1, R^1, R^2, R^3, R^6, R, -Cy-, and n is as defined above and described in embodiments herein, both singly and in combination.

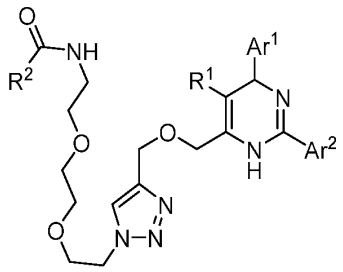
[00210] In some embodiments, the present invention provides a compound of Formulae **XI** or **XII**:



XI **XII**

or a tautomer or pharmaceutically acceptable salt thereof, wherein each of Ar^1, Ar^2, X, L^1, R^1, R^2, R^3, R^6, R, -Cy-, and n is as defined above and described in embodiments herein, both singly and in combination.

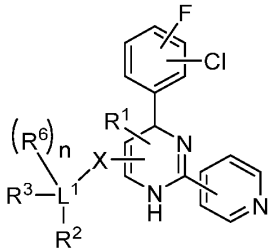
[00211] In some embodiments, the present invention provides a compound of Formula **XIII**:



XIII

or a tautomer or pharmaceutically acceptable salt thereof, wherein each of Ar^1, Ar^2, R^1, R^2, R^6, R, and -Cy- is as defined above and described in embodiments herein, both singly and in combination.

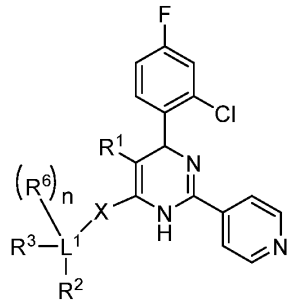
[00212] In some embodiments, the present invention provides a compound of Formula **XIV**:



XIV

or a tautomer or pharmaceutically acceptable salt thereof, wherein each of X, L^1, R^1, R^2, R^3, R^6, R, -Cy-, and n is as defined above and described in embodiments herein, both singly and in combination.

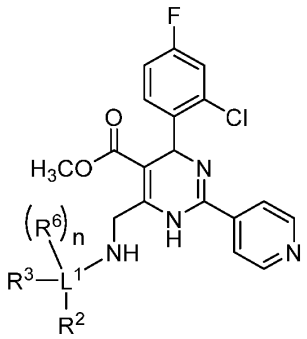
[00213] In some embodiments, the present invention provides a compound of Formula **XV**:



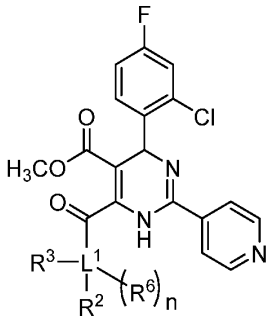
XV

or a tautomer or pharmaceutically acceptable salt thereof, wherein each of X, L^1, R^1, R^2, R^3, R^6, R, -Cy-, and n is as defined above and described in embodiments herein, both singly and in combination.

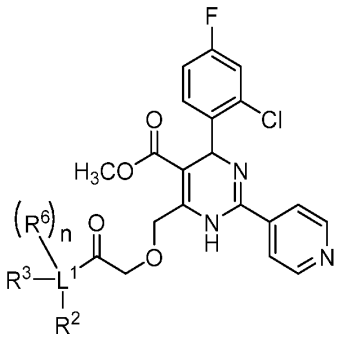
[00214] In some embodiments, the present invention provides a compound of Formulae **XVI**, **XVII**, **XVIII**, or **XIX**:



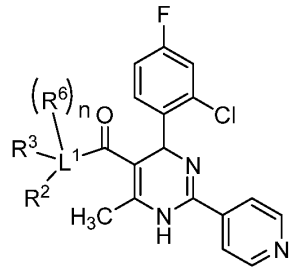
XVI



XVII



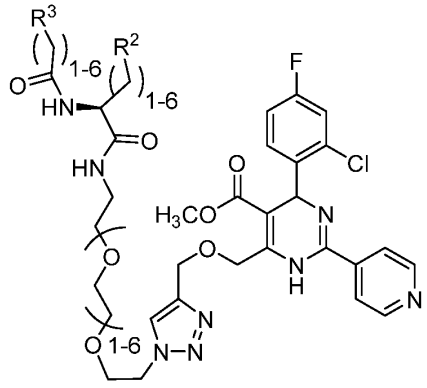
XVIII



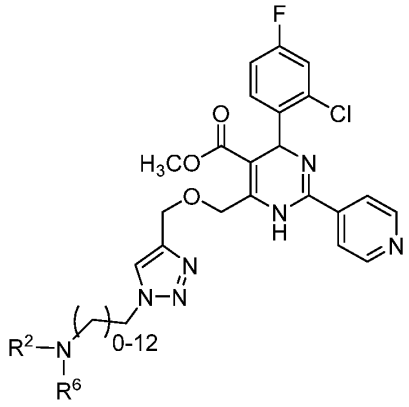
XIX

or a tautomer or pharmaceutically acceptable salt thereof, wherein each of L^1, R^1, R^2, R^3, R^6, R, -Cy-, and n is as defined above and described in embodiments herein, both singly and in combination.

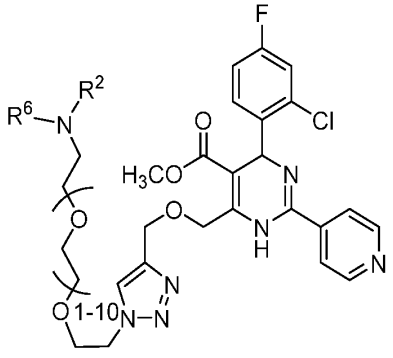
[00215] In some embodiments, the present invention provides a compound of Formulae **XX**, **XXI**, **XXII**, **XXIII**, or **XXIV**:



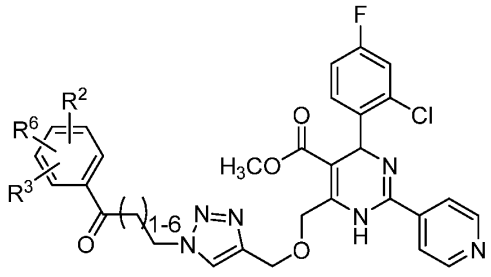
XX



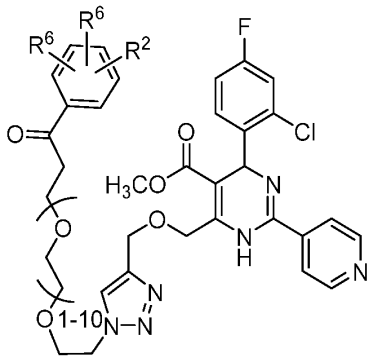
XXI



XXII



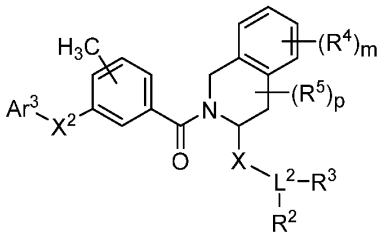
XXIII



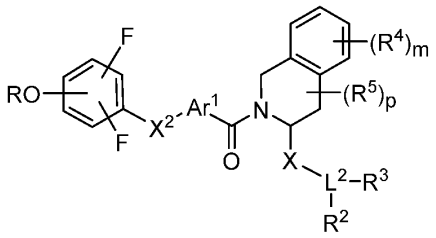
XXIV

or a tautomer or pharmaceutically acceptable salt thereof, wherein each of R^2, R^3, R^6, R, -Cy-, and n is as defined above and described in embodiments herein, both singly and in combination.

[00216] In some embodiments, the present invention provides a compound of Formulae **XXVII** or **XXVIII**:



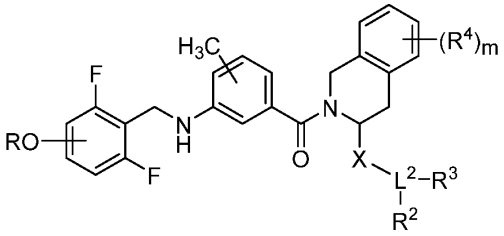
XXVII



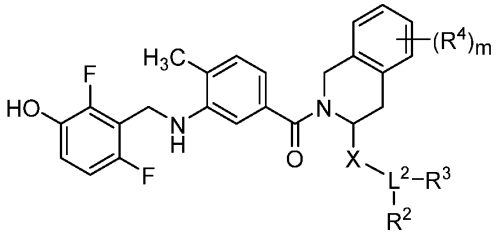
XXVIII

or a pharmaceutically acceptable salt thereof, wherein each of Ar^1, Ar^3, X, X^2, L^2, R^2, R^3, R^4, R^5, R^6, R, -Cy-, m, and p is as defined above and described in embodiments herein, both singly and in combination.

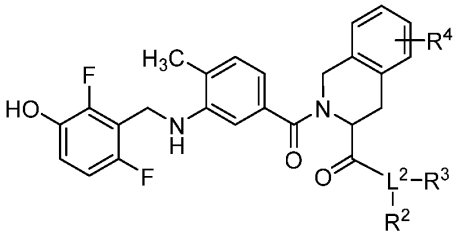
[00217] In some embodiments, the present invention provides a compound of Formulae **XXIX**, **XXX**, **XXXI**, **XXXII**, or **XXXIII**:



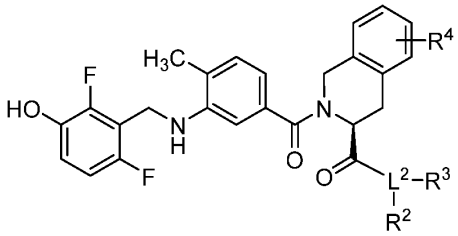
XXIX



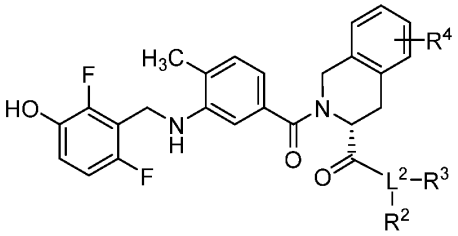
XXX



XXXI



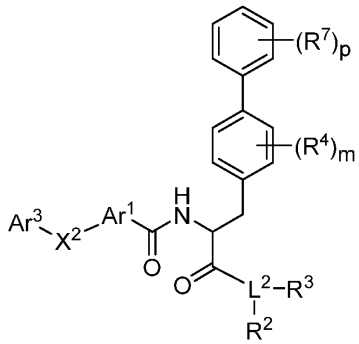
XXXII



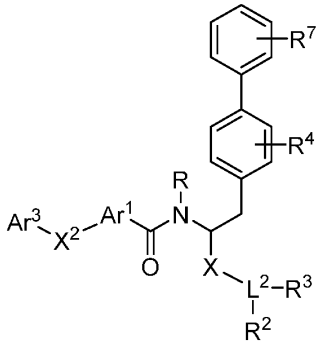
XXXIII

or a pharmaceutically acceptable salt thereof, wherein each of X, X^2, L^2, R^2, R^3, R^4, R^6, R, -Cy-, and m is as defined above and described in embodiments herein, both singly and in combination.

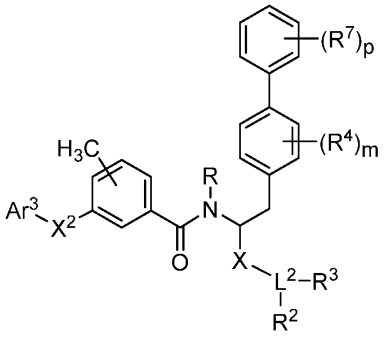
[00218] In some embodiments, the present invention provides a compound of Formulae **XXXIV**, **XXXV**, **XXXVI**, or **XXXVII**:



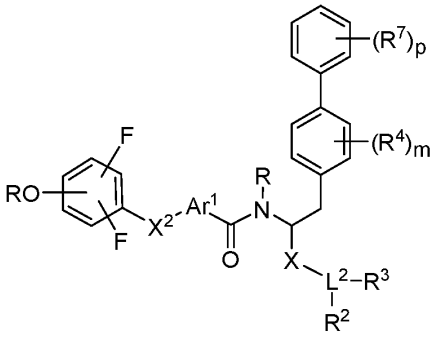
XXXIV



XXXV



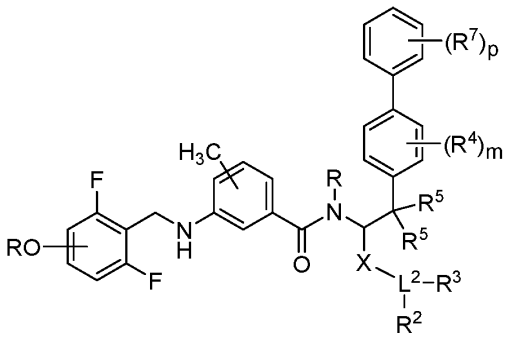
XXXVI



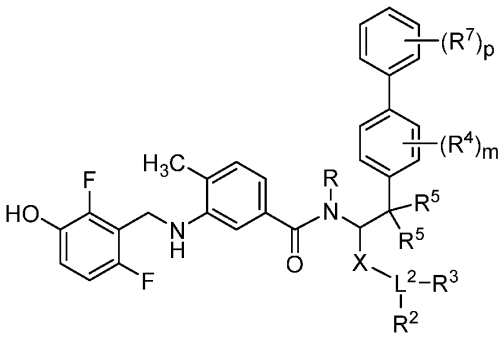
XXXVII

or a pharmaceutically acceptable salt thereof, wherein each of Ar^1, Ar^3, X, X^2, L^2, R^2, R^3, R^4, R^6, R^7, R, -Cy-, m, and p is as defined above and described in embodiments herein, both singly and in combination.

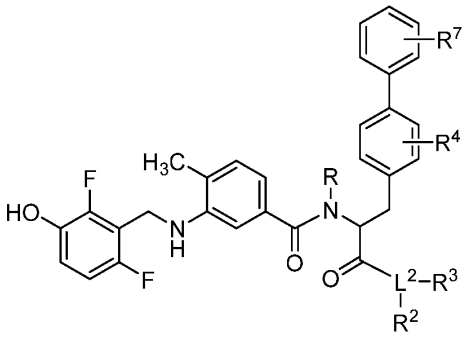
[00219] In some embodiments, the present invention provides a compound of Formulae **XXXVIII**, **XXXIX**, **XL**, **XLI**, or **XLII**:



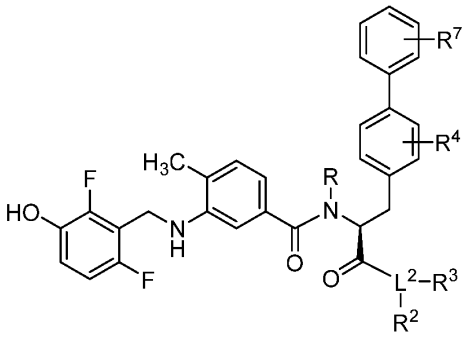
XXXVIII



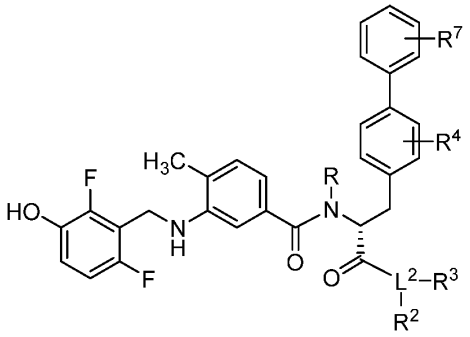
XXXIX



XL



XLI

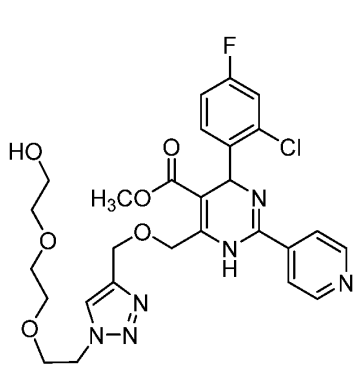


XLII

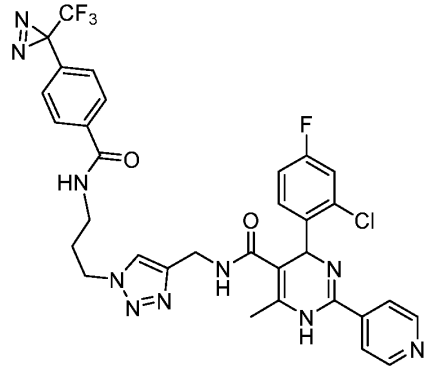
or a pharmaceutically acceptable salt thereof, wherein each of X, L^2, R^2, R^3, R^4, R^6, R^7, R, -Cy-, m, and p is as defined above and described in embodiments herein, both singly and in combination.

[00220] Exemplary compounds of the invention are set forth in Table 5, below.

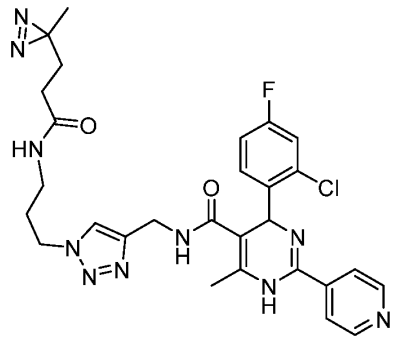
Table 5: Exemplary Compounds



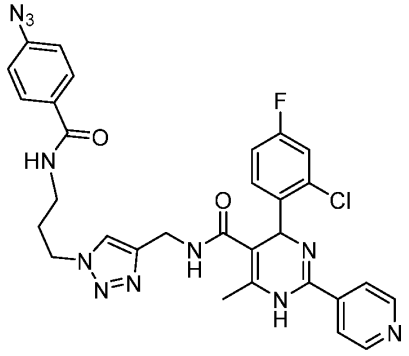
I-1 (ARK-139)



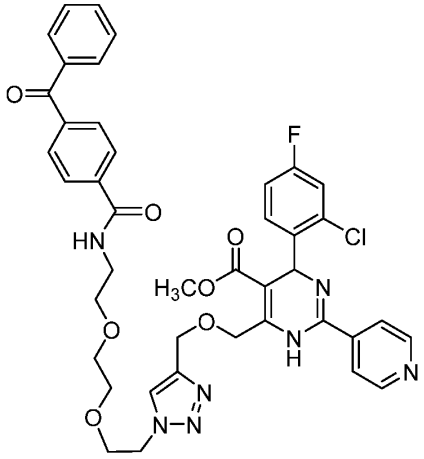
I-2 (ARK-673)



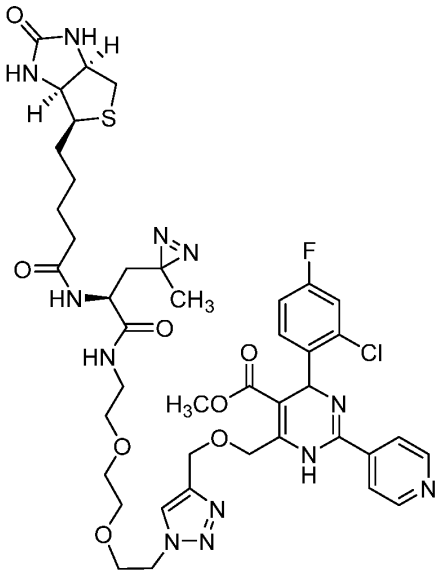
I-3 (ARK-674)



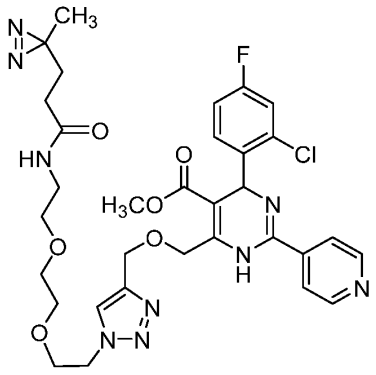
I-4 (ARK-672)



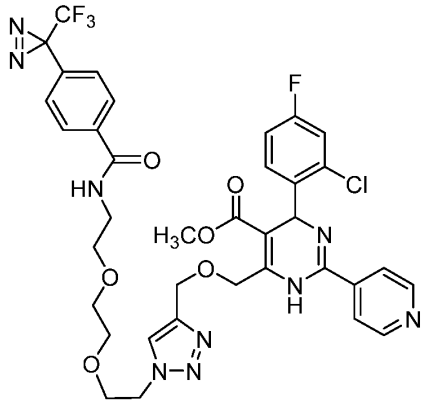
I-5 (ARK-544)



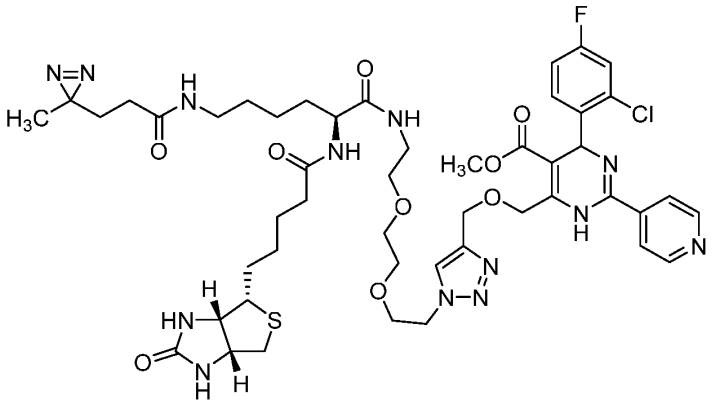
I-6 (ARK-546)



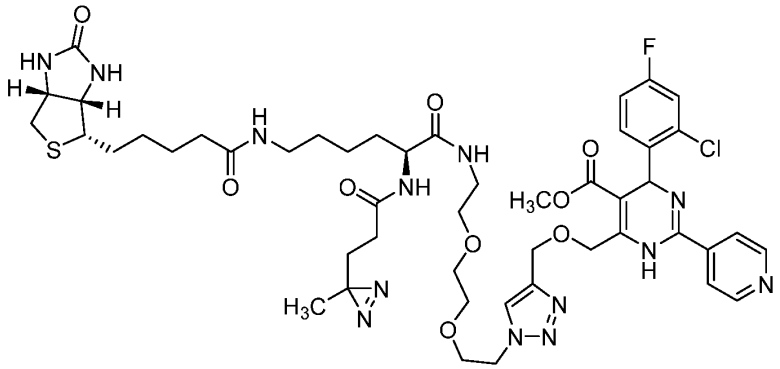
I-7 (ARK-547)



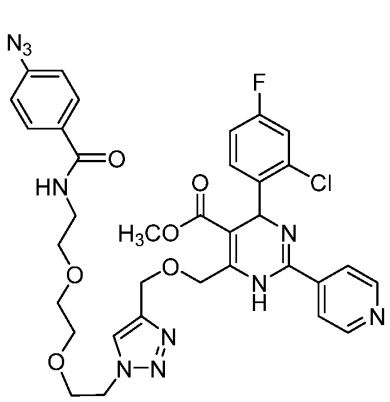
I-8 (ARK-549)



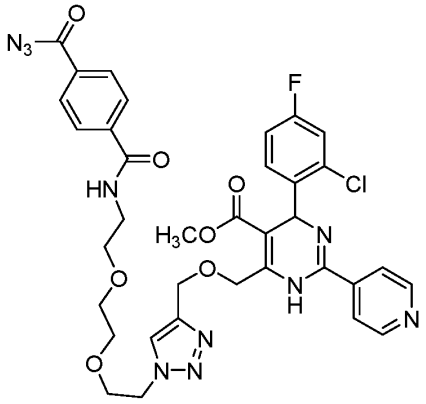
I-9 (ARK-579)



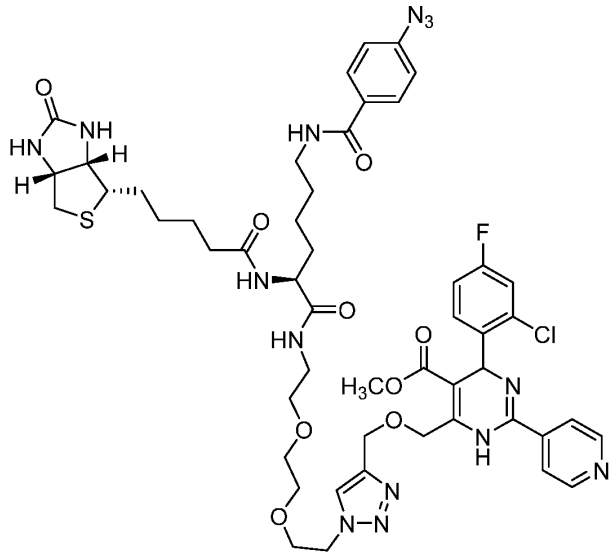
I-10 (ARK-580)



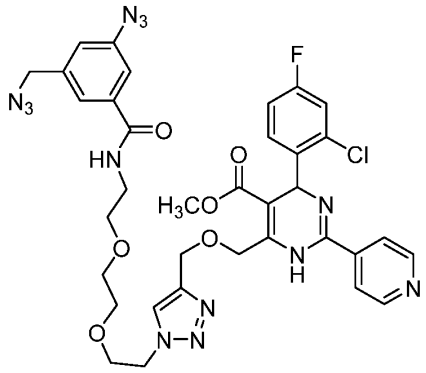
I-11 (ARK-581)



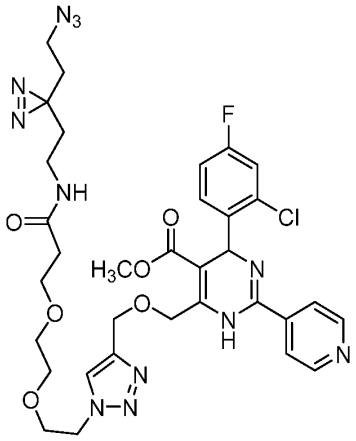
I-12



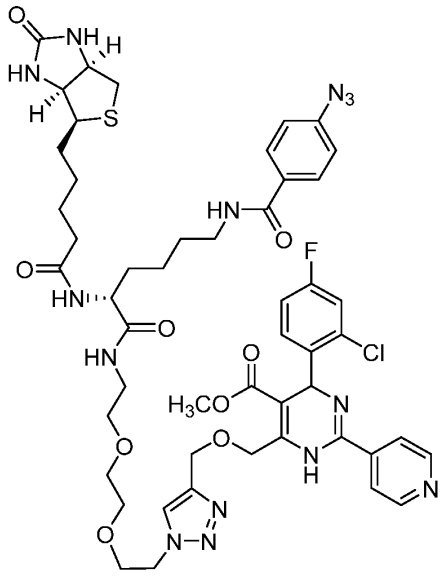
I-13 (ARK-670)



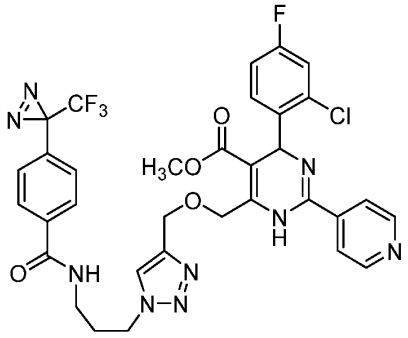
I-14 (ARK-729)



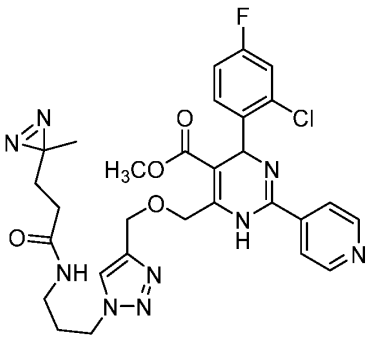
I-15 (ARK-816)



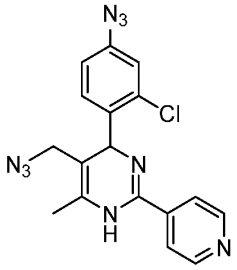
I-16 (ARK-671)



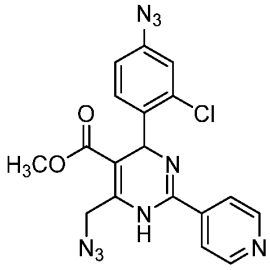
I-17 (ARK-669)



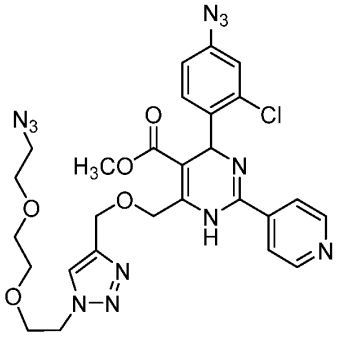
I-18 (ARK-668)



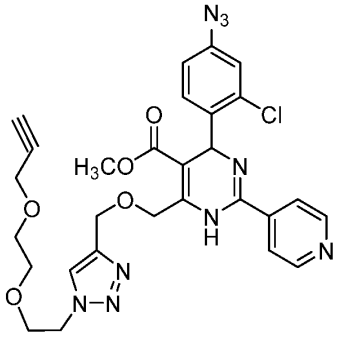
I-19



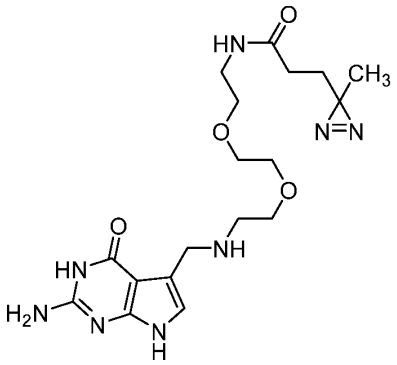
I-20



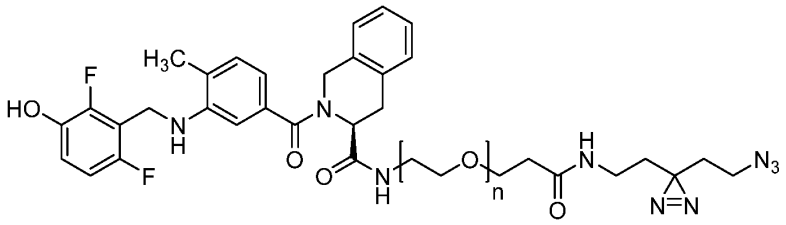
I-21



I-22

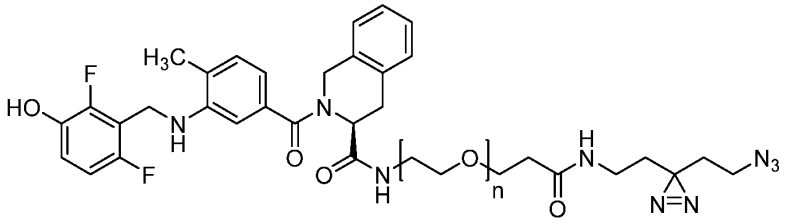


I-23



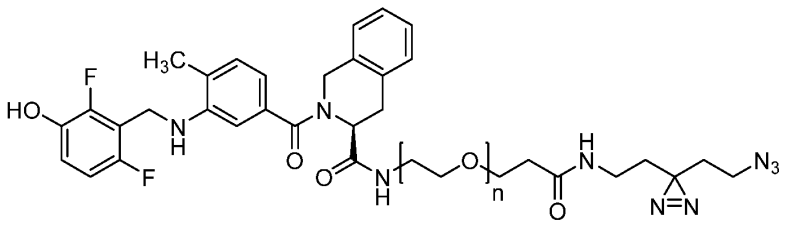
n = 1

I-24



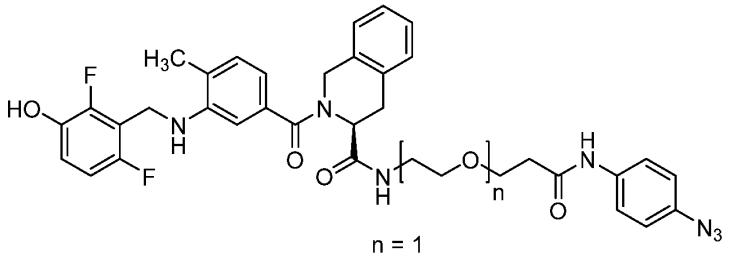
n = 2

I-25

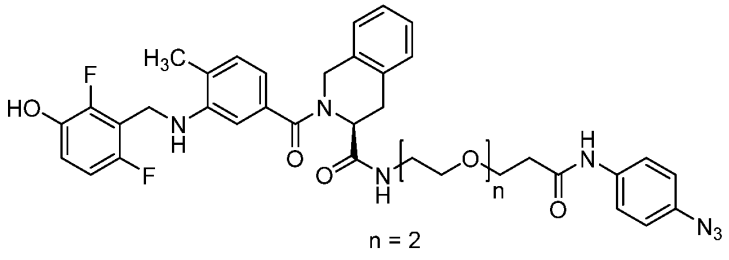


n = 4

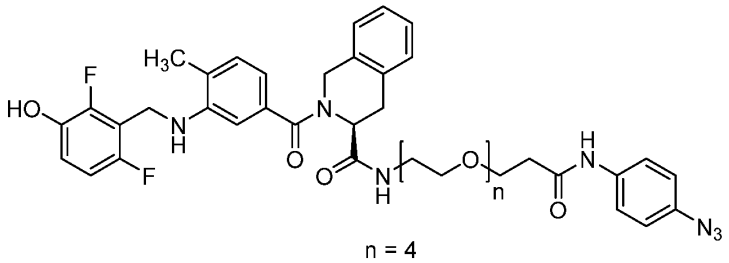
I-26



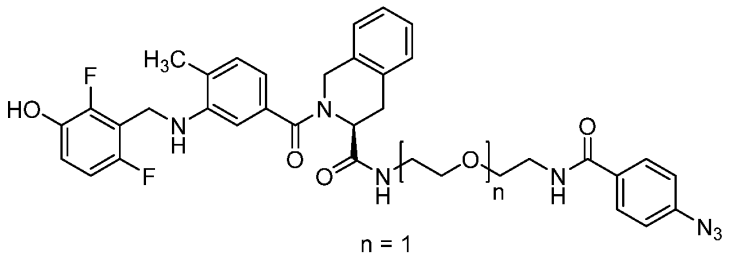
I-27



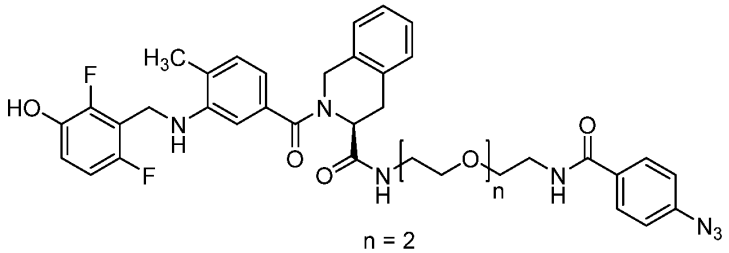
I-28



I-29

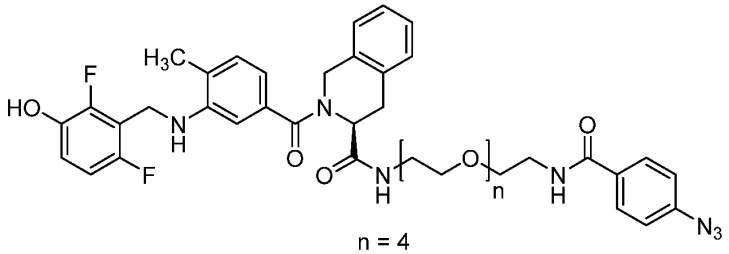


I-30



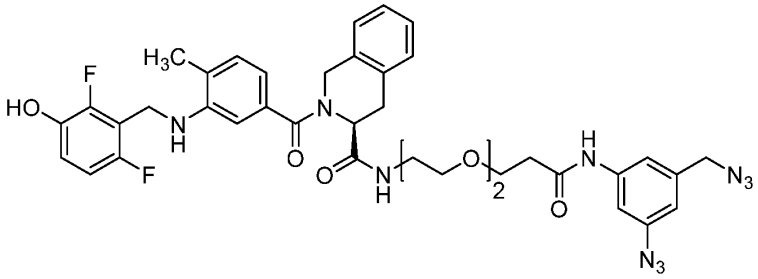
n = 2

I-31

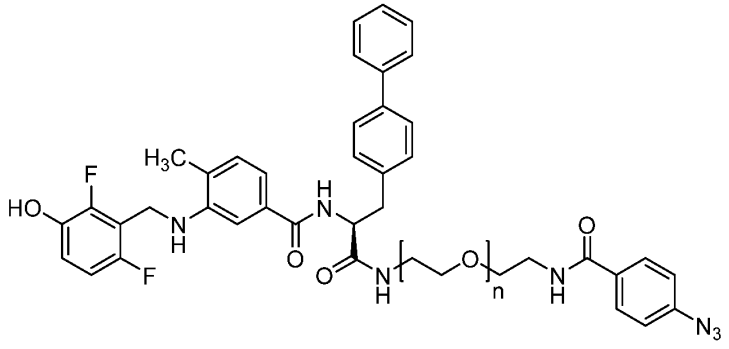


n = 4

I-32

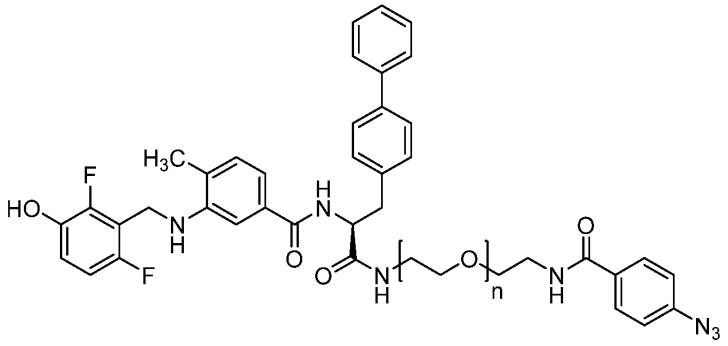


I-33



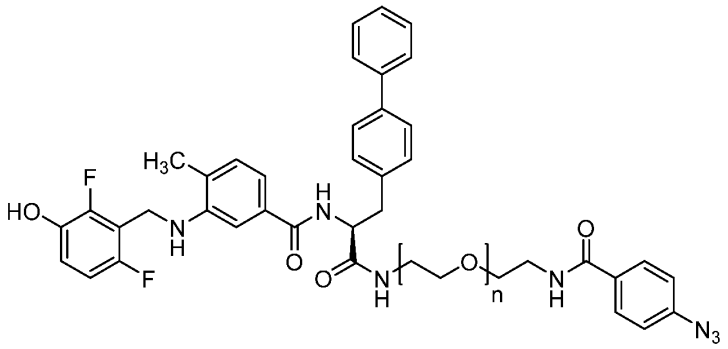
n = 1

I-34



n = 2

I-35



n = 4

I-36

[00221] In some embodiments, the present invention provides a compound set forth in **Table 1**, above, or a pharmaceutically acceptable salt thereof.

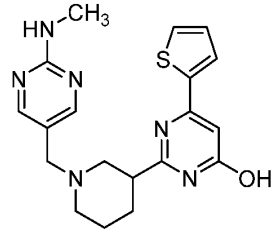
[00222] In some embodiments, the compound or conjugate is selected from those formulae shown in FIGS. 1-44, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof.

Small Molecule RNA Ligands

[00223] The design and synthesis of novel, small molecule ligands capable of binding RNA represents largely untapped therapeutic potential. In some embodiments, the small molecule ligand is selected from a compound known to bind to RNA, such as a heteroaryldihydropyrimidine (HAP), a macrolide (e.g., erythromycin, azithromycin), alkaloid (e.g., berberine, palmatine), aminoglycoside (e.g., paromomycin, neomycin B, kanamycin A), tetracycline (e.g., doxycycline,

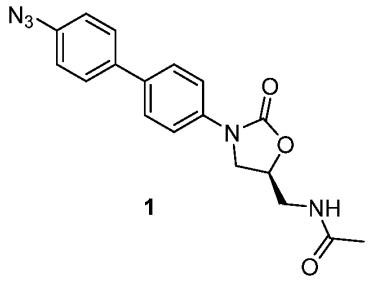
oxytetracycline), a theophylline, ribocil, clindamycin, chloramphenicol, LMI070, a triptycene-based scaffold, an oxazolidinone (e.g., linezolid, tedizolid), or CPNQ.

[00224] In some embodiments, the small molecule ligand is ribocil, which has the following structure:

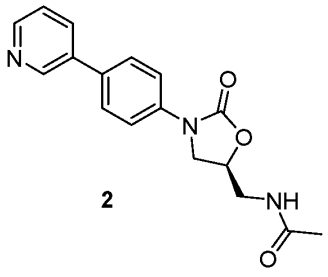


or a pharmaceutically acceptable salt thereof. Ribocil is a a drug-like ligand that binds to the FMN riboswitch (PDB 5KX9) and inhibits riboswitch function (*Nature* **2015**, *526*, 672–677).

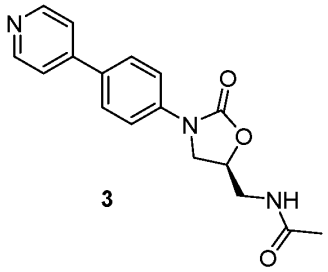
[00225] In some embodiments, the small molecule ligand is an oxazolidinone such as linezolid, tedizolid, eperezolid, or PNU 176798. Exemplary oxazolidinone-based photoprobes are described in, e.g., Matassova, N. B. *et al., RNA* (1999), 5:939–946; Leach, K. L. *et al., Molecular Cell* **2007**, 26, 393–402; Colca, J. R. *et al., J. Biol. Chem.* **2003**, *278 (24)*, 21972–21979; and each of which is hereby incorporated by reference. Such oxazolidinones include the following:



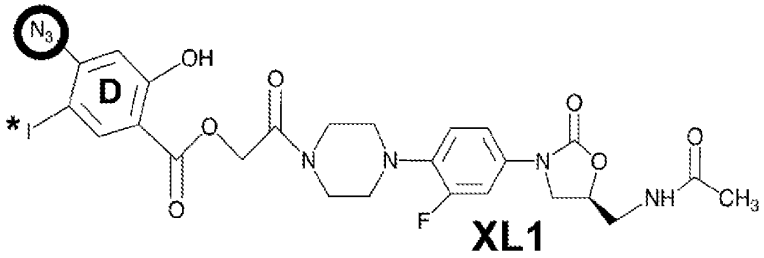
1



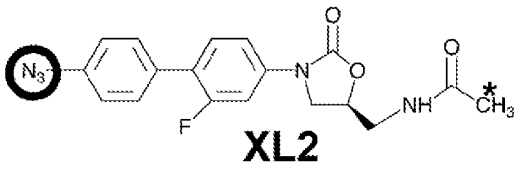
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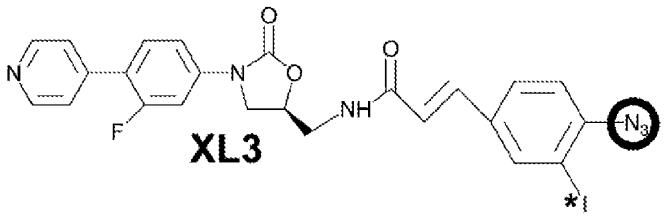
3



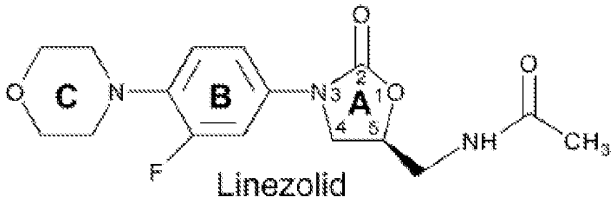
XL1



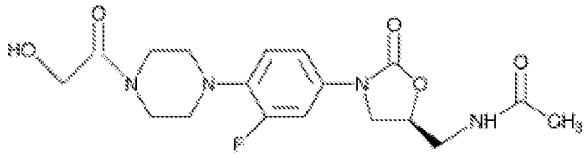
XL2



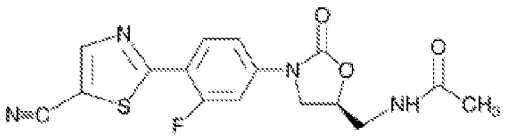
XL3



Linezolid



Eperezolid

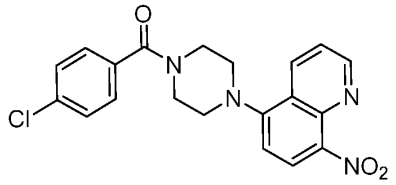


PNU 176798

The foregoing oxazolidinones may be substituted with a photoactivatable group as described herein at any available position, optionally with a tether linking the photoactivatable group with the oxazolidinone. Azide photoactivatable groups are optionally replaced with other photoactivatable groups described herein. The asterisks (*) indicate the position of a ^{125}I or ^{14}C radioligand used in the original reference, and which is optional for use as a pull-down group in the present invention.

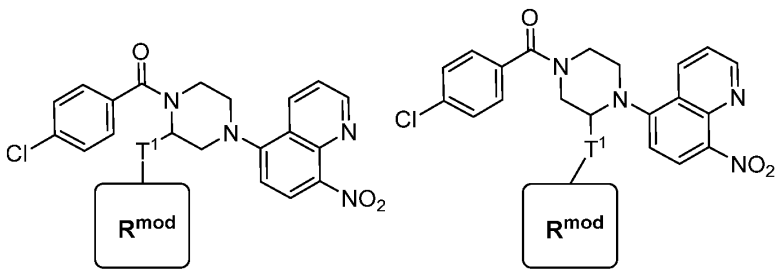
[00226] Aryldiazonium salts, for example the *p*-diazonium anilide of L-5-carboxyspermine and L-2-carboxyputrescine, have also been shown to be useful as photoactivatable probes for RNA mapping and footprinting of RNA/protein interaction. See, e.g., Garcia, A. *et al., Nucleic Acids Res.* **1990**, *18 (1)*, 89-95.

[00227] Furthermore, certain compounds comprising a quinoline core, of which CPNQ is one, are capable of binding RNA. CPNQ has the following structure:



[00228] Accordingly, in some embodiments, the small molecule ligand is selected from CPNQ or a pharmaceutically acceptable salt thereof. In other embodiments, the ligand is selected from a quinoline compound related to CPNQ, such as those provided in any one of FIGS. 36 or 39-43; or a pharmaceutically acceptable salt thereof.

[00229] In some embodiments, CPNQ or a quinoline related to CPNQ is modified at one or more available positions to replace a hydrogen with a tether (-T^1- and/or -T^2-), click-ready group (-R^{CG}), or warhead (-R^{mod}), according to embodiments of each as described herein. For example, CPNQ or a quinoline related to CPNQ may have one of the following formulae:



IX-a **IX-b**

or a pharmaceutically acceptable salt thereof; wherein R^{mod} is optionally substituted with -R^{CG} or -T^2-R^{CG}, and further optionally substituted with a pull-down group. The compound of formulae **IX-a** or **IX-b** may further be optionally substituted with one or more optional substituents, as defined below, such as 1 or 2 optional substituents.

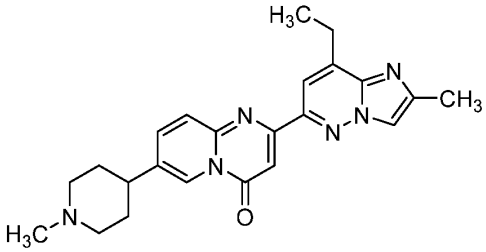
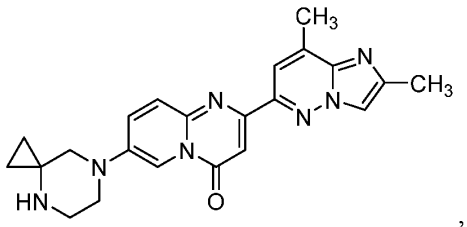
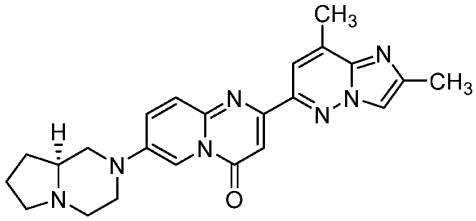
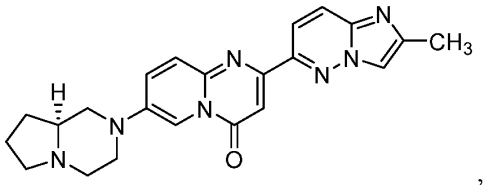
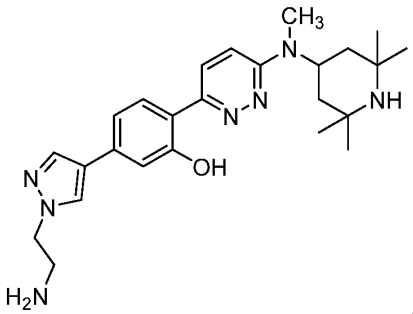
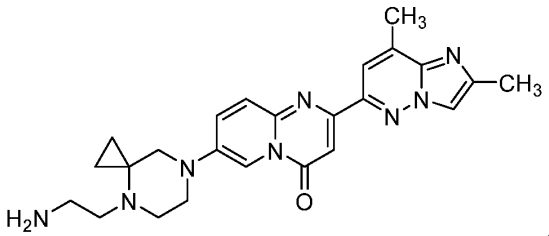
[00230] Organic dyes, amino acids, biological cofactors, metal complexes as well as peptides also show RNA binding ability. It is possible to modulate RNAs such as riboswitches, RNA molecules with expanded nucleotide repeats, and viral RNA elements.

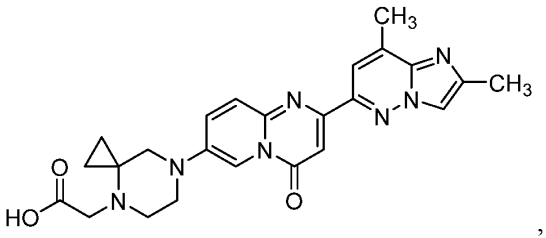
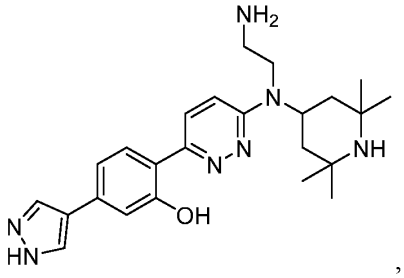
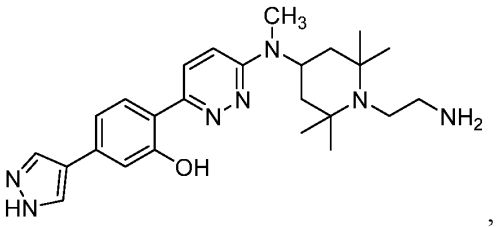
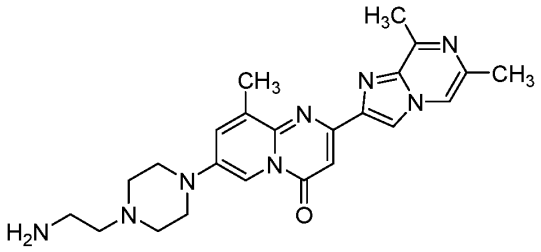
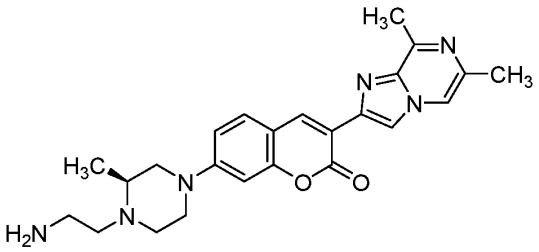
[00231] The term "small molecule that binds a target RNA," "small molecule RNA binder," "affinity moiety," "ligand," or "ligand moiety," as used herein, includes all compounds generally classified as small molecules that are capable of binding to a target RNA with sufficient affinity and specificity for use in a disclosed method, or to treat, prevent, or ameliorate a disease associated with the target RNA. Small molecules that bind RNA for use in the present invention may bind to one or more secondary or tertiary structure elements of a target RNA. These sites include RNA triplexes, hairpins, bulge loops, pseudoknots, internal loops, junctions, and other higher-order RNA structural motifs described or referred to herein.

[00232] Accordingly, in some embodiments, the small molecule that binds to a target RNA (e.g., **Ligand** in Formulae **I-VIII** above) is selected from a heteroaryldihydropyrimidine (HAP), a macrolide, alkaloid, aminoglycoside, a member of the tetracycline family, an oxazolidinone, a SMN2 pre-mRNA ligand such as LMI070 (NVS-SM1), ribocil or an analogue thereof, clindamycin, chloramphenicol, an anthracene, a triptycene, theophylline or an analogue thereof,

or CPNQ or an analogue thereof. In some embodiments, the small molecule that binds to a target RNA is selected from paromomycin, a neomycin (such as neomycin B), a kanamycin (such as kanamycin A), linezolid, tedizolid, pleuromutilin, ribocil, anthracene, triptycene, or CPNQ or an analogue thereof; wherein each small molecule may be optionally substituted with one or more "optional substituents" as defined below, such as 1, 2, 3, or 4, for example 1 or 2, optional substituents. In some embodiments, the small molecule is selected from those shown in FIGS. 1-8 or 18-44, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof.

[00233] In some embodiments, the **Ligand** is selected from


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, or

; or a pharmaceutically acceptable salt thereof.

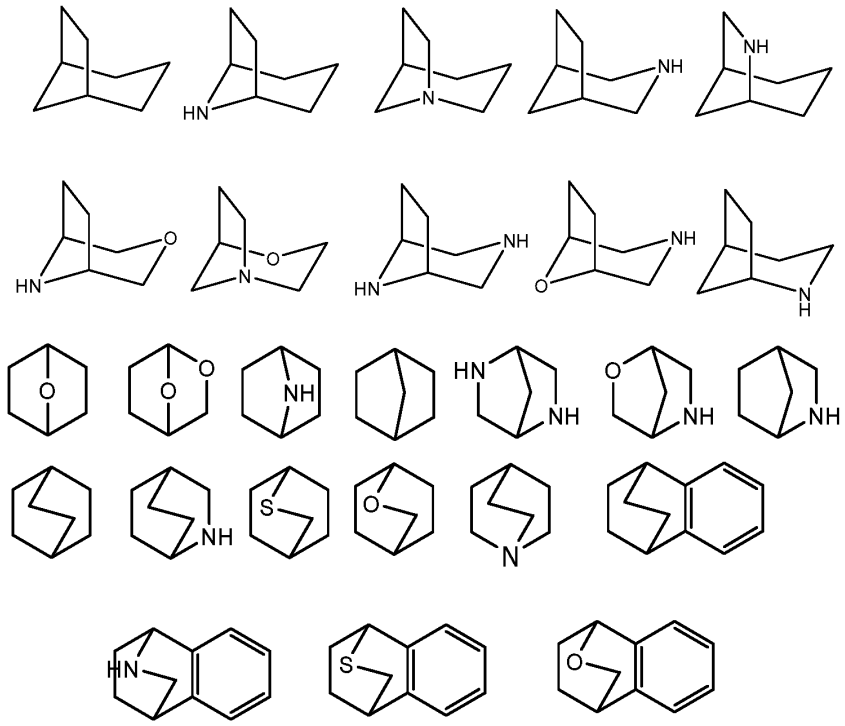
[00234] In some embodiments, the **Ligand** binds to a junction, stem-loop, or bulge in a target RNA. In some embodiments, **Ligand** binds to a nucleic acid three-way junction (3WJ). In some embodiments, the 3WJ is a trans 3WJ between two RNA molecules. In some embodiments, the 3WJ is a trans 3WJ between a miRNA and mRNA. In some embodiments, the **Ligand** binds to DNA, such as a DNA loop or junction.

[00235] Compounds of the present invention include those described generally herein, and are further illustrated by the classes, subclasses, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and

March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[00236] The term "aliphatic" or "aliphatic group," as used herein, means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon or bicyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as "carbocycle," "cycloaliphatic" or "cycloalkyl"), that has a single point of attachment to the rest of the molecule. Unless otherwise specified, aliphatic groups contain 1-6 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-4 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-3 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1-2 aliphatic carbon atoms. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic C_3-C_6 hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[00237] As used herein, the term "bridged bicyclic" refers to any bicyclic ring system, i.e. carbocyclic or heterocyclic, saturated or partially unsaturated, having at least one bridge. As defined by IUPAC, a "bridge" is an unbranched chain of atoms or an atom or a valence bond connecting two bridgeheads, where a "bridgehead" is any skeletal atom of the ring system which is bonded to three or more skeletal atoms (excluding hydrogen). In some embodiments, a bridged bicyclic group has 7-12 ring members and 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Such bridged bicyclic groups are well known in the art and include those groups set forth below where each group is attached to the rest of the molecule at any substitutable carbon or nitrogen atom. Unless otherwise specified, a bridged bicyclic group is optionally substituted with one or more substituents as set forth for aliphatic groups. Additionally or alternatively, any substitutable nitrogen of a bridged bicyclic group is optionally substituted. Exemplary bridged bicyclics include:



[00238] The term "lower alkyl" refers to a C_{1-4} straight or branched alkyl group. Exemplary lower alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[00239] The term "lower haloalkyl" refers to a C_{1-4} straight or branched alkyl group that is substituted with one or more halogen atoms.

[00240] The term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon (including, any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2*H*-pyrrolyl), NH (as in pyrrolidinyl) or NR^+ (as in N-substituted pyrrolidinyl)).

[00241] The term "unsaturated", as used herein, means that a moiety has one or more units of unsaturation.

[00242] As used herein, the term "bivalent C_{1-8} (or C_{1-6}) saturated or unsaturated, straight or branched, hydrocarbon chain," refers to bivalent alkylene, alkenylene, and alkynylene chains that are straight or branched as defined herein.

[00243] The term "alkylene" refers to a bivalent alkyl group. An "alkylene chain" is a polymethylene group, i.e., $-(CH_2)_n-$, wherein n is a positive integer, preferably from 1 to 6, from

1 to 4, from 1 to 3, from 1 to 2, or from 2 to 3. A substituted alkylene chain is a polymethylene group in which one or more methylene hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[00244] The term "alkenylene" refers to a bivalent alkenyl group. A substituted alkenylene chain is a polymethylene group containing at least one double bond in which one or more hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[00245] The term "halogen" means F, Cl, Br, or I.

[00246] The term "aryl" used alone or as part of a larger moiety as in "aralkyl," "aralkoxy," or "aryloxyalkyl," refers to monocyclic or bicyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains 3 to 7 ring members. The term "aryl" may be used interchangeably with the term "aryl ring." In certain embodiments of the present invention, "aryl" refers to an aromatic ring system which includes, but not limited to, phenyl, biphenyl, naphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term "aryl," as it is used herein, is a group in which an aromatic ring is fused to one or more non–aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

[00247] The terms "heteroaryl" and "heteroar–," used alone or as part of a larger moiety, e.g., "heteroaralkyl," or "heteroaralkoxy," refer to groups having 5 to 10 ring atoms, preferably 5, 6, or 9 ring atoms; having 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. The term "heteroatom" refers to nitrogen, oxygen, or sulfur, and includes any oxidized form of nitrogen or sulfur, and any quaternized form of a basic nitrogen. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, and pteridinyl. The terms "heteroaryl" and "heteroar–", as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring. Nonlimiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4*H*–quinolizinyl,

carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[2,3-*b*]-1,4-oxazin-3(4*H*)-one. A heteroaryl group may be mono- or bicyclic. The term "heteroaryl" may be used interchangeably with the terms "heteroaryl ring," "heteroaryl group," or "heteroaromatic," any of which terms include rings that are optionally substituted. The term "heteroaralkyl" refers to an alkyl group substituted with a heteroaryl, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[00248] As used herein, the terms "heterocycle," "heterocyclyl," "heterocyclic radical," and "heterocyclic ring" are used interchangeably and refer to a stable 5– to 7-membered monocyclic or 7–10-membered bicyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, preferably one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes a substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0–3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4–dihydro–2*H*–pyrrolyl), NH (as in pyrrolidinyl), or $^{+}$NR (as in *N*–substituted pyrrolidinyl).

[00249] A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothiophenyl, pyrrolidinyl, piperidinyl, pyrrolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl. The terms "heterocycle," "heterocyclyl," "heterocyclyl ring," "heterocyclic group," "heterocyclic moiety," and "heterocyclic radical," are used interchangeably herein, and also include groups in which a heterocyclyl ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3*H*–indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl. A heterocyclyl group may be mono– or bicyclic. The term "heterocyclylalkyl" refers to an alkyl group substituted with a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

[00250] As used herein, the term "partially unsaturated" refers to a ring moiety that includes at least one double or triple bond. The term "partially unsaturated" is intended to encompass rings

having multiple sites of unsaturation, but is not intended to include aryl or heteroaryl moieties, as herein defined.

[00251] As described herein, compounds of the invention may contain "optionally substituted" moieties. In general, the term "substituted," whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent ("optional substituent") at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[00252] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $–(CH_2)_{0–4}R°$; $–(CH_2)_{0–4}OR°$; $-O(CH_2)_{0-4}R^o$, $–O–(CH_2)_{0–4}C(O)OR°$; $–(CH_2)_{0–4}CH(OR°)_2$; $–(CH_2)_{0–4}SR°$; $–(CH_2)_{0–4}Ph$, which may be substituted with R°; $–(CH_2)_{0–4}O(CH_2)_{0–1}Ph$ which may be substituted with R°; –CH=CHPh, which may be substituted with R°; $–(CH_2)_{0–4}O(CH_2)_{0–1}$-pyridyl which may be substituted with R°; $–NO_2$; –CN; $–N_3$; $-(CH_2)_{0–4}N(R°)_2$; $–(CH_2)_{0–4}N(R°)C(O)R°$; $–N(R°)C(S)R°$; $–(CH_2)_{0–4}N(R°)C(O)NR°_2$; $-N(R°)C(S)NR°_2$; $–(CH_2)_{0–4}N(R°)C(O)OR°$; $–N(R°)N(R°)C(O)R°$; $-N(R°)N(R°)C(O)NR°_2$; $-N(R°)N(R°)C(O)OR°$; $–(CH_2)_{0–4}C(O)R°$; $–C(S)R°$; $–(CH_2)_{0–4}C(O)OR°$; $–(CH_2)_{0–4}C(O)SR°$; $-(CH_2)_{0–4}C(O)OSiR°_3$; $–(CH_2)_{0–4}OC(O)R°$; $–OC(O)(CH_2)_{0–4}SR–$, SC(S)SR°; $–(CH_2)_{0–4}SC(O)R°$; $–(CH_2)_{0–4}C(O)NR°_2$; $–C(S)NR°_2$; –C(S)SR°; –SC(S)SR°, $-(CH_2)_{0–4}OC(O)NR°_2$; -C(O)N(OR°)R°; –C(O)C(O)R°; $–C(O)CH_2C(O)R°$; –C(NOR°)R°; $-(CH_2)_{0–4}SSR°$; $–(CH_2)_{0–4}S(O)_2R°$; $–(CH_2)_{0–4}S(O)_2OR°$; $–(CH_2)_{0–4}OS(O)_2R°$; $–S(O)_2NR°_2$; $-(CH_2)_{0–4}S(O)R°$; $-N(R°)S(O)_2NR°_2$; $–N(R°)S(O)_2R°$; –N(OR°)R°; –C(NH)NR°$_2$; $–P(O)_2R°$; $-P(O)R°_2$; $-OP(O)R°_2$; $–OP(O)(OR°)_2$; $SiR°_3$; $–(C_{1–4}$ straight or branched alkylene)O–$N(R°)_2$; or $–(C_{1–4}$ straight or branched alkylene)C(O)O–$N(R°)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, $C_{1–6}$ aliphatic, $–CH_2Ph$, $–O(CH_2)_{0–}$

$_1$Ph, -CH_2-(5-6 membered heteroaryl ring), or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[00253] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $–(CH_2)_{0–2}R^•$, –(halo$R^•$), $–(CH_2)_{0–2}OH$, $–(CH_2)_{0–2}OR^•$, $–(CH_2)_{0–2}CH(OR^•)_2$; -O(halo$R^•$), –CN, $–N_3$, $–(CH_2)_{0–2}C(O)R^•$, $–(CH_2)_{0–2}C(O)OH$, $–(CH_2)_{0–2}C(O)OR^•$, $–(CH_2)_{0–2}SR^•$, $–(CH_2)_{0–2}SH$, $–(CH_2)_{0–2}NH_2$, $–(CH_2)_{0–2}NHR^•$, $–(CH_2)_{0–2}NR^•_2$, $–NO_2$, $–SiR^•_3$, $–OSiR^•_3$, -C(O)S$R^•$, –($C_{1–4}$ straight or branched alkylene)C(O)O$R^•$, or –SS$R^•$ wherein each $R^•$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from $C_{1–4}$ aliphatic, $–CH_2Ph$, $–O(CH_2)_{0–1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[00254] Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, $=NNR^*_2$, $=NNHC(O)R^*$, $=NNHC(O)OR^*$, $=NNHS(O)_2R^*$, $=NR^*$, $=NOR^*$, $–O(C(R^*_2))_{2–3}O–$, or $–S(C(R^*_2))_{2–3}S–$, wherein each independent occurrence of R^* is selected from hydrogen, $C_{1–6}$ aliphatic which may be substituted as defined below, or an unsubstituted 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: $–O(CR^*_2)_{2–3}O–$, wherein each independent occurrence of R^* is selected from hydrogen, $C_{1–6}$ aliphatic which may be substituted as defined below, or an unsubstituted 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00255] Suitable substituents on the aliphatic group of R^* include halogen, $–R^•$, -(halo$R^•$), -OH, $–OR^•$, –O(halo$R^•$), –CN, –C(O)OH, $–C(O)OR^•$, $–NH_2$, $–NHR^•$, $–NR^•_2$, or $–NO_2$, wherein each $R^•$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens,

and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00256] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include $-R^{\dagger}$, $-NR^{\dagger}_2$, $-C(O)R^{\dagger}$, $-C(O)OR^{\dagger}$, $-C(O)C(O)R^{\dagger}$, $-C(O)CH_2C(O)R^{\dagger}$, $-S(O)_2R^{\dagger}$, $-S(O)_2NR^{\dagger}_2$, $-C(S)NR^{\dagger}_2$, $-C(NH)NR^{\dagger}_2$, or $-N(R^{\dagger})S(O)_2R^{\dagger}$; wherein each $R^{\dagger}$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted –OPh, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^{\dagger}$, taken together with their intervening atom(s) form an unsubstituted 3–12-membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00257] Suitable substituents on the aliphatic group of $R^{\dagger}$ are independently halogen, $-R^{\bullet}$, -(halo$R^{\bullet}$), –OH, $-OR^{\bullet}$, -O(halo$R^{\bullet}$), –CN, –C(O)OH, $-C(O)OR^{\bullet}$, $-NH_2$, $-NHR^{\bullet}$, $-NR^{\bullet}_2$, or $-NO_2$, wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00258] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1–19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid

or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2–hydroxy–ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2–naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3–phenylpropionate, phosphate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p–toluenesulfonate, undecanoate, valerate salts, and the like.

[00259] Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[00260] Unless otherwise stated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, Z and E double bond isomers, and Z and E conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures including the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention. Such compounds are useful, for example, as analytical tools, as probes in biological assays, or as therapeutic agents in accordance with the present invention. In certain embodiments, a warhead moiety, R^1, of a provided compound comprises one or more deuterium atoms.

[00261] As used herein, the term "inhibitor" is defined as a compound that binds to and/or modulates or inhibits a target RNA with measurable affinity. In certain embodiments, an inhibitor has an IC_{50} and/or binding constant of less than about 100 μM, less than about 50 μM, less than about 1 μM, less than about 500 nM, less than about 100 nM, less than about 10 nM, or less than about 1 nM.

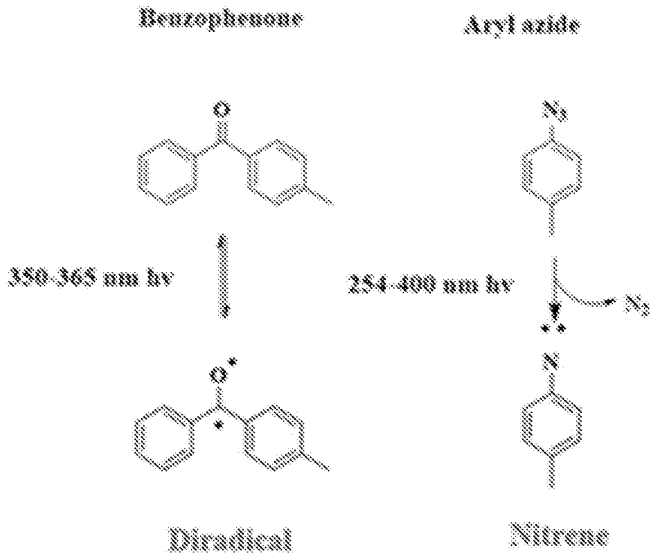
[00262] The terms "measurable affinity" and "measurably inhibit," as used herein, mean a measurable change in a downstream biological effect between a sample comprising a compound of the present invention, or composition thereof, and a target RNA, and an equivalent sample comprising the target RNA, in the absence of said compound, or composition thereof.

[00263] The term "RNA" (ribonucleic acid) as used herein, means naturally-occurring or synthetic oligoribonucleotides independent of source (e.g., the RNA may be produced by a human, animal, plant, virus, or bacterium, or may be synthetic in origin), biological context (e.g., the RNA may be in the nucleus, circulating in the blood, *in vitro*, cell lysate, or isolated or pure form), or physical form (e.g., the RNA may be in single-, double-, or triple-stranded form (including RNA-DNA hybrids), may include epigenetic modifications, native post-transcriptional modifications, artificial modifications (e.g., obtained by chemical or *in vitro* modification), or other modifications, may be bound to, e.g., metal ions, small molecules, protein chaperones, or co-factors, or may be in a denatured, partially denatured, or folded state including any native or unnatural secondary or tertiary structure such as junctions (e.g., *cis* or *trans* three-way junctions (3WJ)), quadruplexes, hairpins, triplexes, hairpins, bulge loops, pseudoknots, and internal loops, etc., and any transient forms or structures adopted by the RNA). In some embodiments, the RNA is 100 or more nucleotides in length. In some embodiments, the RNA is 250 or more nucleotides in length. In some embodiments, the RNA is 350, 450, 500, 600, 750, or 1,000, 2,000, 3,000, 4,000, 5,000, 7,500, 10,000, 15,000, 25,000, 50,000, or more nucleotides in length. In some embodiments, the RNA is between 250 and 1,000 nucleotides in length. In some embodiments, the RNA is a pre-RNA, pre-miRNA, or pretranscript. In some embodiments, the RNA is a non-coding RNA (ncRNA), messenger RNA (mRNA), micro-RNA (miRNA), a ribozyme, riboswitch, lncRNA, lincRNA, snoRNA, snRNA, scaRNA, piRNA, ceRNA, pseudo-gene, viral RNA, or bacterial RNA. The term "target RNA," as used herein, means any type of RNA having a secondary or tertiary structure capable of binding a small molecule ligand described herein. The

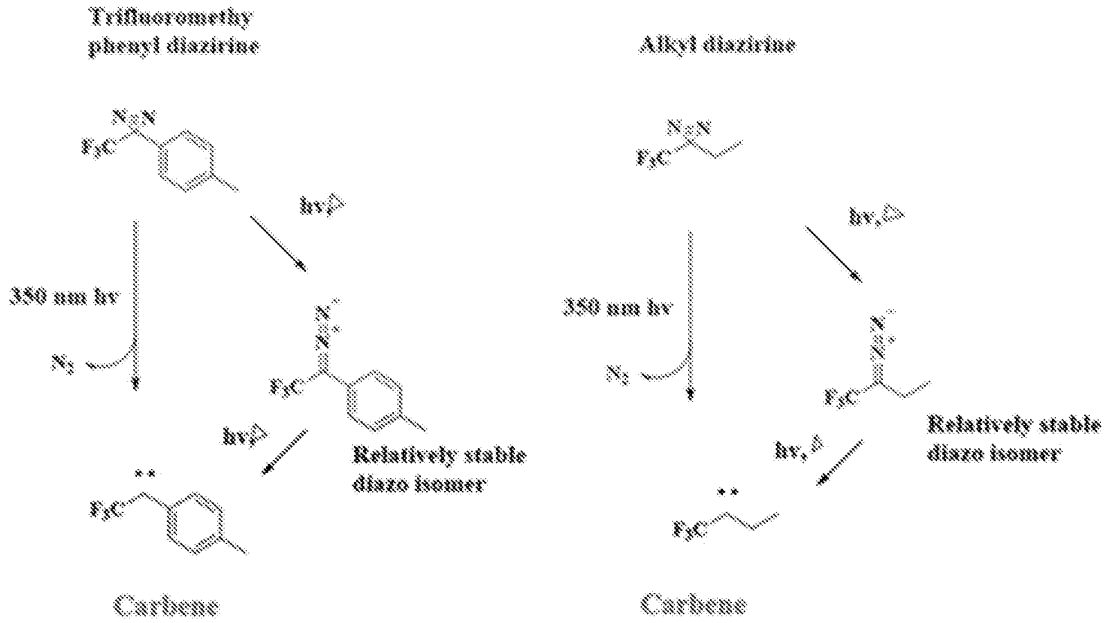
target RNA may be inside a cell, in a cell lysate, or in isolated form prior to contacting the compound.

Photoactivatable Groups

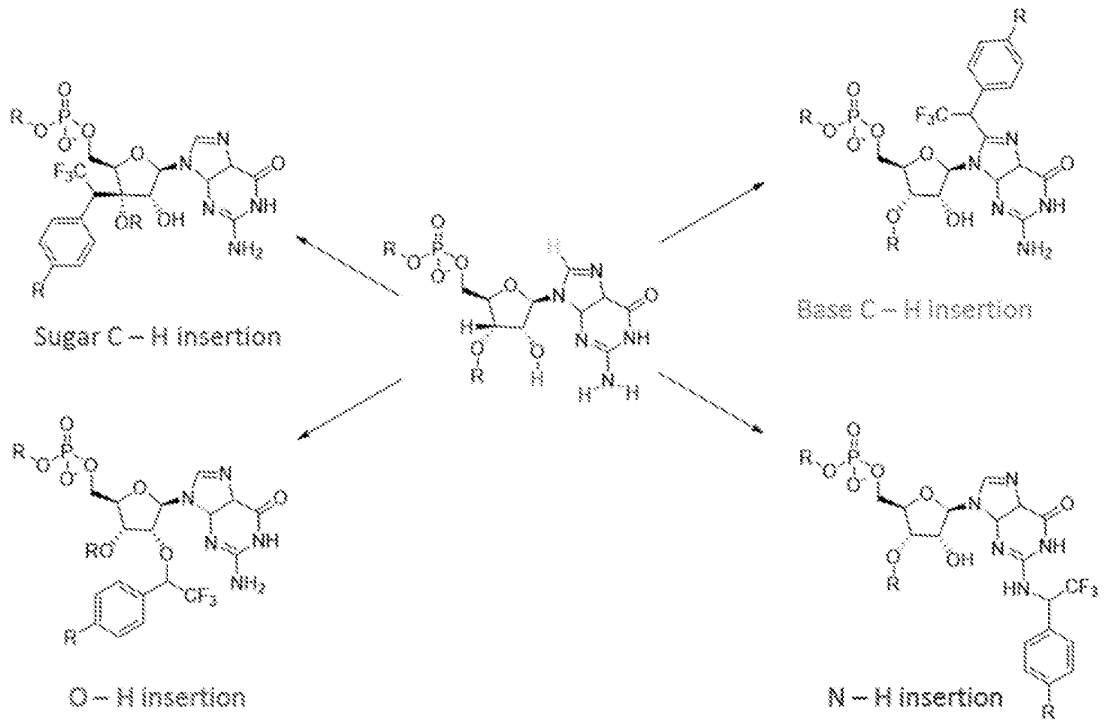
[00264] Suitable covalent modifier moieties (e.g. R^{mod} shown in Formulae **I-VIII** above) for use in the present invention generally include photoactivatable groups that generate a reactive intermediate upon irradiation with visible or ultraviolet light. In some embodiments, the photoactivatable group is a functional group that generates a carbon- or oxygen-centered radical, an aryl or heteroaryl carbocation, a nitrene, or a carbene intermediate upon irradiation with ultraviolet (UV) radiation; and wherein R^{mod} is capable of reacting with a target RNA to which **Ligand** binds to produce a covalent bond with the target RNA. Exemplary photoactivatable chromophores and the reactive species generated after irradiation are shown below.



Scheme 1: Reactions of Benzophenones and Aryl Azides



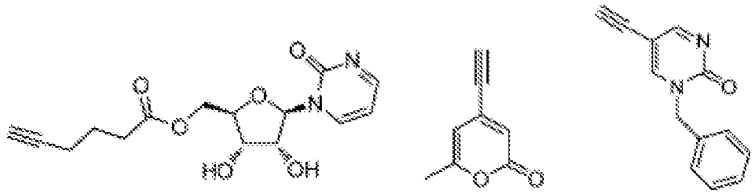
Scheme 2: Reactions of Diazirines



Scheme 3: Exemplary Reactions of Nucleic Acid Purines with Photoactivatable Groups

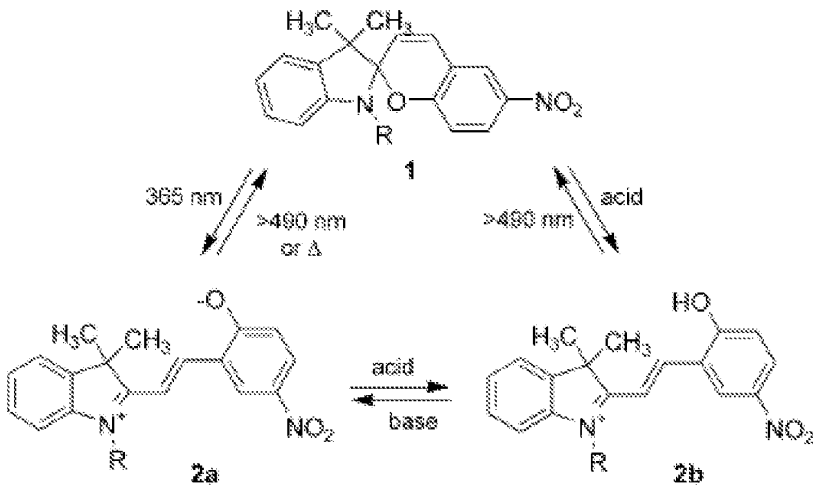
[00265] Since the warhead is unreactive prior to activation, a non-covalent RNA-ligand interaction is required for successful photoactivated modification. The reactive carbene/nitrene intermediates are rapidly quenched by water if a suitably positioned macromolecule is not present.

[00266] α-pyrones and pyrimidones are also photoactivatable groups that may be used in the present invention. For example, Battenberg, O. A. *et al.* (*J. Org. Chem.* **2011**, *76*, 6075–6087) disclose photoprobes such as the following:



In some embodiments, the photoactivatable group is selected from a pyrone or pyrimidone such as those above.

[00267] In other embodiments, the photoactivable group, after irradiation and formation of a covalent bond with the target RNA, undergoes an equilibrium process that optionally includes reversion to its original state. Synthetic molecules that are isomerizable in a reversible fashion under light irradiation of different wavelengths and which may be used as photoactivatable groups include diazobenzenes, dihydropyrenes, spirooxazines, anthracenes, fulgides, and spiropyrans. An exemplary spiropyran and its irradiation-induced equilibrium process are shown in the scheme below.



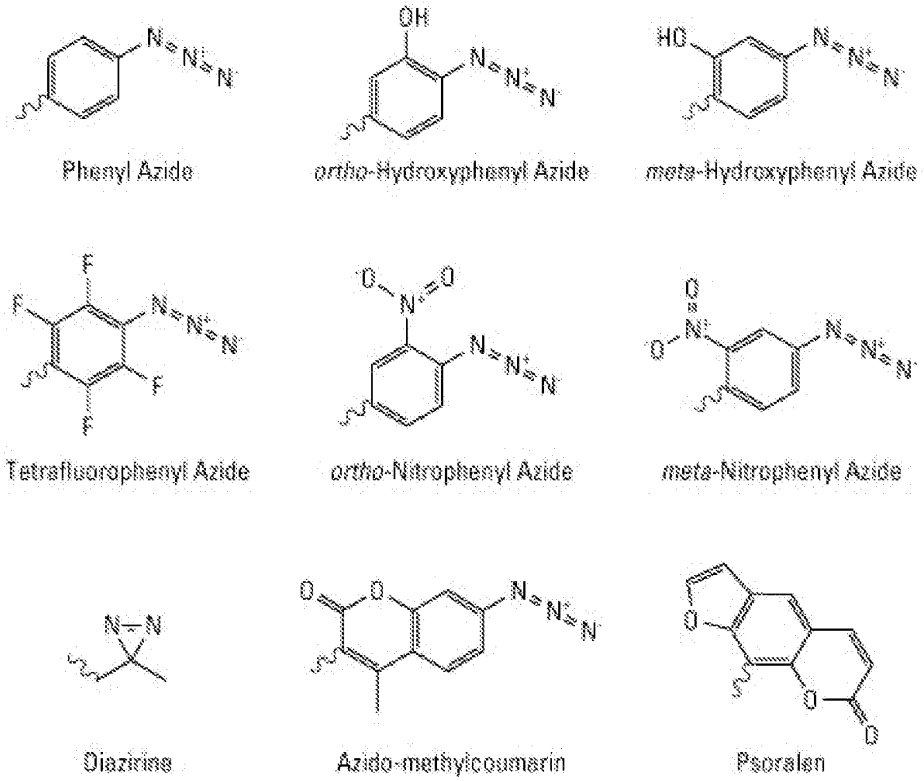
Scheme 4: Exemplary Spiropyran Photoprobe

wherein R = $-(CH_2)_2O_2C(CH_2)_2NH_2$ and **1** above may be optionally substituted; the spiropyran may be linked to a small molecule ligand and optional pull-down group by a covalent bond or tether as described herein. One characteristic of spiropyran **1** in comparison with other reversible systems lies in its photochemical switching being virtually complete (> 95% of **2a** after UV irradiation at 365 nm) because of the distinctively different absorption maxima of **1** (350 nm) and **2a** (563 nm), unlike diazobenzenes, which reach a photostationary state of 70–90% *cis* when exposed to UV light of 365 nm. The equilibrium of **2a** and **2b** can also be influenced by pH. See, e.g., Young, D. D. *et al.*, *ChemBioChem* **2008**, *9*, 1225-1228.

[00268] In some embodiments, the photoactivatable group is selected from an optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic azide or 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl azide, optionally substituted benzoyl azide or 5-8 membered heteroaroyl azide or 8-10 membered heteroaroyl azide wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic diazonium salt, optionally substituted 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl diazonium salt wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted C_{2-6} aliphatic diazo functional group, optionally substituted C_{2-6} aliphatic diazirine, or optionally substituted diphenyl or 8-10-membered diheteroaryl ketone wherein 1-3 atoms of the ring atoms are selected from

nitrogen, sulfur, or oxygen, optionally substituted dihydropyrene, optionally substituted spirooxazine, optionally substituted anthracene, optionally substituted fulgide, optionally substituted spiropyran, optionally substituted α-pyrone or optionally substituted pyrimidone.

[00269] In some embodiments, the photoactivatable group is selected from, e.g.



[00270] Photoactivatable groups can be conjugated to small molecule ligands or tethers by conventional coupling reactions known to those of ordinary skill in the art and as described herein. For example, photoactivatable amino acids such as D- or L- photoleucine, photomethionine, photolysine, *para*-benzoylphenylalanine, and others can serve as convenient means of introducing a photoactivatable group into a compound in accordance with the present invention.

[00271] In some embodiments, the photoactivatable group is an aroyl or heteroaroyl azide, such as nicotinoyl azide (NAz). Such compounds form nitrene intermediates upon irradiation, and have been used to study solvent accessibility of nucleic acids such as the SAM-1 riboswitch and rRNA in cells. NAz probes react, for example, with the C8 position of accessible purines.

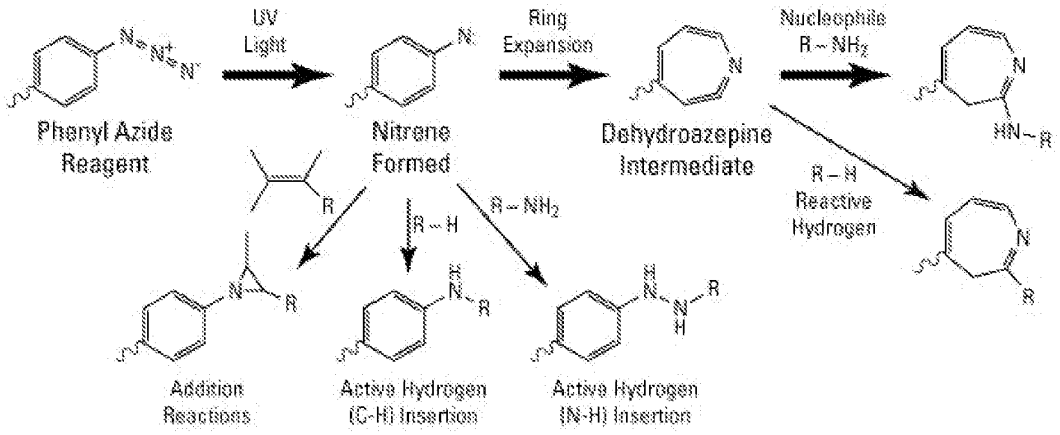
[00272] The term "covalent modifier moiety" or "warhead" as used herein, means any photoactivatable group capable of forming a covalent bond with an available nucleotide of a RNA to produce a modified RNA (such as a C8-modified purine or 2′-O-modified RNA) after irradiation with visible or UV light.

[00273] The wavelength of visible or UV light for activating the photoactivatable group is generally selected to generate the reactive intermediate such as a nitrene without substantially degrading the biological system under investigation or causing off-target reactivity. The wavelength is generally a wavelength known to function to generate the reactive intermediate for each specific protoactivatable group. In some embodiments, the wavelenegth is about 252 nm, 302 nm, or 365 nm; or 254 nm, 265-275 nm, 365 nm, 300-460 nm, or about 250 nm to about 350 nm.

[00274] When an aryl azide is exposed to UV light (250 to 350 nm), it forms a nitrene group that can initiate addition reactions with double bonds, insertion into C–H and N–H sites, or subsequent ring expansion to react with a nucleophile (e.g., primary amines). The latter reaction path dominates when primary amines are present in the sample.

[00275] Thiol-containing reducing agents (e.g., DTT or 2-mercaptoethanol) must be avoided in the sample solution during all steps before and during photo-activation, because they reduce the azide functional group to an amine, preventing photo-activation. Reactions can be performed in a variety of amine-free buffer conditions. If working with heterobifunctional photoreactive crosslinkers, use buffers compatible with both reactive chemistries involved. In general, experiments are performed in subdued light and/or with reaction vessels covered in foil until photoreaction is intended. Typically, photo-activation is accomplished with a hand-held UV lamp positioned close to the reaction solution and shining directly on it (i.e., not through glass or polypropylene) for several minutes.

[00276] Examples of aryl azides include simple phenyl azides, hydroxyphenyl azides, and nitrophenyl azides. Generally, short-wavelength UV light (e.g., 254 nm; 265 to 275 nm) is needed to efficiently activate simple phenyl azides, while long-UV light (e.g, 365 nm; 300 to 460 nm) is sufficient for nitrophenyl azides.



Covalent Modification of RBP Proteins and Other Proximate Biomolecules

[00277] In some embodiments, the photoactivatable group reacts with a protein in proximity to the small molecule ligand binding site on a target RNA. RNA binding proteins (RBPs) are frequently associated with target RNAs of interest. In some cases, a RBP is associated with or otherwise in proximity to the targeted RNA sub-structure to which a disclosed photoactivatable compound binds. Accordingly, an advantage of the photoaffinity warheads is that they are agnostic about covalent modification of RNAs or proteins such as RBPs proximal to the photoactivatable group that is attached to the small molecule ligand. Thus, in some embodiments, a disclosed compound covalently modifies either a target RNA or a RBP associated with the target RNA. This in turn yields insight into which RBPs are bound to a target RNA and in which cells or tissues of an organism, as well as the effect of the small molecule binding on RBP binding.

[00278] Thus, in one aspect, the present invention provides a method of determining the presence of or association/binding of a RNA binding protein (RBP) with a target RNA, comprising the step of contacting the target RNA with a disclosed compound and irradiating the compound with visible or UV light, and optionally performing one or more assays to determine whether a RBP has been covalently modified by the photoactivatable group of the compound.

[00279] The highly reactive and thus relatively indiscriminate carbenes, nitrenes, diradicals, and other intermediates produced by activation of the photoaffinity warheads thus have the advantage of covalently modifying a target RNA or any biomolecule in proximity, unlike previously known methods.

Tethering Groups (Linkers)

[00280] The present invention contemplates the use of a wide variety of tethering groups (tethers; e.g., variables T^1 and T^2 as shown in Formulae **I-VIII** above) to provide optimal binding and reactivity toward nucleotides or RBPs proximal to the binding site of a target RNA. In some embodiments, T^1 and T^2 are selected from those shown in FIGS. 10-17. For example, in some embodiments, T^1 and/or T^2 is a polyethylene glycol (PEG) group of, e.g., 1-10 ethylene glycol subunits. In some embodiments, T^1 and/or T^2 is an optionally substituted C_{1-12} aliphatic group or a peptide comprising 1-8 amino acids.

[00281] In some embodiments, T^1 and T^2 are each independently selected from L^1 or L^2, as L^1 and L^2 are defined in embodiments herein.

[00282] In some embodiments, the physical properties such as the length, rigidity, hydrophobicity, and/or other properties of the tether are selected to optimize the pattern of proximity-induced covalent bond formation between the target RNA or an associated RBP and the photoactivatable group (warhead). In some embodiments, the physical properties of the tether (such as those above) are selected so that, upon binding of the compound to the active or allosteric sites of a target RNA, the modifying moiety selectively reacts with a an available functionality of the target RNA such as a purine C8 carbon or 2′-OH group of the target RNA proximal to the active site or allosteric sites, or reacts with a proximal amino acid of a RBP.

Click-Ready Groups

[00283] A variety of bioorthogonal reaction partners (e.g., R^{CG} in Formulae **I-VIII** or R^2 in Formulae above) may be used in the present invention to couple a compound described herein with a pull-down moiety. The term "bioorthogonal chemistry" or "bioorthogonal reaction," as used herein, refers to any chemical reaction that can take place in living systems without interfering with native biochemical processes. Accordingly, a "bioorthogonal reaction partner" is a chemical moiety capable of undergoing a bioorthogonal reaction with an appropriate reaction partner to couple a compound described herein to a pull-down moiety. In some embodiments, a bioorthogonal reaction partner is covalently attached to the chemical modifying moiety or the tethering group. In some embodiments, the bioorthogonal reaction partner is selected from a click-

ready group or a group capable of undergoing a nitrone/cyclooctyne reaction, oxime/hydrazone formation, a tetrazine ligation, an isocyanide-based click reaction, or a quadricyclane ligation.

[00284] In some embodiments, the bioorthogonal reaction partner is a click-ready group. The term "click-ready group" refers to a chemical moiety capable of undergoing a click reaction, such as an azide or alkyne.

[00285] Click reactions tend to involve high-energy ("spring-loaded") reagents with well-defined reaction coordinates, that give rise to selective bond-forming events of wide scope. Examples include nucleophilic trapping of strained-ring electrophiles (epoxide, aziridines, aziridinium ions, episulfonium ions), certain carbonyl reactivity (e.g., the reaction between aldehydes and hydrazines or hydroxylamines), and several cycloaddition reactions. The azide-alkyne 1,3-dipolar cycloaddition and the Diels-Alder cycloaddition are two such reactions.

[00286] Such click reactions (i.e., dipolar cycloadditions) are associated with a high activation energy and therefore require heat or a catalyst. Indeed, use of a copper catalyst is routinely employed in click reactions. However, in certain instances where click chemistry is particularly useful (e.g., in bioconjugation reactions), the presence of copper can be detrimental (See Wolbers, F. et al.; Electrophoresis 2006, 27, 5073). Accordingly, methods of performing dipolar cycloaddition reactions were developed without the use of metal catalysis. Such "metal free" click reactions utilize activated moieties in order to facilitate cycloaddition. Therefore, the present invention provides click-ready groups suitable for metal-free click chemistry.

[00287] Certain metal-free click moieties are known in the literature. Examples include 4-dibenzocyclooctynol (DIBO) (from Ning et al; *Angew Chem Int Ed*, 2008, 47, 2253); *gem*-difluorinated cyclooctynes (DIFO or DFO) (from Codelli, et al.; *J. Am. Chem. Soc.* 2008, 130, 11486-11493.); biarylazacyclooctynone (BARAC) (from Jewett et al.; *J. Am. Chem. Soc.* 2010, 132, 3688.); or bicyclononyne (BCN) (From Dommerholt, et al.; *Angew Chem Int Ed*, 2010, 49, 9422-9425).

[00288] As used herein, the phrase "a moiety suitable for metal-free click chemistry" refers to a functional group capable of dipolar cycloaddition without use of a metal catalyst. Such moieties include an activated alkyne (such as a strained cyclooctyne), an oxime (such as a nitrile oxide precursor), or oxanorbornadiene, for coupling to an azide to form a cycloaddition product (e.g., triazole or isoxazole).

[00289] Thus, in certain embodiments, the click-ready group is selected from an azide, an alkyne, 4-dibenzocyclooctynol (DIBO) *gem*-difluorinated cyclooctynes (DIFO or DFO), biarylazacyclooctynone (BARAC), bicyclononyne (BCN), a strained cyclooctyne, an oxime, or oxanorbornadiene.

[00290] In some embodiments, the click-ready group is selected from those shown in FIG. 9.

Pull-down Groups

[00291] A number of pull-down groups (R^{PD} in, for example, Formulae **I-VIII** above) may be used in the present invention. In some embodiments, pull-down groups contain a bioorthogonal reaction partner that reacts with a click-ready group to attach the pull-down group to the rest of the compound, as well as an appropriate functional group or affinity group such as a hapten (e.g., biotin) or a radiolabel allowing for selective isolation or detection of the pulled-down compound. For example, use of avidin or streptavidin to interact with a pull-down group would allow isolation of only those RNAs that had been covalently modified, as explained in further detail below.

[00292] In some embodiments, a pull-down group is covalently attached to a disclosed compound as described above and in formulae described herein, before binding to a target RNA and before irradiation to activate the photoactivatable group. In other embodiments, a pull-down group is attached to a compound or RNA conjugate after the photoactivatable group has been irradiated and covalent modification of a target RNA has taken place. In some embodiments, such attachment is achieved by a bioorthogoal reaction between a click-ready group on the compound or RNA conjugate and an appropriate reaction partner that is part of the pull-down group. Accordingly, in some embodiments, the pull-down group comprises a click-ready group attached via a tether (as described elsewhere herein) to an affinity group or bioorthogonal functional group. In some embodiments, the affinity group or bioorthogonal functional group is a hapten (e.g., biotin) or a radiolabel such as ^{125}I, ^{14}C, ^{32}P, or ^{3}H.

3. General Methods of Providing the Present Compounds

[00293] The compounds of this invention may be prepared or isolated in general by synthetic and/or semi-synthetic methods known to those skilled in the art for analogous compounds and by methods described in detail in the Examples and Figures, herein.

[00294] In the schemes and chemical reactions depicted in the detailed description, Examples, and Figures, where a particular protecting group ("PG"), leaving group ("LG"), or transformation condition is depicted, one of ordinary skill in the art will appreciate that other protecting groups, leaving groups, and transformation conditions are also suitable and are contemplated. Such groups and transformations are described in detail in *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, M. B. Smith and J. March, 5th Edition, John Wiley & Sons, 2001, *Comprehensive Organic Transformations*, R. C. Larock, 2nd Edition, John Wiley & Sons, 1999, and *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of each of which is hereby incorporated herein by reference.

[00295] As used herein, the phrase "leaving group" (LG) includes, but is not limited to, halogens (e.g. fluoride, chloride, bromide, iodide), sulfonates (e.g. mesylate, tosylate, benzenesulfonate, brosylate, nosylate, triflate), diazonium, and the like.

[00296] As used herein, the phrase "oxygen protecting group" includes, for example, carbonyl protecting groups, hydroxyl protecting groups, etc. Hydroxyl protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Examples of suitable hydroxyl protecting groups include, but are not limited to, esters, allyl ethers, ethers, silyl ethers, alkyl ethers, arylalkyl ethers, and alkoxyalkyl ethers. Examples of such esters include formates, acetates, carbonates, and sulfonates. Specific examples include formate, benzoyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, pivaloate (trimethylacetyl), crotonate, 4-methoxy-crotonate, benzoate, p-benzylbenzoate, 2,4,6-trimethylbenzoate, carbonates such as methyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(phenylsulfonyl)ethyl, vinyl, allyl, and p-nitrobenzyl. Examples of such silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl, and other trialkylsilyl ethers. Alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, allyl, and allyloxycarbonyl ethers or derivatives. Alkoxyalkyl ethers include acetals such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzyloxymethyl, beta-(trimethylsilyl)ethoxymethyl, and tetrahydropyranyl ethers. Examples of arylalkyl ethers include

benzyl, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, and 2- and 4-picolyl.

[00297] Amino protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Suitable amino protecting groups include, but are not limited to, aralkylamines, carbamates, cyclic imides, allyl amines, amides, and the like. Examples of such groups include t-butyloxycarbonyl (BOC), ethyloxycarbonyl, methyloxycarbonyl, trichloroethyloxycarbonyl, allyloxycarbonyl (Alloc), benzyloxocarbonyl (CBZ), allyl, phthalimide, benzyl (Bn), fluorenylmethylcarbonyl (Fmoc), formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, phenylacetyl, trifluoroacetyl, benzoyl, and the like.

[00298] One of skill in the art will appreciate that various functional groups present in compounds of the invention such as aliphatic groups, alcohols, carboxylic acids, esters, amides, aldehydes, halogens and nitriles can be interconverted by techniques well known in the art including, but not limited to reduction, oxidation, esterification, hydrolysis, partial oxidation, partial reduction, halogenation, dehydration, partial hydration, and hydration. "March's Advanced Organic Chemistry," 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001, the entirety of which is incorporated herein by reference. Such interconversions may require one or more of the aforementioned techniques, and certain methods for synthesizing compounds of the invention are described below in the Exemplification and Figures.

4. Uses, Formulation and Administration

Pharmaceutically acceptable compositions

[00299] According to another embodiment, the invention provides a composition comprising a compound of this invention or a pharmaceutically acceptable derivative thereof and a pharmaceutically acceptable carrier, adjuvant, or vehicle. The amount of compound in compositions of this invention is such that is effective to measurably inhibit or modulate a target RNA, or a mutant thereof, in a biological sample or in a patient. In certain embodiments, the amount of compound in compositions of this invention is such that is effective to measurably inhibit or modulate a target RNA, in a biological sample or in a patient. In certain embodiments,

a composition of this invention is formulated for administration to a patient in need of such composition. In some embodiments, a composition of this invention is formulated for oral administration to a patient.

[00300] The term "patient," as used herein, means an animal, preferably a mammal, and most preferably a human.

[00301] The term "pharmaceutically acceptable carrier, adjuvant, or vehicle" refers to a non-toxic carrier, adjuvant, or vehicle that does not destroy the pharmacological activity of the compound with which it is formulated. Pharmaceutically acceptable carriers, adjuvants or vehicles that may be used in the compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[00302] A "pharmaceutically acceptable derivative" means any non-toxic salt, ester, salt of an ester or other derivative of a compound of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorily active metabolite or residue thereof.

[00303] Compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously. Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable

vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

[00304] For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[00305] Pharmaceutically acceptable compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[00306] Alternatively, pharmaceutically acceptable compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[00307] Pharmaceutically acceptable compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[00308] Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

[00309] For topical applications, provided pharmaceutically acceptable compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, provided pharmaceutically acceptable compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[00310] For ophthalmic use, provided pharmaceutically acceptable compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutically acceptable compositions may be formulated in an ointment such as petrolatum.

[00311] Pharmaceutically acceptable compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[00312] Most preferably, pharmaceutically acceptable compositions of this invention are formulated for oral administration. Such formulations may be administered with or without food. In some embodiments, pharmaceutically acceptable compositions of this invention are administered without food. In other embodiments, pharmaceutically acceptable compositions of this invention are administered with food.

[00313] The amount of compounds of the present invention that may be combined with the carrier materials to produce a composition in a single dosage form will vary depending upon the

host treated, the particular mode of administration. Preferably, provided compositions should be formulated so that a dosage of between 0.01 - 100 mg/kg body weight/day of the inhibitor can be administered to a patient receiving these compositions.

[00314] It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of a compound of the present invention in the composition will also depend upon the particular compound in the composition.

Uses of Compounds and Pharmaceutically Acceptable Compositions

[00315] Compounds and compositions described herein are generally useful for the modulation of a target RNA to retreat an RNA-mediated disease or condition.

[00316] The activity of a compound utilized in this invention to modulate a target RNA may be assayed *in vitro*, *in vivo* or in a cell line. *In vitro* assays include assays that determine modulation of the target RNA. Alternate *in vitro* assays quantitate the ability of the compound to bind to the target RNA. Detailed conditions for assaying a compound utilized in this invention to modulate a target RNA are set forth in the Examples below.

[00317] As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence.

[00318] Provided compounds are modulators of a target RNA and are therefore useful for treating one or more disorders associated with or affected by (e.g., downstream of) the target RNA. Thus, in certain embodiments, the present invention provides a method for treating an RNA-mediated disorder comprising the step of administering to a patient in need thereof a compound of the present invention, or pharmaceutically acceptable composition thereof.

[00319] As used herein, the terms "RNA-mediated" disorders, diseases, and/or conditions as used herein means any disease or other deleterious condition in which RNA, such as an overexpressed, underexpressed, mutant, misfolded, pathogenic, or ongogenic RNA, is known to play a role. Accordingly, another embodiment of the present invention relates to treating or lessening the severity of one or more diseases in which RNA, such as an overexpressed, underexpressed, mutant, misfolded, pathogenic, or ongogenic RNA, is known to play a role.

[00320] In some embodiments, the present invention provides a method for treating one or more disorders, diseases, and/or conditions wherein the disorder, disease, or condition includes, but is not limited to, a cellular proliferative disorder.

Cellular Proliferative Disorders

[00321] The present invention features methods and compositions for the diagnosis and prognosis of cellular proliferative disorders (e.g., cancer) and the treatment of these disorders by modulating a target RNA. Cellular proliferative disorders described herein include, e.g., cancer, obesity, and proliferation-dependent diseases. Such disorders may be diagnosed using methods known in the art.

Cancer

[00322] Cancer includes, in one embodiment, without limitation, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (e.g., Hodgkin's disease or non-Hodgkin's disease), Waldenstrom's macroglobulinemia, multiple myeloma, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor,

cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma). In some embodiments, the cancer is melanoma or breast cancer.

[00323] Cancers includes, in another embodiment, without limitation, mesothelioma, hepatobilliary (hepatic and billiary duct), bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, ovarian cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, gastrointestinal (gastric, colorectal, and duodenal), uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, testicular cancer, chronic or acute leukemia, chronic myeloid leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, non-Hodgkins's lymphoma, spinal axis tumors, brain stem glioma, pituitary adenoma, adrenocortical cancer, gall bladder cancer, multiple myeloma, cholangiocarcinoma, fibrosarcoma, neuroblastoma, retinoblastoma, or a combination of one or more of the foregoing cancers.

[00324] In some embodiments, the present invention provides a method for treating a tumor in a patient in need thereof, comprising administering to the patient any of the compounds, salts or pharmaceutical compositions described herein. In some embodiments, the tumor comprises any of the cancers described herein. In some embodiments, the tumor comprises melanoma cancer. In some embodiments, the tumor comprises breast cancer. In some embodiments, the tumor comprises lung cancer. In some embodiments the the tumor comprises small cell lung cancer (SCLC). In some embodiments the the tumor comprises non-small cell lung cancer (NSCLC).

[00325] In some embodiments, the tumor is treated by arresting further growth of the tumor. In some embodiments, the tumor is treated by reducing the size (e.g., volume or mass) of the tumor by at least 5%, 10%, 25%, 50 %, 75%, 90% or 99% relative to the size of the tumor prior to treatment. In some embodiments, tumors are treated by reducing the quantity of the tumors in the

patient by at least 5%, 10%, 25%, 50 %, 75%, 90% or 99% relative to the quantity of tumors prior to treatment.

Other Proliferative Diseases

[00326] Other proliferative diseases include, e.g., obesity, benign prostatic hyperplasia, psoriasis, abnormal keratinization, lymphoproliferative disorders (e.g., a disorder in which there is abnormal proliferation of cells of the lymphatic system), chronic rheumatoid arthritis, arteriosclerosis, restenosis, and diabetic retinopathy. Proliferative diseases that are hereby incorporated by reference include those described in U.S. Pat. Nos. 5,639,600 and 7,087,648.

Inflammatory Disorders and Diseases

[00327] Compounds of the invention are also useful in the treatment of inflammatory or allergic conditions of the skin, for example psoriasis, contact dermatitis, atopic dermatitis, alopecia areata, erythema multiforma, dermatitis herpetiformis, scleroderma, vitiligo, hypersensitivity angiitis, urticaria, bullous pemphigoid, lupus erythematosus, systemic lupus erythematosus, pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus, epidermolysis bullosa acquisita, acne vulgaris, and other inflammatory or allergic conditions of the skin.

[00328] Compounds of the invention may also be used for the treatment of other diseases or conditions, such as diseases or conditions having an inflammatory component, for example, treatment of diseases and conditions of the eye such as ocular allergy, conjunctivitis, keratoconjunctivitis sicca, and vernal conjunctivitis, diseases affecting the nose including allergic rhinitis, and inflammatory disease in which autoimmune reactions are implicated or having an autoimmune component or etiology, including autoimmune hematological disorders (e.g. hemolytic anemia, aplastic anemia, pure red cell anemia and idiopathic thrombocytopenia), systemic lupus erythematosus, rheumatoid arthritis, polychondritis, scleroderma, Wegener granulamatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (e.g. ulcerative colitis and Crohn's disease), irritable bowel syndrome, celiac disease, periodontitis, hyaline membrane disease, kidney disease, glomerular disease, alcoholic liver disease, multiple sclerosis, endocrine opthalmopathy, Grave's disease, sarcoidosis, alveolitis, chronic hypersensitivity pneumonitis, multiple sclerosis, primary biliary cirrhosis, uveitis (anterior and posterior), Sjogren's syndrome, keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic

arthritis, systemic juvenile idiopathic arthritis, cryopyrin-associated periodic syndrome, nephritis, vasculitis, diverticulitis, interstitial cystitis, glomerulonephritis (with and without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minal change nephropathy), chronic granulomatous disease, endometriosis, leptospiriosis renal disease, glaucoma, retinal disease, ageing, headache, pain, complex regional pain syndrome, cardiac hypertrophy, musclewasting, catabolic disorders, obesity, fetal growth retardation, hyperchlolesterolemia, heart disease, chronic heart failure, mesothelioma, anhidrotic ecodermal dysplasia, Behcet's disease, incontinentia pigmenti, Paget's disease, pancreatitis, hereditary periodic fever syndrome, asthma (allergic and non-allergic, mild, moderate, severe, bronchitic, and exercise-induced), acute lung injury, acute respiratory distress syndrome, eosinophilia, hypersensitivities, anaphylaxis, nasal sinusitis, ocular allergy, silica induced diseases, COPD (reduction of damage, airways inflammation, bronchial hyperreactivity, remodeling or disease progression), pulmonary disease, cystic fibrosis, acid-induced lung injury, pulmonary hypertension, polyneuropathy, cataracts, muscle inflammation in conjunction with systemic sclerosis, inclusion body myositis, myasthenia gravis, thyroiditis, Addison's disease, lichen planus, Type 1 diabetes, or Type 2 diabetes, appendicitis, atopic dermatitis, asthma, allergy, blepharitis, bronchiolitis, bronchitis, bursitis, cervicitis, cholangitis, cholecystitis, chronic graft rejection, colitis, conjunctivitis, Crohn's disease, cystitis, dacryoadenitis, dermatitis, dermatomyositis, encephalitis, endocarditis, endometritis, enteritis, enterocolitis, epicondylitis, epididymitis, fasciitis, fibrositis, gastritis, gastroenteritis, Henoch-Schonlein purpura, hepatitis, hidradenitis suppurativa, immunoglobulin A nephropathy, interstitial lung disease, laryngitis, mastitis, meningitis, myelitis myocarditis, myositis, nephritis, oophoritis, orchitis, osteitis, otitis, pancreatitis, parotitis, pericarditis, peritonitis, pharyngitis, pleuritis, phlebitis, pneumonitis, pneumonia, polymyositis, proctitis, prostatitis, pyelonephritis, rhinitis, salpingitis, sinusitis, stomatitis, synovitis, tendonitis, tonsillitis, ulcerative colitis, uveitis, vaginitis, vasculitis, or vulvitis.

[00329] In some embodiments the inflammatory disease which can be treated according to the methods of this invention is an disease of the skin. In some embodiments, the inflammatory disease of the skin is selected from contact dermatitits, atompic dermatitis, alopecia areata, erythema multiforma, dermatitis herpetiformis, scleroderma, vitiligo, hypersensitivity angiitis, urticaria, bullous pemphigoid, pemphigus vulgaris, pemphigus foliaceus, paraneoplastic

pemphigus, epidermolysis bullosa acquisita, and other inflammatory or allergic conditions of the skin.

[00330] In some embodiments the inflammatory disease which can be treated according to the methods of this invention is selected from acute and chronic gout, chronic gouty arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Juvenile rheumatoid arthritis, Systemic jubenile idiopathic arthritis (SJIA), Cryopyrin Associated Periodic Syndrome (CAPS), and osteoarthritis.

[00331] In some embodiments the inflammatory disease which can be treated according to the methods of this invention is a TH17 mediated disease. In some embodiments the TH17 mediated disease is selected from Systemic lupus erythematosus, Multiple sclerosis, and inflammatory bowel disease (including Crohn's disease or ulcerative colitis).

[00332] In some embodiments the inflammatory disease which can be treated according to the methods of this invention is selected from Sjogren's syndrome, allergic disorders, osteoarthritis, conditions of the eye such as ocular allergy, conjunctivitis, keratoconjunctivitis sicca and vernal conjunctivitis, and diseases affecting the nose such as allergic rhinitis.

Metabolic Disease

[00333] In some embodiments the invention provides a method of treating a metabolic disease. In some embodiments the metabolic disease is selected from Type 1 diabetes, Type 2 diabetes, metabolic syndrome or obesity.

[00334] The compounds and compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for treating or lessening the severity of a cancer, an autoimmune disorder, a proliferative disorder, an inflammatory disorder, a neurodegenerative or neurological disorder, schizophrenia, a bone-related disorder, liver disease, or a cardiac disorder. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular agent, its mode of administration, and the like. Compounds of the invention are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any

particular patient or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed, and like factors well known in the medical arts. The term "patient," as used herein, means an animal, preferably a mammal, and most preferably a human.

[00335] Pharmaceutically acceptable compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments, the compounds of the invention may be administered orally or parenterally at dosage levels of about 0.01 mg/kg to about 50 mg/kg and preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect.

[00336] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[00337] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition,

sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[00338] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00339] In order to prolong the effect of a compound of the present invention, it is often desirable to slow the absorption of the compound from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the compound then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered compound form is accomplished by dissolving or suspending the compound in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the compound in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of compound to polymer and the nature of the particular polymer employed, the rate of compound release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the compound in liposomes or microemulsions that are compatible with body tissues.

[00340] Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[00341] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating

agents such as agar--agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[00342] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

[00343] The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

[00344] Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

[00345] According to one embodiment, the invention relates to a method of modulating the activity of a target RNA in a biological sample comprising the step of contacting said biological sample with a compound of this invention, or a composition comprising said compound.

[00346] According to another embodiment, the invention relates to a method of modulating the activity of a target RNA in a biological sample comprising the step of contacting said biological sample with a compound of this invention, or a composition comprising said compound. In certain embodiments, the invention relates to a method of irreversibly inhibiting the activity of a target RNA in a biological sample comprising the step of contacting said biological sample with a compound of this invention, or a composition comprising said compound.

[00347] The term “biological sample”, as used herein, includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from a mammal or extracts thereof; and blood, saliva, urine, feces, semen, tears, cerebrospinal fluid, or other body fluids or extracts thereof.

[00348] Another embodiment of the present invention relates to a method of modulating the activity of a target RNA in a patient comprising the step of administering to said patient a compound of the present invention, or a composition comprising said compound.

[00349] According to another embodiment, the invention relates to a method of inhibiting the activity of a target RNA in a patient comprising the step of administering to said patient a compound of the present invention, or a composition comprising said compound. According to certain embodiments, the invention relates to a method of irreversibly inhibiting the activity of a

target RNA in a patient comprising the step of administering to said patient a compound of the present invention, or a composition comprising said compound. In other embodiments, the present invention provides a method for treating a disorder mediated by a target RNA in a patient in need thereof, comprising the step of administering to said patient a compound according to the present invention or pharmaceutically acceptable composition thereof. Such disorders are described in detail herein.

EXEMPLIFICATION

[00350] As depicted in the Examples below, exemplary compounds are prepared according to the following general procedures and used in biological assays and other procedures described generally herein. It will be appreciated that, although the general methods depict the synthesis of certain compounds of the present invention, the following general methods, and other methods known to one of ordinary skill in the art, can be applied to all compounds and subclasses and species of each of these compounds, as described herein. Similarly, assays and other analyses can be adapted according to the knowledge of one of ordinary skill in the art.

Example 1: Application of Photoprobes to Locate and Quantify Sites of Modifications in RNA

[00351] As discussed above, a variety of RNA molecules play important regulatory roles in cells. RNA secondary and tertiary structures are critical for these regulatory activities. Various tools are available for determining RNA structure. One of the most effective methods is SHAPE (selective 2′-hydroxyl acylation and primer extension). This methodology takes advantage of the characteristic that the ribose group in all RNAs has a 2′-hydroxyl whose reactivity is affected by local nucleotide flexibility and accessability to solvent. This 2′-hydroxyl is reactive in regions of the RNA that are single-stranded and flexible, but is unreactive at nucleotides that are base-paired. In other words, SHAPE reactivity is inversely proportional to the probability that a nucleotide is base paired within an RNA secondary structure. Reagents that chemically modify the RNA at this 2′-hydroxyl can be used as probes to discern RNA structure. SHAPE reagents include small-molecules such as 1-methyl-7-nitroisatoic anhydride (1M7) and benzoyl cyanide (BzCN) that react with the 2′-hydroxyl group of flexible nucleotides to form a 2′-O-adduct. Other acylation

electrophiles such as 2-methylnicotinic acid imidazolide (NAI) and 2-methyl-3-furoic acid imidazolide (FAI) can be utilized.

[00352] One useful aspect of the present invention is the tethering of a RNA-binding small molecule ligand to a photoprobe. This links the photoactivation-mediated covalent modification event with the ligand binding event such that the photoprobe is most likely to react with a portion of the RNA that is proximal (e.g., near in space) to the binding site of the ligand. Thus, the modification pattern on the RNA will be decisively altered because the activity of the photoactivatable agent will be constrained to nucleotides proximal to ligand binding pockets on the RNA. Thus, one can infer the existence and the location of ligand binding pockets from the altered reactivity pattern, as revealed in appropriate analytical methods such as sequencing.

[00353] The SHAPE-MaP approach exploits conditions that cause reverse transcriptase to misread SHAPE-modified nucleotides and incorporate a nucleotide non-complementary to the original sequence into the newly synthesized cDNA. The positions and relative frequencies of SHAPE adducts are recorded as mutations in the cDNA primary sequence. In a SHAPE-MaP experiment, the RNA is treated with a SHAPE reagent or treated with solvent only, and the RNA is modified. RNA from each experimental condition is reverse-transcribed, and the resulting cDNAs are then sequenced. Reactive positions are identified by subtracting data for the treated sample from data obtained for the untreated sample and by normalizing to data for a denatured (unfolded) control RNA.

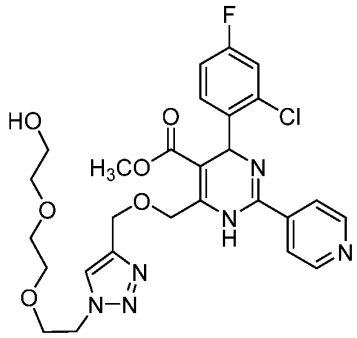
[00354] SHAPE-MaP can be performed and analyzed according to detailed published methods (Martin *et al., RNA* 2012; 18:77-87; McGuinness *et al., J. Am. Chem. Soc.* 2012; 134:6617-6624; Siegfried *et al., Nature Methods* 2014; 11:959-965; Lavender *et al., PLoS Comput. Biol.* 2015; 11(5)e1004230; McGuinness *et al., Proc. Natl. Acad. Sci. USA* 2015; 112:2425-2430). The SHAPE-MaP sequence data can be analyzed using ShapeFinder (Vasa *et al., RNA* 2008; 14:1979-1990) or ShapeMapper (Siegfried *et al., Nature Methods* 2014; 11:959-965) or other software. Each of the foregoing publications is hereby incorporated by reference.

[00355] PEARL-seq (Proximity-Enhanced Activation via RNA Ligation-sequencing) departs from SHAPE and SHAPE-MaP in that it uses a tether to link the acylation event to a ligand binding event, thus decisively altering the acylation pattern, which is observed as ‘mutations’ in the sequencing, because only riboses proximal to ligand binding pockets will be acylated. From this

one infers the existence of small-molecule binding sites on the targeted RNA as well as the location of those ligand binding sites across the transcriptome. Those RNA ligand/tether/warhead constructs ('hooks') that also bear a click functional group can be pulled down by clicking to a clickable biotin and then complexing with streptavidin on beads. This click/pull-down protocol enables sequencing of only those RNAs that have been covalently modified by a 'hook'. SHAPE-MaP & RING-MaP protocols carried out separately on the targeted RNAs enable the building of structural models of targeted RNAs as a framework that will enhance the interpretation of "covalent affinity transcriptomics" sequence data. Success is measured by bioactivities of free ligands in cells.

[00356] Libraries for use in the present invention will contain small molecules ("RNA ligands") tethered to electrophilic warheads that selectively form covalent bonds with nucleotides proximal to the binding site in the target RNA. The library's diversity encompasses variation in RNA ligand structure, tether structure, and warhead structure.

[00357] The RNA ligands are designed based on hypotheses about the structural determinant of RNA affinity and then synthesized and attached to the tether and warhead. As an example, Lau and coworkers used SELEX (systematic evolution of ligands by exponential enrichment) to evolve a short RNA sequence termed Aptamer-21 as a high-affinity RNA aptamer (K_d = 50 nM) against a heteroaryldihydropyrimidine structure, compound **1b** (**I-1** herein) below:



I-1 (compound 1b in Lau et al.)

(Lau, J. L.; Baksh, M. M.; Fiedler, J. D.; Brown, S. D.; Kussrow, A.; Bornhop, D. J.; Ordoukhanian, P.; Finn, M. G. *ACS Nano*, **2011**, *5*, 7722 – 7729; for more information on SELEX, see also, e.g., a) S. E. Osborne, A. D. Ellington, *Chem. Rev.* 1997, 97, 349–370; b) L. Gold, D. Brown, Y. Y. He, T. Shtatland, B. S. Singer, Y. Wu, *Proc. Natl. Acad. Sci. USA* 1997, 94, 59–64;

c) L. Gold, B. Polisky, O. Uhlenbeck, M. Yarus, *Annu. Rev. Biochem.* 1995, 64, 763–797.) This structure was chosen as a representative drug-like molecule with no cross-reactivity with mammalian or bacterial cells. The authors also embedded Aptamer 21, its weaker-binding variants, and a known aptamer against theophylline in a longer RNA sequence that was encapsidated inside a virus-like particle by an expression technique. These nucleoprotein particles were shown by backscattering interferometry to bind to the small-molecule ligands with affinities similar to those of the free (nonencapsidated) aptamers. Compound **I-1** is water-soluble, nontoxic, and sufficiently dissimilar in structure to native biological molecules to minimize off-target binding. It features a 1,4- triazole linkage installed with copper-catalyzed azide-alkyne cycloaddition (CuAAC) chemistry, which enables convenient connection of the ligand to other molecules of interest. Other HAP variants described herein are expected to have similarly advantageous properties. Furthermore, use of the Aptamer 21 RNA as a starting point offers the advantage of a well-characterized RNA of known structure and whose binding mode with **I-1** can be verified by reference to the original publication. Aptamer 21 has the following sequence: GGGUAGGCCAGGCAGCCAACUAGCGAGAGCUUAAAUCUCUGAGCCCGAGAGGGU UCAGUGCUGCUUAUGUGGACGGCU (SEQ. ID:25).

[00358] Alternatively, the RNA ligands are selected from commercially available sources based on their similarity to known RNA ligands or complementarity to RNA binding pockets, purchased, and subjected to further synthesis to attach to the tether and warhead. Examples include but are not limited to: tetracycline antibiotics, aminoglycoside antibiotics, theophylline and similar structures (e.g., xanthines), ribocil and similar structures, linezolid and similar structures. In a third and complementary approach, libraries of RNA ligands are prepared using combinatorial chemistry techniques. Specifically, the tethers of choice are affixed to polymers that support organic synthesis, and through a series of synthetic chemistry steps, compounds are made in a one-bead-one-compound format. These steps lead to the incorporation in the final RNA ligand a wide range of fragments and reactants connected by a wide range of functional groups. Those compounds are released and the final off-bead step is attachment of the RNA warhead.

[00359] As a key element of the library's functional outcome, for each RNA ligand and RNA warhead, a number of structurally diverse tethers are incorporated in order to optimize tether length, tether flexibility, and the ability to tolerate additional functionality (in particular, click

functional groups). Specific tethers that are explored include oligoethylene glycols containing one to six ethylene units, oligopeptides that are highly flexible (e.g., oligoglycines or oligo-N-methylglycines containing one to six amino acids) or more rigid (e.g., oligoprolines or oligo-4-hydroxyprolines containing one to six amino acids). Incorporation of click functional groups into the oligoethylene glycol tethers requires insertion of an amino acid, bearing a clickable functional group, at either the RNA ligand or the RNA warhead end of the tether. Incorporation of click functional groups into the oligopeptides tethers simply requires replacing any one of the amino acid residues with an amino acid bearing the clickable functional group.

[00360] The RNA warheads are selected from known or modified photoactivatable functional groups. Additional warheads will be identified by (1) synthetic modifications to the aforementioned warheads to establish the structure/activity relationship for RNA warheads as well as (2) screening commercially available photoactivatable groups.

[00361] Click functional groups are selected from the standard 'toolkit' of published click reagents and reactants. The present work focuses on azides, alkynes (both terminal and strained), dienes, tetrazines, and dienophiles.

[00362] Further details of the SHAPE, SHAPE-MaP, and PEARL-seq methods, including alternate reagents, conditions, and data analysis are described in WO 2017/136450, WO 2015/054247, US 2014/0154673, U.S. 7,745,614, and U.S. 8,313,424, each of which is hereby incorporated by reference in its entirety.

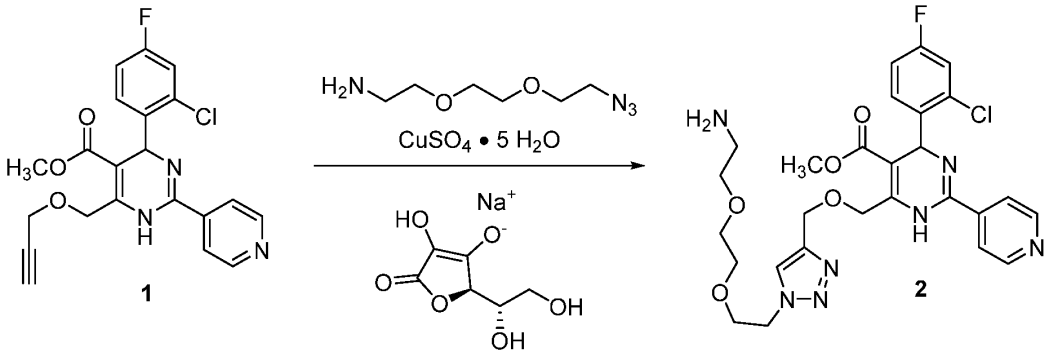
Example 2: Preparation of CPNQ Analogues and Other Quinoline-Based Ligands

[00363] Exemplary small molecule ligands based on CPNQ and other quinoline scaffolds may be prepared based on the synthetic schemes shown in WO2017/136450, which is hereby incorporated by reference in its entirety.

Example 3: Synthesis of Exemplary Photoprobe Compounds

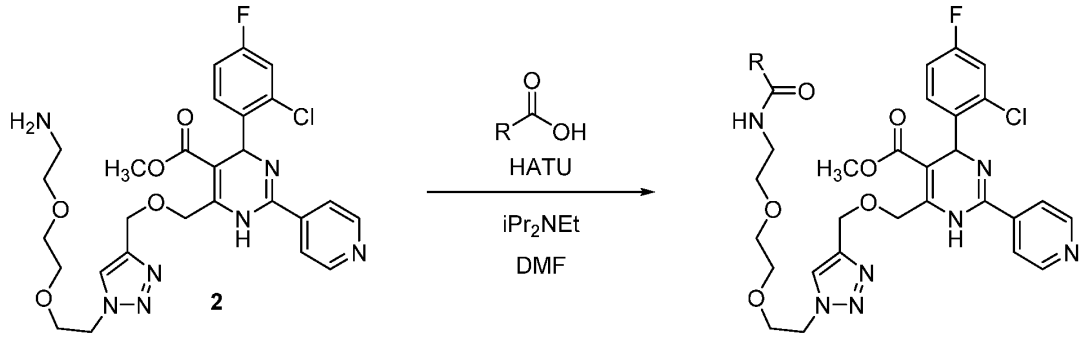
[00364] **General**: Unless otherwise noted, all reactions were conducted under an N_2 atmosphere. All solvents and reagents were used as received without further purification. Compound **1** was synthesized as previously described in Lau, J. L.; Baksh, M. M.; Fiedler, J. D.; Brown, S. D.; Kussrow, A.; Bornhop, D. J.; Ordoukhanian, P.; Finn, M. G. *ACS Nano*, **2011**, *5*,

7722–7729. 3-azido-5-(azidomethyl)benzoic acid was prepared as previously described in Yoshida, S.; Misawa, Y.; Hosoya, T. *Eur. J. Org. Chem.*, **2014**, *19*, 3991–3995. Rotary evaporation was performed under 30 torr with a bath temperature below 40 °C. Analytical LC-MS data were obtained on a Waters Acquity UPLC system equipped with an Acquity BEH 1.7 µm C_{18} column (2.1 x 50 mm) and an elution gradient of 90 : 10 A : B to 40 : 60 A : B over 2.3 min, where A = 0.1% formic acid in water and B = 0.1% formic acid in acetonitrile and with a flow rate of 0.80 mL min^{-1}. Column chromatography was performed on a Teledyne Isco Combiflash Rf+ system using pre-packed RediSep Rf+ Gold silica gel (Teledyne Isco) or 40 – 60 µm spherical C_{18} silica gel columns (Agela). NMR spectra were obtained on a Bruker 400 MHz spectrometer; chemical shifts are referenced to the residual mono-^{1}H-isotopomer of the solvent: CHD_2OD = 3.31 ppm; CD_3SOCD_2H = 2.49 ppm; $CHCl_3$ = 7.26 ppm.

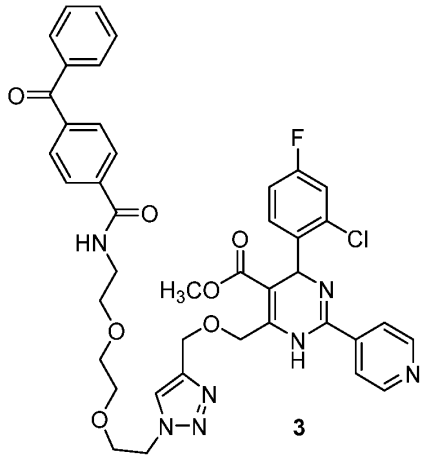


[00365] Compound **2**: To a solution of MeOH (4 mL) and H_2O (1 mL) at room temperature was added **1** (100 mg, 242 µmol, 1 eq.), 2-[2-(2-azidoethoxy)ethoxy]ethylamine (42 mg, 242 µmol, 1.0 eq.), $CuSO_4$•$5H_2O$ (60 mg, 242 µmol, 1.0 eq.) and sodium ascorbate (96 mg, 483 µmol, 2.0 eq.). The mixture was stirred at 20 °C for 0.5 h. The mixture was diluted with a saturated aqueous solution of $NaHCO_3$ (30 mL), then the mixture was extracted with EtOAc (3 x 50 mL). The combined extracts were washed with saturated aqueous NaCl solution, then were dried over Na_2SO_4. The solids were filtered and the filtrate was concentrated under reduced pressure to afford the crude product as a yellow solid. The residue was purified by preparatory HPLC using a Phenomenex Synergi C_{18} column (150 x 25 x10 µm), eluting with a gradient of 10 – 30% CH_3CN in water containing 0.05% HCl to afford **2** as yellow solid (HCl salt, 65 mg, 43% yield). LCMS: t_R: 1.749 min; (M+H) = 588.2. ^{1}H NMR (400 MHz, METHANOL-d_4) ppm = 9.12 (br s, 2H), 8.21 (br d, J=17.1 Hz, 3H), 7.76 (dd, J=6.0, 8.7 Hz, 1H), 7.42 (dd, J=2.4, 8.6 Hz, 1H), 7.26 (dt, J=2.6,

8.3 Hz, 1H), 6.42 (s, 1H), 5.02 - 4.93 (m, 2H), 4.84 (s, 2H), 4.65 (t, J=5.0 Hz, 2H), 3.94 (t, J=5.0 Hz, 2H), 3.75 - 3.55 (m, 9H), 3.10 (t, J=4.8 Hz, 2H).

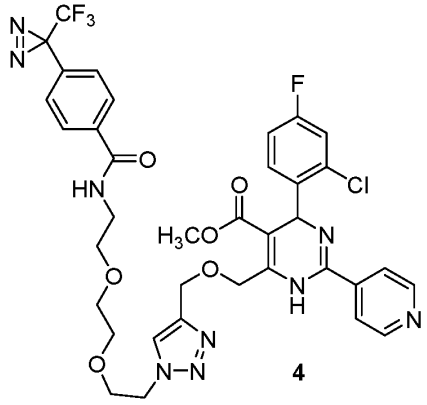


[00366] General procedure A – HATU-mediated acid-amine coupling reactions: To a stirring solution of carboxylic acid (1.2 eq.) in DMF (0.05 M) at room temperature was added HATU (2.0 eq.), *N*,*N*-diisopropylethylamine (4.0 eq.), and primary amine (1 eq.). The resulting solution was incubated at room temperature for 2 – 16 h, then the reaction mixture was loaded directly onto a pre-packed C_{18}-silica gel column for purification. Fractions containing the desired product were combined, frozen at -78 °C (acetone/CO_2), and lyophilized to afford the final photoprobe compounds as solids.



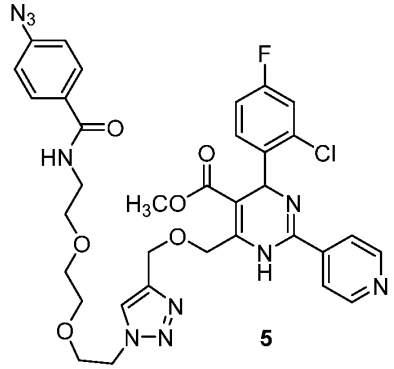
[00367] Compound **3**: Compound **3** was prepared according to General Procedure A above from **2** (30 mg, 51 µmol) and 4-benzoylbenzoic acid. The crude product mixture was purified by reverse-phase chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1%

formic acid. Probe **3** was isolated as a light tan solid (formate salt, 39 mg, 83% yield). LC-MS: t_R: 1.49 min; $[M+H]^+$ 796.2.



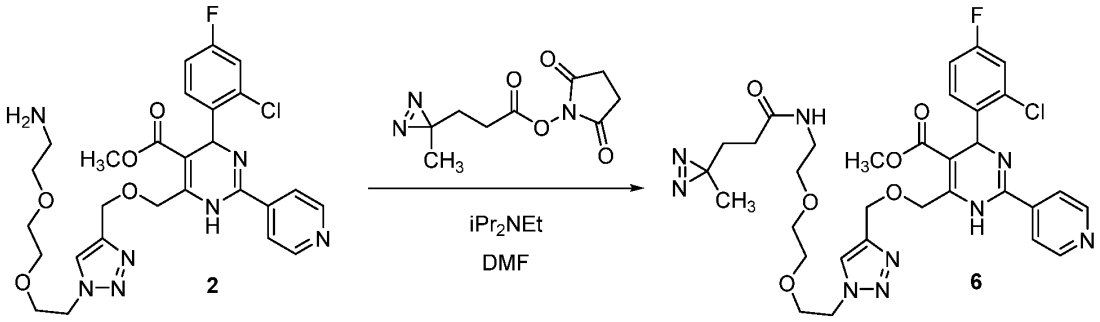
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[00368] Compound **4**: Compound **4** was prepared according to General Procedure A above from **2** (30 mg, 51 μmol) and 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid. The crude product mixture was purified by reverse-phase chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Compound **4** was isolated as a bright yellow solid (formate salt, 19 mg, 44% yield). LC-MS: t_R: 1.56 min; $[M+H]^+$ 800.2.



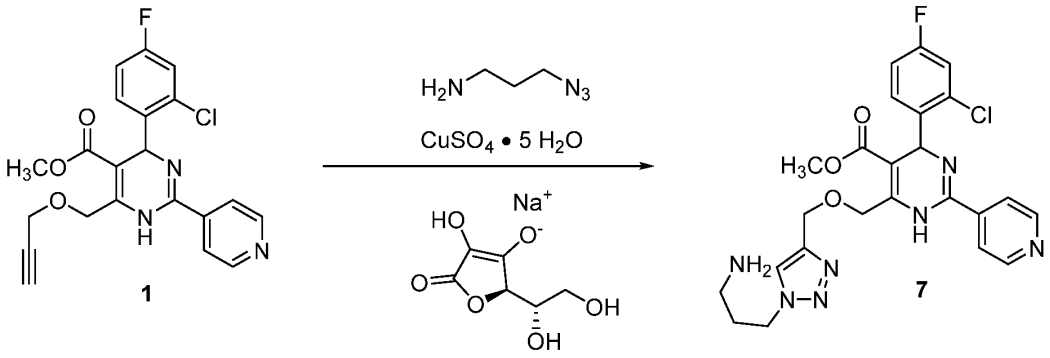
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[00369] Compound **5**: Compound **5** was prepared according to General Procedure A above from **2** (50 mg, 85 μmol) and 4-azidobenzoic acid (as a 0.2 M solution in methyl *tert*-butyl ether). After stirring at room temperature for 3 h, the crude product mixture was partially concentrated to remove methyl *tert*-butyl ether, then the resulting product solution (in DMF) was purified by reverse-phase chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Compound **5** was isolated as a bright yellow solid (formate salt, 32 mg, 49% yield). LC-MS: t_R: 1.45 min; $[M+H]^+$ 733.2.



2 6

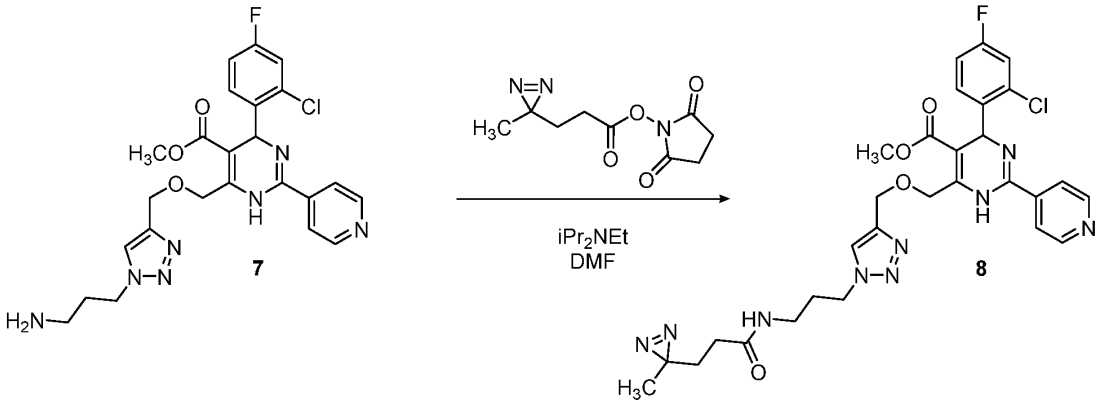
[00370] Compound **6**: To a stirring solution of **2** (30 mg, 51 μmol, 1 eq.) in DMF (2.0 mL) at room temperature was added *N*,*N*-diisopropylethylamine (27 μL, 153 μmol, 3.0 eq.) and 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (12 mg, 54 μmol, 1.05 eq.). The resulting bright yellow reaction mixture was allowed to stir at room temperature for 2 h, then was purified immediately by flash column chromatography over C18-silica gel, eluting with 0 – 60% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product were concentrated under reduced pressure to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford the desired product **6** as a bright yellow solid (formate salt, 16.0 mg, 42% yield). LC-MS: t_R: 1.40 min; $[M+H]^+$ 698.3.



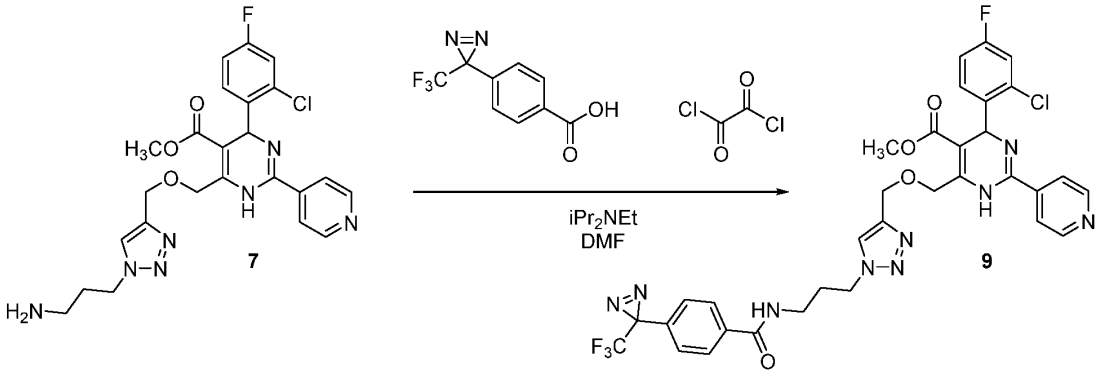
1 7

[00371] Compound **7**: To a stirring suspension of **1** (321 mg, 0.78 mmol, 1 eq.) in *t*-BuOH (4.0 mL) and water (4.0 mL) was added 3-azidopropylamine (93 mg, 0.93 mmol, 1.2 eq.) and $CuSO_4 \cdot 5H_2O$ (15 mg, 62 μmol, 0.08 eq.). To the resulting suspension was added a solution of sodium ascorbate (46 mg, 0.23 mmol, 0.30 eq.) in water (1.0 mL). The resulting suspension was allowed to stir vigorously at room temperature for 2 h, then was concentrated under reduced pressure to remove *t*-BuOH. The crude product solution that remained was directly purified by flash column chromatography over C_{18}-silica gel, eluting with 0 – 30% CH_3CN in water containing

0.1% NH_3. Fractions containing the desired product were frozen at -78 °C and lyophilized to afford compound **7** as a bright yellow solid (305 mg, 77% yield). LCMS: t_R: 1.19 min; $[M+H]^+$ 514.2.

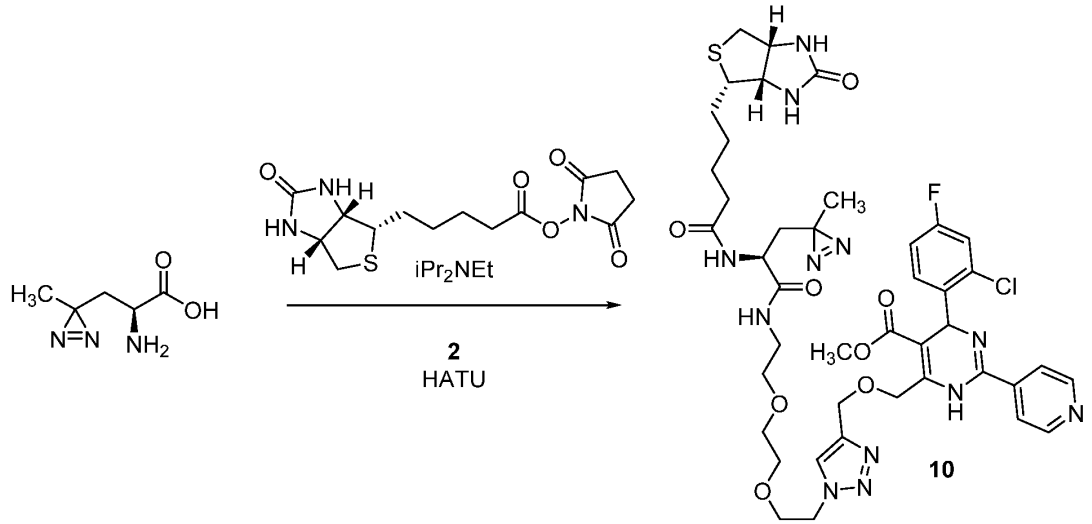


[00372] Compound **8**: To a stirring solution of **7** (30 mg, 58 μmol, 1 eq.) in DMF (2.0 mL) at room temperature was added *N,N*-diisopropylethylamine (41 μL, 233 μmol, 4.0 eq.) and 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3*H*-diazirin-3-yl)propanoate (14 mg, 64 μmol, 1.10 eq.). The resulting bright yellow reaction mixture was allowed to stir at room temperature for 2 h, then was purified immediately by flash column chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product were concentrated under reduced pressure to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford the desired product as a bright yellow solid (formate salt, 17.0 mg, 44% yield). LC-MS: t_R: 1.40 min; $[M+H]^+$ 624.2.

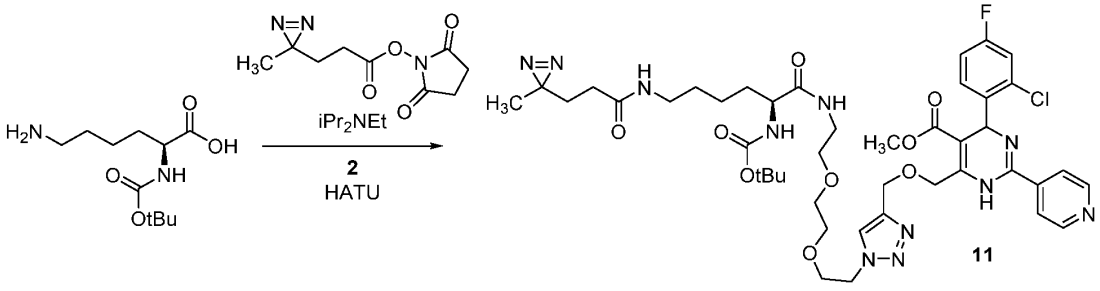


[00373] Compound **9**: To a stirring suspension of 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid (27 mg, 116 μmol, 1.2 eq.) in CH_2Cl_2 (2.0 mL) at room temperature was added DMF (ca. 10 μL) and a 2.0 M solution of oxalyl chloride in CH_2Cl_2 (49 μL, 98 μmol, 1.0 eq.). The resulting clear, colorless mixture was allowed to stir at room temperature for 1 h, then **7** (50 mg, 97 μmol, 1 eq.) and *N*,*N*-diisopropylethylamine (68 μL, 0.39 mmol, 4.0 eq.) were added. The mixture was allowed to stir at room temperature for 3 h, then was concentrated under reduced pressure. The crude product was purified by reverse-phase flash column chromatography on C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product were combined and partially evaporated, then were frozen at -78 °C and lyophilized to afford the desired product as a yellow solid (formate salt, 32 mg, 47% yield). LC-MS: t_R: 1.57 min; $[M+H]^+$ 726.0.

[00374] **General Procedure B – Three component coupling of an amino acid, carboxylic acid NHS ester, and an amine:** To a suspension of amino acid (1 eq.) in DMF (2.0 mL) at room temperature was added *N*,*N*-diisopropylethylamine (4.0 eq.) and carboxylic acid *N*-hydroxysuccinimidyl ester (1.05 eq.). The resulting suspension was allowed to stir at room temperature for 12 – 48 h. Once formation of the amide was complete (as determined by LC-MS analysis), 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (2.0 eq.) and amine (1.2 eq.) were added and the resulting bright yellow mixture was allowed to stir at room temperature for 3 h. The mixture was directly purified by column chromatography over C_{18} silica gel. Fractions containing the desired product were combined and partially evaporated to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford the desired products as solids.

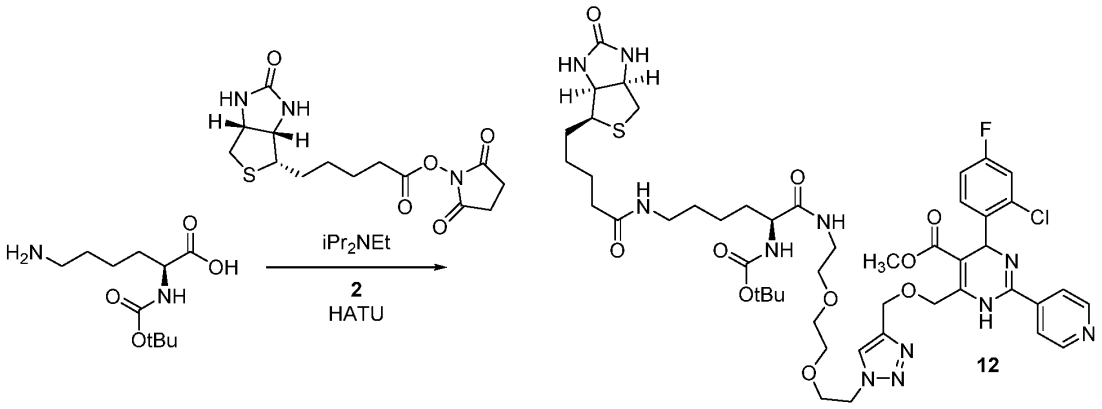


[00375] Compound **10**: Compound **10** was prepared according to General Procedure B above from L-photoleucine (25 mg, 0.17 mmol, 1 eq.), biotin *N*-hydroxysuccinimidyl ester (62 mg, 0.18 mmol, 1.05 eq.), and **2** (122 mg, 0.21 mmol, 1.2 eq.). The mixture was directly purified by column chromatography over C_{18} silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product were combined and partially evaporated to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford the desired product as a yellow solid (formate salt, 97 mg, 57% yield). LC-MS: t_R: 1.35 min; $[M+H]^+$ 939.3.

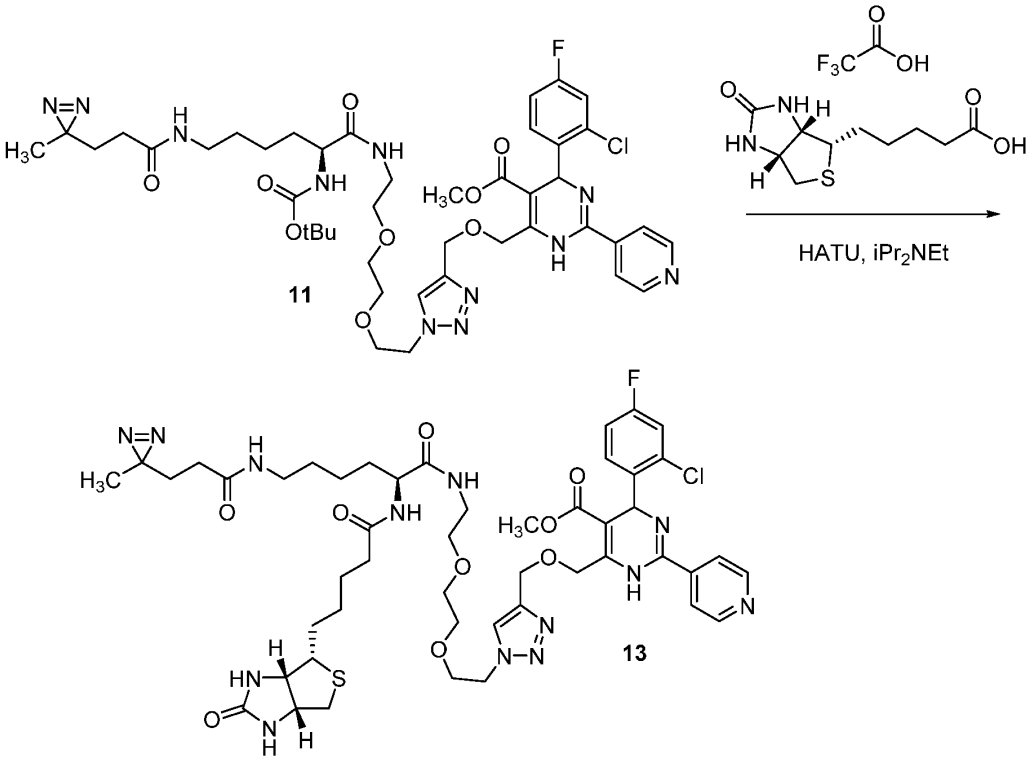


[00376] Compound **11**: Compound **11** was prepared according to General Procedure B above from N^{α}-*tert*-butoxycarbonyl-L-lysine (50 mg, 0.20 mmol, 1 eq.), 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (48 mg, 0.21 mmol, 1.05 eq.), and **2** (142 mg, 0.24 mmol, 1.2 eq.). The mixture was directly purified by column chromatography over C_{18} silica gel, eluting with 0 – 50% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product

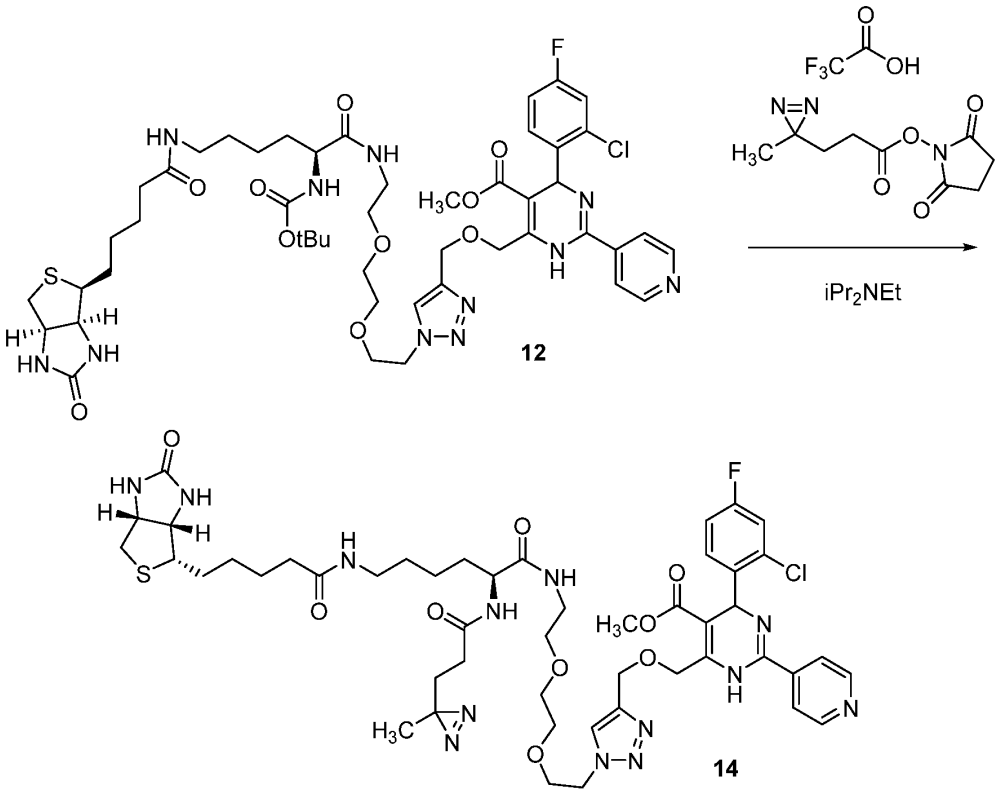
were combined and partially evaporated to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford the desired product as a yellow solid (formate salt, 120 mg, 61% yield). LC-MS: t_R: 1.46 min; $[M+H]^+$ 926.4.



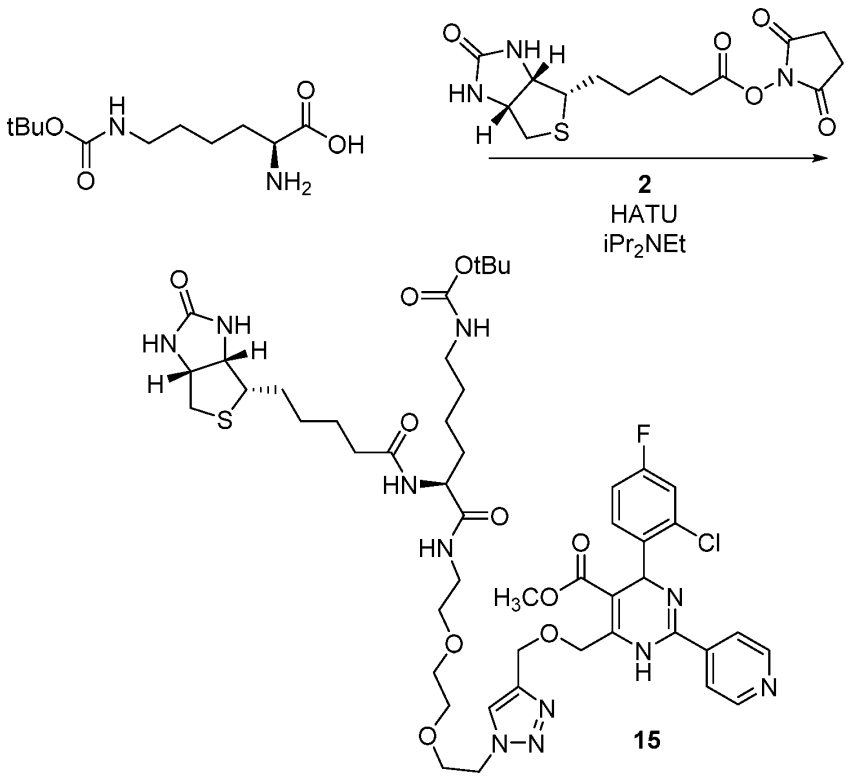
[00377] Compound **12**: Compound **12** was prepared according to General Procedure B above from N^{α}-*tert*-butoxycarbonyl-L-lysine (50 mg, 0.20 mmol, 1 eq.), biotin *N*-hydroxysuccinimidyl ester (72 mg, 0.21 mmol, 1.05 eq.), and **2** (142 mg, 0.24 mmol, 1.2 eq.). The mixture was directly purified by column chromatography over C_{18} silica gel, eluting with 0 – 50% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product were combined and partially evaporated to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford the desired product as a yellow solid (formate salt, 105 mg, 48% yield). LC-MS: t_R: 1.38 min; $[M+H]^+$ 1042.4.



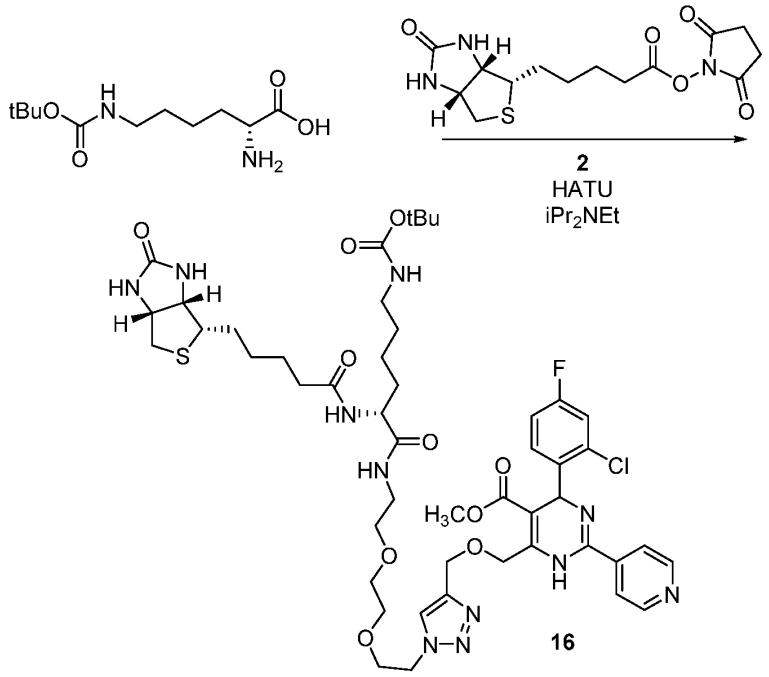
[00378] Compound **13**: A stirring solution of **11** (40 mg, 41 μmol, 1 eq.) in CH_2Cl_2 (1.0 mL) at 0 °C was treated dropwise with neat trifluoroacetic acid (250 μL). The resulting mixture was allowed to warm to room temperature, then was allowed to stir at room temperature for 15 min. The mixture was concentrated to dryness under reduced pressure to afford the crude primary amine. The crude amine was resuspended in DMF (1.0 mL), then was treated with *N*,*N*-diisopropylethylamine (72 μL, 0.41 mmol, 10.0 eq.), biotin, (15 mg, 62 μmol, 1.5 eq.), and HATU (31 mg, 82 μmol, 2.0 eq.). The resulting bright yellow mixture was maintained at room temperature for 2 h, then was immediately loaded onto a C_{18}-silica gel column, eluting with 0 – 70% CH_3CN in water containing 0.1% formic acid). Fractions containing the desired product were combined and partially evaporated under reduced pressure to remove CH_3CN, then were frozen and lyophilized to afford the desired product as a yellow solid (formate salt, 27 mg, 60% yield). LC-MS: t_R: 1.38 min; $[M+H]^+$ 1052.4.



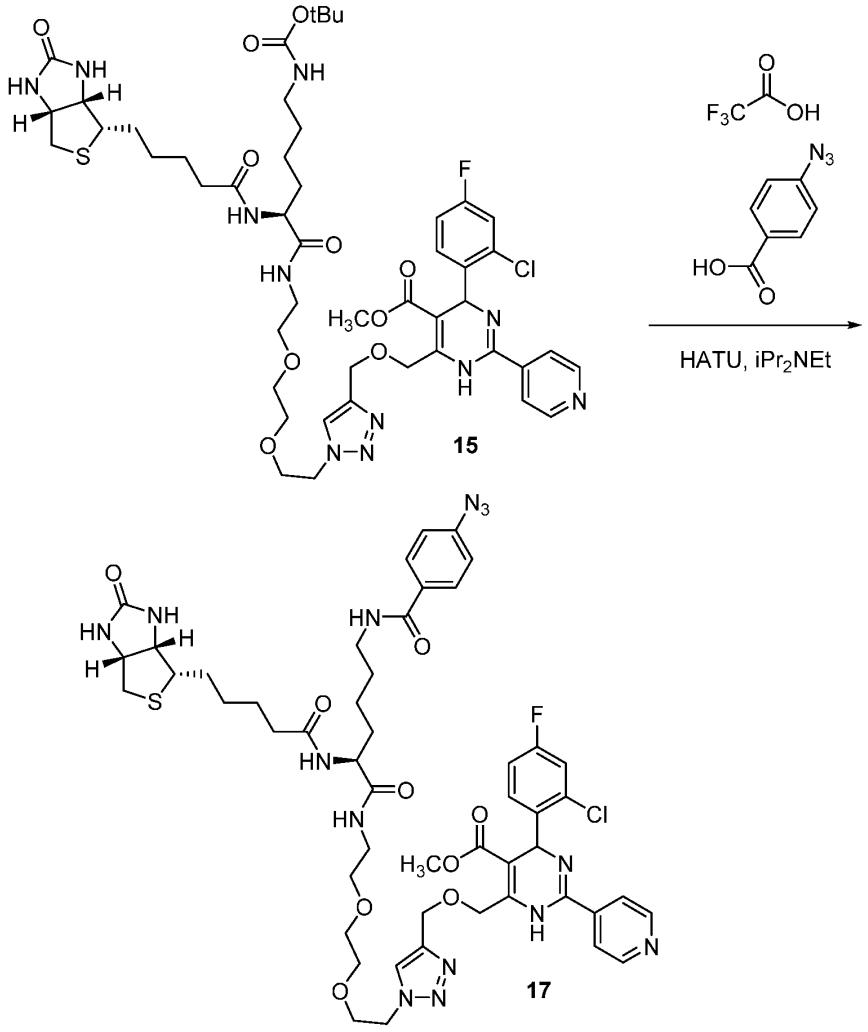
[00379] Compound **14**: A stirring solution of **12** (40 mg, 37 μmol, 1 eq.) in CH_2Cl_2 (1.0 mL) at 0 °C was treated dropwise with neat trifluoroacetic acid (250 μL). The resulting mixture was allowed to warm to room temperature, then was allowed to stir at room temperature for 55 min. The mixture was concentrated to dryness under reduced pressure to afford the crude primary amine. The crude amine was resuspended in DMF (1.0 mL), then was treated with *N*,*N*-diisopropylethylamine (64 μL, 0.37 mmol, 10.0 eq.), and 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (10 mg, 44 μmol, 1.2 eq.). The resulting bright yellow mixture was maintained at room temperature for 16 h, then was immediately loaded onto a C_{18}-silica gel column, eluting with 0 – 70% CH_3CN in water containing 0.1% formic acid). Fractions containing the desired product were combined and partially evaporated under reduced pressure to remove CH_3CN, then were frozen and lyophilized to afford the desired product as a yellow solid (formate salt, 22 mg, 54% yield). LC-MS: t_R: 1.37 min; $[M+H]^+$ 1052.4.



[00380] Compound **15**: Compound **15** was prepared according to General Procedure B above using N^{ε}-*tert*-butoxycarbonyl-L-lysine (175 mg, 0.71 mmol, 1 eq.), biotin *N*-hydroxysuccinimidyl ester (252 mg, 0.74 mmol, 1.05 eq.), and **2** (500 mg, 0.85 mmol, 1.2 eq.). Following completion of the reaction (as determined by LC-MS analysis), the reaction mixture was diluted with EtOAc (150 mL) and was washed with 4 x 40 mL portions of water. The organic phase was dried over Na_2SO_4, the solids filtered, and the filtrate was concentrated to afford the crude product as a yellow gum. The crude product was triturated once with diethyl ether : pentane (1 : 1, 10 mL), then the resulting solid was dissolved in methanol (5 mL) and was cooled to -50 °C. Diethyl ether (20 mL) was added, then the resulting solid was collected by filtration to afford **15** as a yellow solid (380 mg, 43% yield). LC-MS: t_R:1.40 min; $[M+H]^+$ 1042.1.

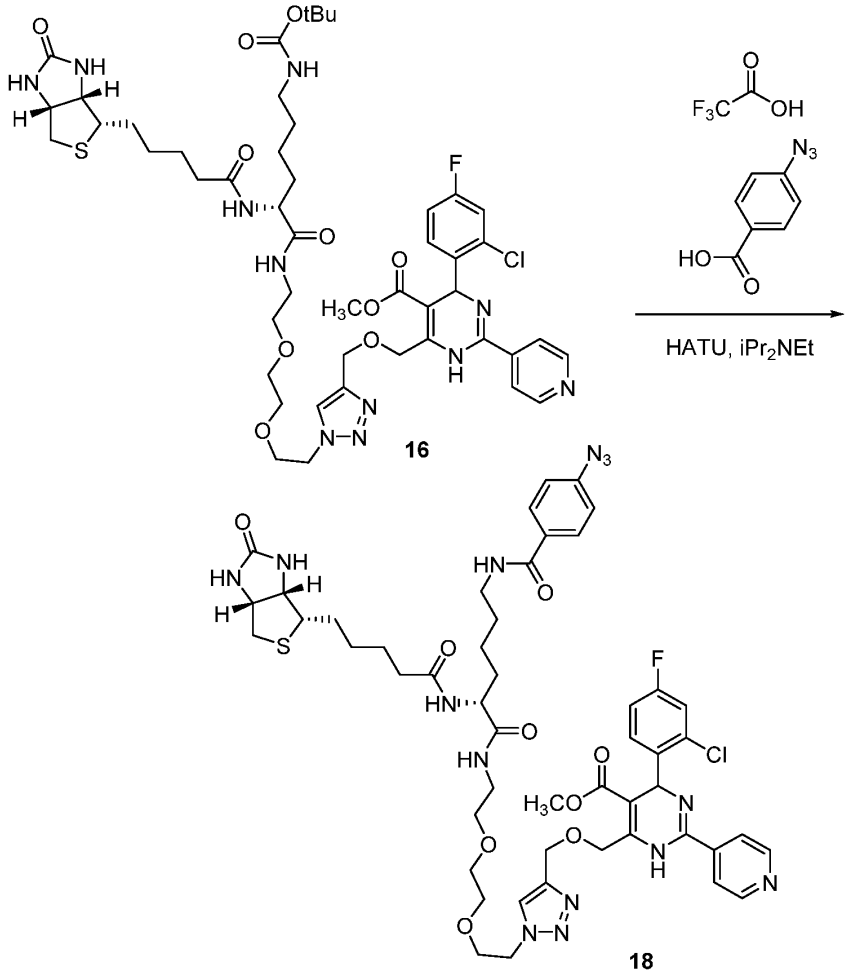


[00381] Compound **16**: Compound **16** was prepared analogously to compound **15**, with the exception that N^{ε}-*tert*-butoxycarbonyl-D-lysine (900 mg, 1.90 mmol, 1 eq.) was used in place of N^{ε}-*tert*-butoxycarbonyl-L-lysine as the starting amino acid. Compound **16** was obtained as a yellow solid (1.03 g, 52% yield). LC-MS: t_R: 1.40 min; $[M+H]^+$ 1042.1.



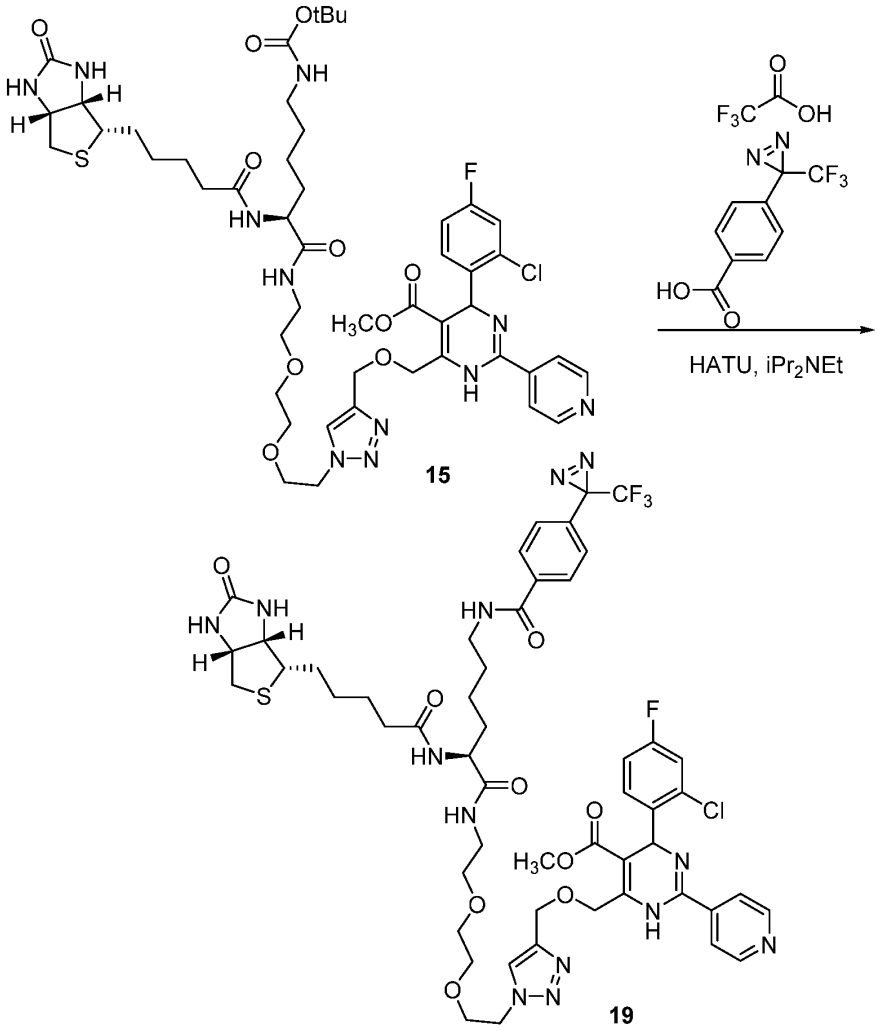
[00382] Compound **17**: A solution of **15** (55 mg, 53 μmol, 1 eq.) in CH_2Cl_2 (1.0 mL) at 0 °C was treated with trifluoroacetic acid (0.25 mL). The resulting mixture was allowed to warm to room temperature over 15 minutes. After this time the mixture was concentrated to dryness under reduced pressure. The resulting crude amine was dissolved in DMF (2.0 mL), then was treated with a 0.2 M solution of 4-azidobenzoic acid in methyl *tert*-butyl ether (0.53 mL, 0.11 mmol, 2.0 eq.), HATU (40 mg, 0.11 mmol, 2.0 eq.), and *N*,*N*-diisopropylethylamine (37 μL, 0.21 mmol, 4.0 eq.). The resulting mixture was allowed to stir at room temperature overnight, then was partially concentrated to remove methyl *tert*-butyl ether. The resulting product solution (in DMF) was directly purified by column chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in

water containing 0.1% formic acid. Fractions containing the desired product were combined and partially evaporated to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford **17** as a yellow solid (formate salt, 35 mg, 58% yield). LC-MS: t_R: 1.41 min; $[M+H]^+$ 1087.4.



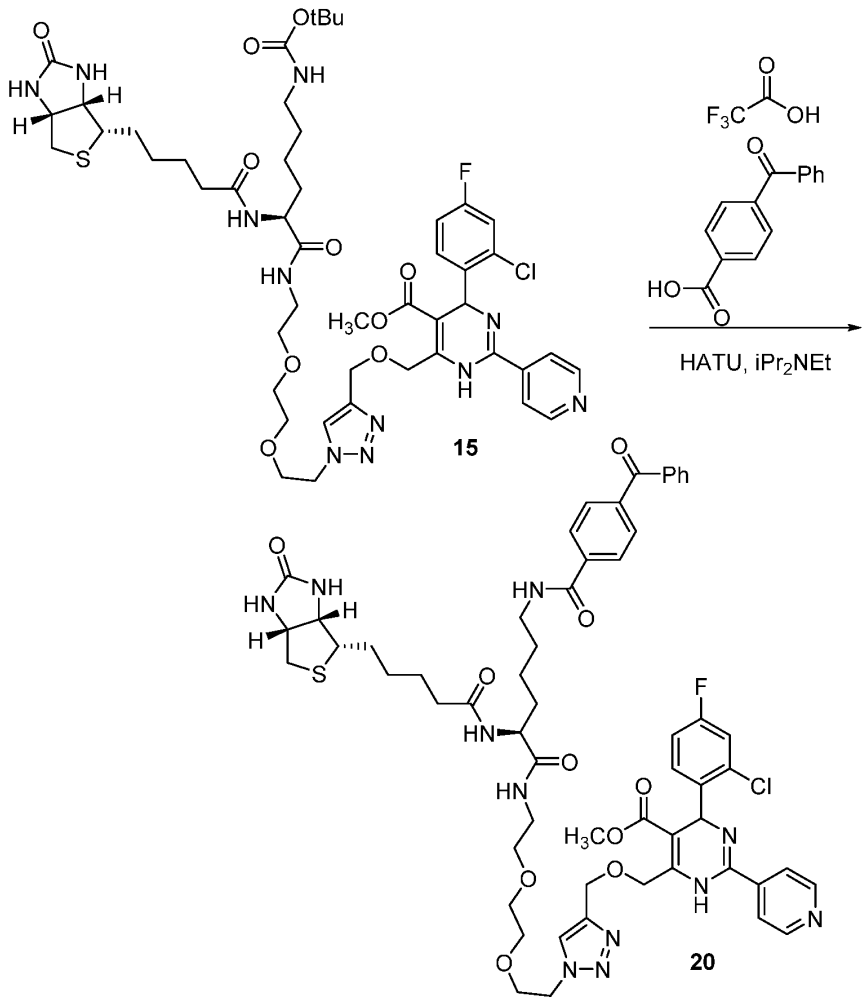
[00383] Compound **18**: A solution of **16** (55 mg, 53 µmol, 1 eq.) in CH_2Cl_2 (1.0 mL) at 0 °C was treated with trifluoroacetic acid (0.25 mL). The resulting mixture was allowed to warm to room temperature over 15 minutes. After this time the mixture was concentrated to dryness under reduced pressure. The resulting crude amine was dissolved in DMF (2.0 mL), then was treated with a 0.2 M solution of 4-azidobenzoic acid in methyl *tert*-butyl ether (0.53 mL, 0.11 mmol, 2.0 eq.), HATU (40 mg, 0.11 mmol, 2.0 eq.), and *N*,*N*-diisopropylethylamine (37 µL, 0.21 mmol, 4.0 eq.). The resulting mixture was allowed to stir at room temperature overnight, then was partially

concentrated to remove methyl *tert*-butyl ether. The resulting product solution (in DMF) was directly purified by column chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product were combined and partially evaporated to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford **18** as a yellow solid (formate salt, 38 mg, 63% yield). LC-MS: t_R: 1.41 min; $[M+H]^+$ 1087.4.



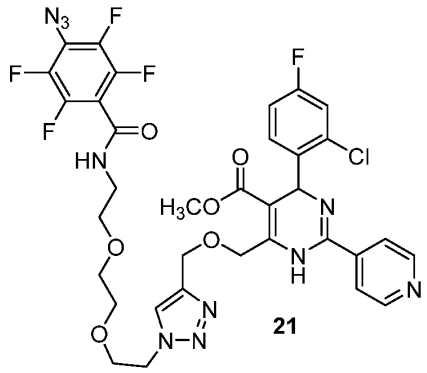
[00384] Compound **19**: A solution of **15** (55 mg, 53 µmol, 1 eq.) in CH_2Cl_2 (1.0 mL) at 0 °C was treated with trifluoroacetic acid (0.25 mL). The resulting mixture was allowed to warm to room temperature over 15 minutes. After this time the mixture was concentrated to dryness under reduced pressure. The resulting crude amine was dissolved in DMF (2.0 mL), then was treated

with 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid (24 mg, 0.11 mmol, 2.0 eq.), HATU (40 mg, 0.11 mmol, 2.0 eq.), and *N*,*N*-diisopropylethylamine (37 µL, 0.21 mmol, 4.0 eq.). The resulting mixture was allowed to stir at room temperature overnight, then the resulting product solution (in DMF) was directly purified by column chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product were combined and partially evaporated to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford **19** as a yellow solid (formate salt, 15 mg, 24% yield). LC-MS: t_R: 1.48 min; $[M+H]^+$ 1155.6.



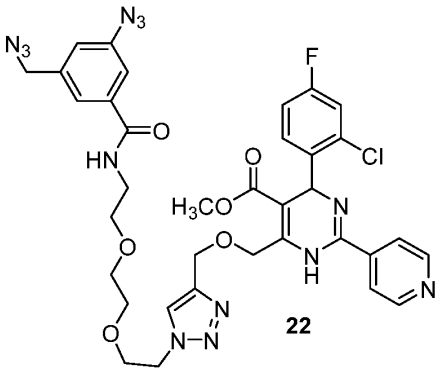
[00385] Compound **20**: A solution of **15** (55 mg, 53 µmol, 1 eq.) in CH_2Cl_2 (1.0 mL) at 0 °C was treated with trifluoroacetic acid (0.25 mL). The resulting mixture was allowed to warm to

room temperature over 15 minutes. After this time the mixture was concentrated to dryness under reduced pressure. The resulting crude amine was dissolved in DMF (2.0 mL), then was treated with a 4-benzoylbenzoic acid (24 mg, 0.11 mmol, 2.0 eq.), HATU (40 mg, 0.11 mmol, 2.0 eq.), and *N*,*N*-diisopropylethylamine (37 µL, 0.21 mmol, 4.0 eq.). The resulting mixture was allowed to stir at room temperature overnight, then the resulting product solution (in DMF) was directly purified by column chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Fractions containing the desired product were combined and partially evaporated to remove CH_3CN, then were frozen at -78 °C and lyophilized to afford **20** as a yellow solid (formate salt, 32 mg, 52% yield). LC-MS: t_R: 1.43 min; $[M+H]^+$ 1151.7.



21

[00386] Compound **21**: Compound **21** was prepared according to General Procedure A above from **2** (37 mg, 63 µmol) and 4-azido-2,3,5,6-tetrafluorobenzoic acid. After stirring at room temperature for 3 h, the crude product mixture was purified by reverse-phase chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Compound **21** was isolated as a bright yellow solid (formate salt, 26 mg, 52% yield). LC-MS: t_R: 1.51 min; $[M+H]^+$ 806.6.

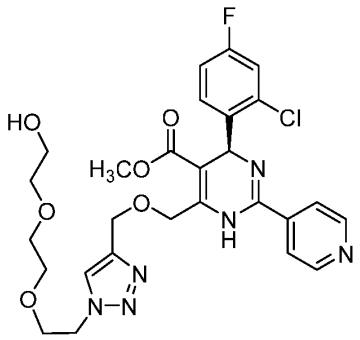


22

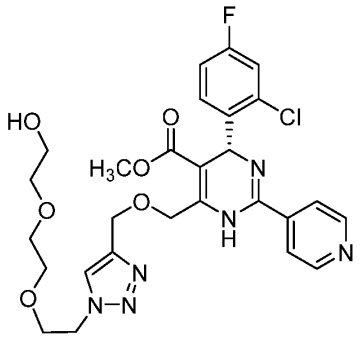
[00387] Compound **22**: Compound **22** was prepared according to General Procedure A above from **2** (37 mg, 63 μmol) and 3-azido-5-(azidomethyl)benzoic acid. After stirring at room temperature for 3 h, the crude product mixture was purified by reverse-phase chromatography over C_{18}-silica gel, eluting with 0 – 100% CH_3CN in water containing 0.1% formic acid. Compound **22** was isolated as a bright yellow solid (formate salt, 26 mg, 52% yield). LC-MS: t_R: 1.51 min; $[M+H]^+$ 789.5.

Example 4: SFC Separation of I-1 (ARK-139) Enantiomers and Determination of Absolute Stereochemistry of Active Isomer

[00388] Racemic **I-1** (ARK-139) was subjected to SFC chiral separation (ChiralPak AD column, isocratic elution of 70%A/30%B, phase A for supercritical CO_2, phase B for MeOH, total flowrate of 60 g/min, cycle time 3 min), and two peaks were separated and collected.



I-1a (S-enantiomer) ARK-702



I-1b (R-enantiomer) ARK-701

[00389] Separation of the two enantiomers of **I-1** allows preparation of non-racemic versions of each of the compounds of Table 5 and Example 3 and other HAP compounds disclosed herein.

In some embodiments, the present invention accordingly provides such compounds that are enantioenriched at the HAP stereocenter.

[00390] (*R*) -methyl 4-(2-chloro-4-fluorophenyl)-6-(((1-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-1H-1,2,3- triazol-4-yl)methoxy)methyl)- 2-(pyridin-4-yl)-1,4-dihydropyrimidine-5-carboxylate (**I-1b** above). Yellow oil. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.68 (q, *J* = 1.6, 3.2 Hz, 2 H), 8.54 (br s, 1H), 7.87 (s, 1H), 7.70 (q, *J* = 1.6, 3.2 Hz, 2H), 7.31 (dd, *J* = 6.0, 2.4 Hz, 1H), 7.13 (dd, *J* = 2.4, 6.0 Hz, 1H), 6.95-6.90 (dt, *J* = 4.2, 8.4 Hz, 1H), 6.21 (s, 1H), 4.98 (d, *J* = 1.0, 2H), 4.83 (s, 2H), 4.59 (t, *J* = 4.8 Hz, 2H), 3.90 (t, *J* = 4.8 Hz, 2H), 3.71 (t, *J* = 4.8 Hz, 2H), 3.65-3.60 (m, 7H), 3.56 (t, t, *J* = 4.8 Hz, 1H), 2.70 (br s, 1H). LCMS, calcd $C_{27}H_{30}ClFN_6O_6$ (M+H) = 589.02, found = 589.4. Retention time in chiral LCMS: 1.74 min, Optical rotation: $[\alpha]_D^{25}$ + 57.4 (c 0.7, MeOH).

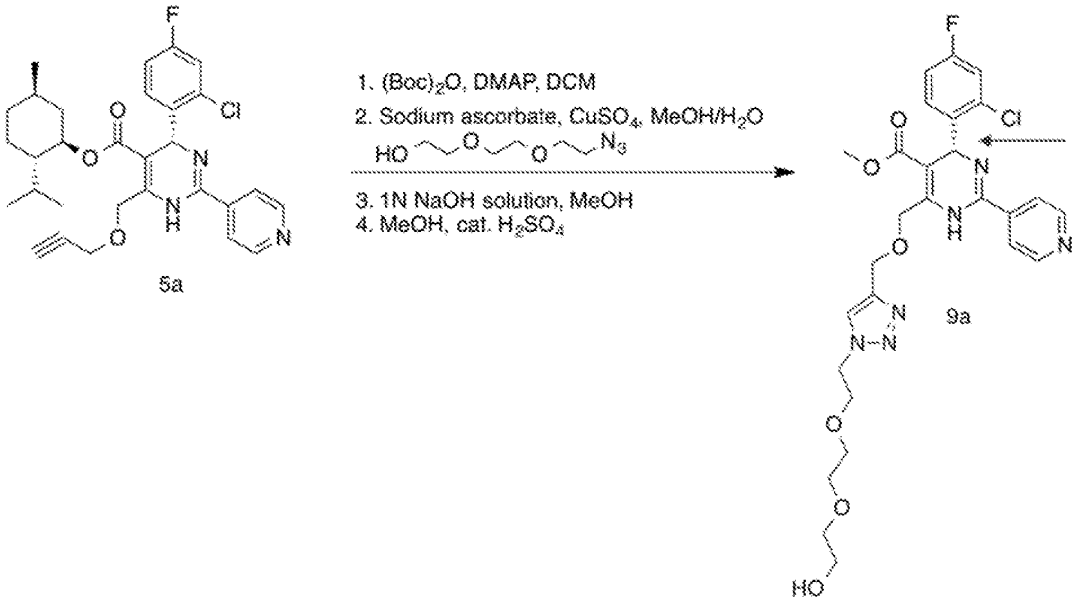
[00391] (*S*)-methyl 4-(2-chloro-4-fluorophenyl)-6-(((1-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-1H-1,2,3- triazol-4-yl)methoxy)methyl)- 2-(pyridin-4-yl)-1,4-dihydropyrimidine-5-carboxylate (**I-1a** above). Yellow oil. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.68 (q, *J* = 1.6, 3.2 Hz, 2 H), 8.54 (br s, 1H), 7.87 (s, 1H), 7.70 (q, *J* = 1.6, 3.2 Hz, 2H), 7.31 (dd, *J* = 6.0, 2.4 Hz, 1H), 7.13 (dd, *J* = 2.4, 6.0 Hz, 1H), 6.95-6.90 (dt, *J* = 4.2, 8.4 Hz, 1H), 6.21 (s, 1H), 4.98 (s, 2H), 4.83 (s, 2H), 4.59 (t, *J* = 4.8 Hz, 2H), 3.90 (t, *J* = 4.8 Hz, 2H), 3.71 (t, *J* = 4.8 Hz, 2H), 3.65-3.60 (m, 7H), 3.56 (t, t, *J* = 4.8 Hz, 1H), 2.62 (br s, 1H). LCMS, calcd $C_{27}H_{30}ClFN_6O_6$ (M+H) = 589.02, found = 589.4. Retention time in chiral LCMS: 1.93 min, Optical rotation: $[\alpha]_D^{25}$ – 60.7 (c 0.7, MeOH).

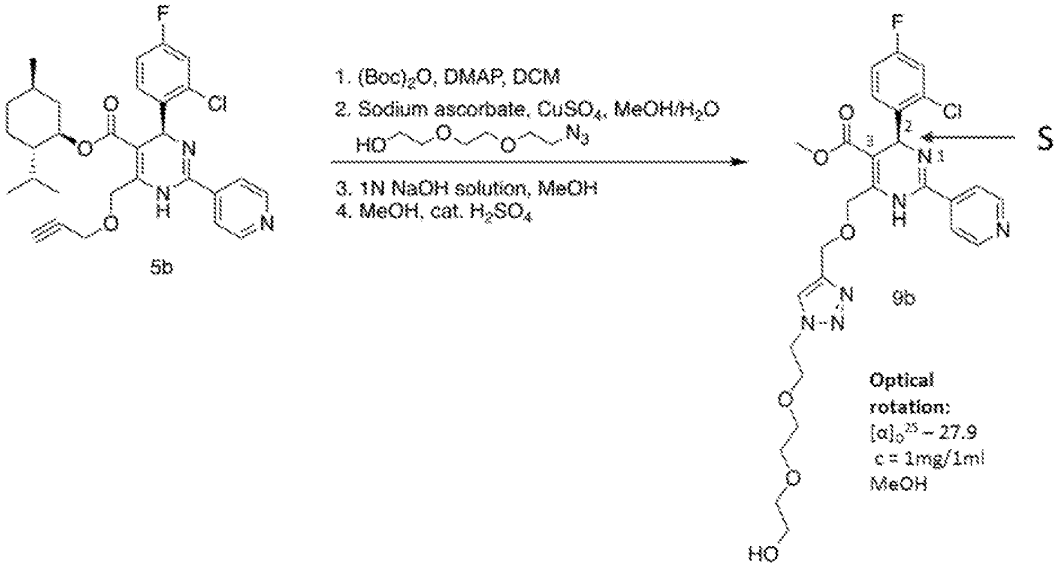
SFC Separation Conditions

Instrument	Waters 80Q SFC	
Column	ChiralPak AD column, 250×25mm I.D., 10um particle size;	
Mobile Phase	Phase A for Supercritical CO_2	Phase B for Methanol (neutral))
Isocratic elution	30% Phase B (70% Phase A)	
Total flow rate	60 g/min	
Cycle time	3 min	
Back Pressure	100 bar to keep the CO_2 in Supercritical flow	
Detector	UV 220nm	

Sample preparation	Material (about 80 mg) was dissolved in 25 mL MeOH
Injection	1 mL

[00392] Further experiments (SPR) showed that ARK-702 (I-1a) did not bind at 10 uM to Aptamer 21. On the other hand, ARK-701 (I-1b) did bind and is therefore the sole active isomer. Absolute stereochemistry was determined as shown below and using SFC to confirm.





Example 5: Procedure for SEC/MS Analyses

[00393] Online SEC-LCMS has been used to study the binding to Aptamer 21 RNA against **I-1** (ARK-139).

[00394] The RNA concentration was 1 μM and the ligand concentration ranged from 0.1 μM to 10 μM in an appropriate buffer (TRIS-HCl 20 mM pH 8.0, $MgCl_2$ 3 mM, KCl 100 mM).

[00395] The RNA/ligand mixtures were incubated at room temperature for 20 minutes in a 96-well plate format, then this plate was loaded into an autosampler linked to the chromatography system fitted with a reusable SEC column for rapid separation of target/ligand complexes from unbound components. RNA/ligand complexes in SEC eluent were monitored by UV detection, and an automated valving system directed the RNA peak to a reverse phase chromatography column for dissociation, desalting, and elution of any ligands into an ESI-MS system for identification.

[00396] All liquid chromatographic components were HP1200 modules. The mass spectrometer was the Waters LCT premier instrument operated in positive electrospray (ES+) TOF-MS mode. The mass range acquired was 80-1000 m/z in 0.25 s. The SEC column was 50 x 4.6 mm polyhydroxyethyl column. The SEC mobile phase was 50 mM phosphate buffer with 200 mM NaCl, pH=7.0. The LC column was a 2.1 x 50 mm C18 Phenomenex column. The gradient LC mobile phase A was 0.1% formic acid in water and B was acetonitrile/water with 0.1 % formic acid. The run time was 7.9 min.

[00397] For more information on SEC/LCMS, see, e.g., Blom, K. F. *et al., J. Comb. Chem.* **1999**, *1*, 82-90.

Results

[00398] Binding of **I-1** to Aptamer 21 was measured. SEC/LCMS conditions for this experiment were: [**I-1**] = 5 uM, [RNA] = 1 uM, Buffer = Tris-HCl 20 mM, KCl 100 mM, 3 mM $MgCl_2$.

Table 6: Binding of I-1 to Aptamer 21

ARK-000139 conc (μM)	Peak area SEC-LCMS	Peak area LCMS	% of binding
0.1	26.81	36.99	72.5
0.5	75.34	206.33	36.5

1	151.55	451.51	33.6
3	372.39	1665.87	22.4
10	471.84	5305.54	8.9

[00399] The experiment was duplicated and showed good reproducibility between the 2 experiments. The start of saturation was between 1 and 3 μM. SEC/LCMS was also used to measure **I-1** binding to Aptamer 21-E. Consistent with SPR data and the published literature, **I-1** does not bind Aptamer 21-E. SEC/LCMS represents a promising homogeneous method for assessing affinity.

Example 6: PEARL-seq Photoprobe Assay

General methods

Sequences

[00400] The following sequences were employed in the assays described herein.

Table 7: Nucleic Acid Sequences

Name	Sequence
PEARLv2_RT	CTTTCCCTACACGACGCTCTTCCGATCTTAGATCATTGATGG TGCCTACAG (SEQ ID NO:26)
PEARLv2_2nd_adapter	AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC (SEQ ID NO:27)
PEARLv2_for_pri (where <index> refers to the 6 bp Illumina index)	CAAGCAGAAGACGGCATACGAGAT <index> GTGACTGGAGTTCAGACGTGTGCTC (SEQ ID NO:28)
PEARLv2_rev_pri	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG ATCT (SEQ ID NO:29)
PEARLv3_1st_linker (where rN refers to a random mixture of the A, G, C and U RNA residues)	/5Phos/rArUrArUrArGrGrNrNrNrNrNrArGrArUrCrGrGrArArGrArGrCrArCrArCrGrUr CrUrGrArArCrUrC/3SpC3/ (SEQ ID NO:30)

PEARLv3_2nd_adapter	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT/3SpC3/ (SEQ ID NO:31)
PEARLv3_RT	GAGTTCAGACGTGTGCTCTTCCGATCT (SEQ ID NO:32)
PEARLv3_for_pri (where <i5> refers to the 6 bp Illumina i5 index)	AATGATACGGCGACCACCGAGATCTACAC <i5> ACACTCTTTCCCTACACGAC (SEQ ID NO:33)
PEARLv3_rev_pri (where <i7> refers to the 6 bp Illumina i7 index)	CAAGCAGAAGACGGCATACGAGAT <i7> GTGACTGGAGTTCAGACGTGTGCTC (SEQ ID NO:34)

Table 8: Illumina Index Sequences

Index ID	Sequence
1	CGTGAT
2	ACATCG
3	GCCTAA
4	TGGTCA
5	CACTGT
6	ATTGGC
7	GATCTG
8	TCAAGT
9	CTGATC
10	AAGCTA
11	GTAGCC
12	TACAAG
13	TTGACT
14	GGAACT
15	TGACAT
16	GGACGG
18	GCGGAC
19	TTTCAC
20	GGCCAC
21	CGAAAC
22	CGTACG
23	CCACTC
25	ATCAGT
27	AGGAAT

Folding RNA

[00401] A solution of RNA at 2 to 5 μM in nuclease-free water was heated to 95 °C for 3 min and then cooled on ice for 2 min. This solution was diluted with ½ volume of 3X RNA folding buffer (60 mM TrisHCl pH 8, 300 mM KCl, 9 mM $MgCl_2$) and incubated at 37 °C for 20 min. Folding was carried out immediately before use of the RNA in each experiment. In cases where a mixture of RNA molecules was used *in vitro* the RNAs were folded separately and then combined prior to the probing experiment.

Reverse transcription drop-off gel assay

Cross-linking

[00402] Folded RNA (1 μM) was incubated with the photoaffinity probe (10 μM) in total 25 μL of buffer (20 mM TrisHCl pH 8, 100 mM KCl, 3 mM $MgCl_2$) with 2.5% DMSO for 30 min at 37 °C to allow binding to come to equilibrium. The sample was then irradiated with long wave UV light (~365 nm) for 30 min in a UV crosslinker (Fisher Scientific).

Reverse transcription

[00403] A 3.75 μL aliquot of 10 μM reverse transcription primer was added to the cross-linked RNA sample, which was then incubated for 5 min at 65 °C and then cooled on ice. The sample was then diluted to a final volume of 60 μL and final buffer concentration of 1X Protoscript-II buffer (New England Biolabs), 0.5 mM dNTPs, 10 mM DTT, 0.4 U/μL RNase Inhibitor (New England Biolabs), and 10 U/μL Protoscript-II (New England Biolabs). The reaction was incubated at 45 °C for 2 h, 65 °C for 20 min, and then 4 °C.

Isolation of cDNA

[00404] The RNA was hydrolyzed by the addition of 4.8 μL of 2.5 M NaOH and heating at 95 °C for 5 min. The reaction was quenched by the addition of 0.9 μL of acetic acid. The cDNA was precipitated by the addition of 6.6 μL of 3 M sodium acetate pH 5 and 181 μL ethanol, cooling to -80 °C for 1 h, and then centrifuging at 20,500xg at 4 °C for 30 min. The cDNA pellet was washed twice with 500 μL of 70% ethanol and then air dried for 5 min.

Polyacrylamide gel analysis

[00405] The cDNA pellet was resuspended in 12 μL of 1X TBE-urea sample loading buffer (Bio-Rad) and heated to 95 °C for 5 min. The sample was then run on a pre-cast 8.6 x 6.7 cm TBE-

urea 10% PAGE gel (Bio-Rad) at 120 V until the lower bromophenol blue dye reached the bottom of the gel. The gel was stained with 1X SYBR-gold (Invitrogen) in 1X TBE buffer at room temperature for 20 min covered from light. The gel fluorescence was imaged on an Azure c600 instrument.

LC-MS analysis of cross-linked RNA

Generating cross-linked RNA

[00406] Folded RNA (1.33 μM) was incubated with or without the photoaffinity probe (20 μM) in total 100 μL of buffer (20 mM TrisHCl pH 8, 100 mM KCl, 3 mM $MgCl_2$) with 2% DMSO for 30 min at 37 °C to allow binding to come to equilibrium. The sample was then irradiated with long wave UV light (~365 nm) for 30 min in a UV crosslinker (Fisher Scientific).

[00407] The RNA was then precipitated by adding 10 μL of 3 M sodium acetate pH 5 and 275 μL ethanol, cooling to -80 °C for 1 h, and then centrifuging at 20,500xg at 4 °C for 30 min. The RNA pellet was washed with 500 μL of 70% ethanol, air dried for 5 min, and then resuspended in 120 μL of water.

LC-MS analysis

[00408] 50 μL of RNA sample was injected onto Clarity 2.6 μm Oligo X-T column (50 * 2.1 mm, 60 °C), and the gradient and flowrate were as follows: 95% A/5% B to 75% A/25% B over 5 min, flowrate: 400 μL/min; 75% A/25% B to 30% A/70% B over 1 min, flowrate: 400 μL/min; 100% D over 1 min, flowrate: 500 μL/min; 95% A/5% B over 2 min, flowrate: 500 μL/min (A: 1% HFIPA (hexafluoroisopropyl alcohol), 0.1% DIEA (diisopropylethylamine), 1 μM EDTA (ethylenediamine tetraacetic acid) in H_2O; B: 0.075% HFIPA, 0.0375% DIEA, 1 μM EDTA in 65/35 MeCN/H_2O; D: 40/40/20% MeOH/MeCN/H_2O); The LCMS instrument was a Thermo Finnigan LTQ, and ProMass deconvolution software coupled with Xcaliber were used for all data processing.

Next generation sequencing analysis of ARK-547 cross-linked RNA

Generating Crosslinked RNA

[00409] A 9.5 μL sample of folded RNA (2.6 μM) in buffer (20 mM TrisHCl pH 8, 100 mM KCl, 3 mM $MgCl_2$) was added to a 0.5 μL aliquot of 1 mM ARK-547 in DMSO. The reaction was incubated for 10 min at 37 °C, irradiated with long wave UV light (~365 nm) for 30 min in a UV crosslinker (Fisher Scientific), and then kept on ice.

Linker Ligation

[00410] The samples of cross-linked RNA were diluted to a final volume of 20 μL and final reaction conditions of 1X T4 RNA ligase buffer (New England Biolabs), 5 U/μL T4 RNA ligase 2, truncated KQ (New England Biolabs), 16.5% PEG-8000, and 0.5 μM universal miRNA cloning linker (New England Biolabs). The reaction was incubated in a thermal cycler for 2.5 h cycling between 5 min at 16 °C and 3 min at 25 °C. The RNA was then purified using Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer. The RNA was eluted with 20 μL of nuclease-free water.

Reverse Transcription

A 10 μL aliquot of the eluted RNA sample was mixed with a 1 μL aliquot of 2 μM PEARLv2_RT primer, heated to 65 °C for 5 min, and then cooled on ice. An 8 μL sample of 2.5X mutagenic reverse transcription buffer (125 mM TrisHCl pH 8, 187.5 mM KCl, 25 mM DTT, 1.25 mM dNTPs) was added to the sample and it was heated to 42 °C for 2 min, followed by addition of 1 μL of SuperScript II enzyme (Thermo), and then incubation at 42 °C for 3 h and 70 °C for 15 min.

[00411] To remove excess primer, the sample was mixed with 4.5 μL of ExoSAP-IT, incubated at 37 °C for 15 min, and then quenched with 1 μL of 0.5 M EDTA pH 8. The RNA was then degraded by adding 2.08 μL of 2.5 M NaOH, incubating at 95 °C for 5 min, and quenching with 3.25 μL of 10% acetic acid. The remaining cDNA was purified by Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 30 μL of nuclease-free water. The concentration was determined by absorbance at 260 nm.

Second Adaptor Ligation

[00412] Second adaptor ligation was carried out using the CircLigase ssDNA ligase system (Epicentre). A 0.8 pmol aliquot of purified cDNA was brought to a final volume of 20 μL and final concentration of 83.5 μM PEARLv2_2nd_adapter oligo, 1X CircLigase buffer, 50 μM ATP, 2.5 mM $MnCl_2$, and 5 U/μL enzyme. The reaction was incubated at 60 °C for 2 h and then 80 °C for 10 min. The adaptor-ligated cDNA was purified by Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 30 μL of nuclease-free water.

Polymerase Chain Reaction

[00413] The adaptor-ligated cDNA was PCR amplified using Q5 high-fidelity DNA polymerase (New England Biolabs) to install the Illumina adaptor sequences. A 5 μL aliquot of the adaptor-ligated cDNA was brought to a final volume of 50 μL and final concentration of 0.2 μM PEARLv2_for_pri primer, 0.2 μM PEARLv2_rev_pri primer, and 1X Q5 master mix. The polymerase chain reaction was carried out with heating to 98 °C for 30 seconds; 5 cycles of 98 °C for 10 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds; 15 cycles of 98 °C for 10 seconds, and 72 °C for 30 seconds; 72 °C for 2 min. A different Illumina barcode was installed in each PCR product via the forward primer. PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 30 μL of nuclease-free water.

Next Generation Sequencing

[00414] The concentrations of different PCR products were measured using the Denovix dsDNA fluorescence quantitation kit (Denovix) and multiplexed at equal concentrations with a 20% PhiX spike-in. Sequencing was performed on an Illumina MiSeq with 150 bp paired-end reads using the standard manufacturer protocol.

Capture of Aptamer 21 from a Defined Mixture of RNAs

Generating Crosslinked RNA

[00415] A sample of folded RNA was prepared containing 1 μM each of Myc_HP_PA, Aptamer 21, FMN, MYC_3WJ-HP_N3G, and PreQ1 RNAs in folding buffer (20 mM TrisHCl pH 8, 100 mM KCl, 3 mM $MgCl_2$). A 95 μL sample of this solution was added to a 5 μL aliquot of 0.2 mM ARK-670 (probe) or ARK-139 (control) in DMSO. The reaction was incubated for 30 min at 37 °C, irradiated with long wave UV light (~365 nm) for 30 min in a UV crosslinker (Fisher Scientific), and then cooled on ice. The excess probe and buffer was then removed by passing the sample through an Illustra MicroSpin G-25 spin column (GE Healthcare) according to the manufacturer's protocol.

Avidin Bead Capture

[00416] For each treatment, a 75 μL aliquot of the crosslinked RNA was mixed with 0.75 μL of 10% Tween-20 and 0.45 μL of 0.5 M EDTA pH 8 solution. A 50 μL aliquot of MyOne Streptavidin C1 Dynabead slurry (Thermo) was captured on a magnet, washed twice with 50 μL of 1X bind/wash buffer (20 mM TrisHCl pH 7, 100 mM KCl, 0.1% Tween-20), and then

resuspended in 50 μL of the crosslinked RNA sample. The bead suspension was rotated at room temperature for 60 min and then washed twice with 100 μL of 1X bind/wash solution. To elute the bound RNA, the beads were resuspended in 50 μL of elution buffer (95% formamide, 20 mM EDTA), heated to 95 °C for 5 min, and then the supernatant was removed. The RNA was ethanol precipitated by adding 50 μL of water, 2 uL of 5 mg/mL glycogen, 10 μL 3 M NaOAc pH 5, and 250 μL ethanol, incubating at -80 °C for 1 h, centrifuging at 20,000g for 30 min, washing the pellet twice with 500 μL of 70% ethanol, air drying the pellet, and then resuspending the pellet in 16 μL of nuclease-free water.

Linker ligation

[00417] The samples of bead-eluted RNA were diluted to a final volume of 20 μL and final reaction conditions of 1X T4 RNA ligase buffer (New England Biolabs), 5 U/μL T4 RNA ligase 2, truncated KQ (New England Biolabs), 16.5% PEG-8000, and 0.5 μM universal miRNA cloning linker (New England Biolabs). The reaction was incubated in a thermal cycler for 2.5 h cycling between 5 min at 16 °C and 3 min at 25 °C. The RNA was then purified using Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer. The RNA was eluted with 11 μL of nuclease-free water.

Reverse transcription with SuperScript III

[00418] To the 11 μL RNA sample from linker ligation was added 1 μL of 2 μM PEARLv2_RT primer and 1 μL of 10 mM dNTP mix. The sample was heated to 65 °C for 5 min and then cooled on ice for 1 min. The sample was then mixed with 4 μL of 5X first-strand buffer (Thermo), 1 μL of 0.1 M DTT, 1 μL of RNaseOUT (Thermo), and 1 μL of SuperScript III enzyme (Thermo). The reaction was incubated at 55 °C for 45 min, 70 °C for 15 min, and then cooled to 4 °C.

[00419] To remove excess primer, the sample was mixed with 4.5 μL of ExoSAP-IT, incubated at 37 °C for 15 min, and then quenched with 1 μL of 0.5 M EDTA pH 8. The RNA was then degraded by adding 2.08 μL of 2.5 M NaOH, incubating at 95 °C for 5 min, and quenching with 3.25 μL of 10% acetic acid. The remaining cDNA was purified by Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 15 μL of nuclease-free water. The concentration was determined by absorbance at 260 nm.

Second adapter ligation

[00420] Second adaptor ligation was carried out using the CircLigase ssDNA ligase system (Epicentre). A 0.8 pmol aliquot of purified cDNA was brought to a final volume of 20 μL and final concentration of 83.5 μM PEARLv2_2nd_adapter oligo, 1X CircLigase buffer, 50 μM ATP, 2.5 mM $MnCl_2$, and 5 U/μL enzyme. The reaction was incubated at 60 °C for 2 h and then 80 °C for 10 min. The adaptor-ligated cDNA was purified by Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 30 μL of nuclease-free water.

Polymerase Chain Reaction

[00421] The adaptor-ligated cDNA was PCR amplified using Q5 high-fidelity DNA polymerase (New England Biolabs) to install the Illumina adaptor sequences. A 5 μL aliquot of the adaptor-ligated cDNA was brought to a final volume of 50 μL and final concentration of 0.2 μM PEARLv2_for_pri primer, 0.2 μM PEARLv2_rev_pri primer, and 1X Q5 master mix. The polymerase chain reaction was carried out with heating to 98 °C for 30 seconds; 5 cycles of 98 °C for 10 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds; 15 cycles of 98 °C for 10 seconds, and 72 °C for 30 seconds; 72 °C for 2 min. A different Illumina barcode was installed in each PCR product via the forward primer. PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 30 μL of nuclease-free water.

Next Generation Sequencing

[00422] The concentrations of different PCR products were measured using the Denovix dsDNA fluorescence quantitation kit (Denovix) and multiplexed at equal concentrations with a 20% PhiX spike-in. Sequencing was performed on an Illumina MiSeq with 150 bp paired-end reads using the standard manufacturer protocol.

Capture of Aptamer 21 with Click-Biotinylated Probes

Generating Crosslinked and Click-Biotinylated RNA

[00423] A 50 μL sample of 1 μM folded Aptamer 21 or Aptamer 21-E solution in folding buffer (20 mM TrisHCl pH 8, 100 mM KCl, 3 mM $MgCl_2$) was added to a 0.5 μL aliquot of 0.5 mM ARK-729, ARK-2058, ARK-816, or ARK-2059 in DMSO or a DMSO-only control. The solution was incubated at 37 °C for 1 h and then irradiated with long wave UV light (~365 nm) for 30 min in a UV crosslinker (Fisher Scientific). To this solution was added 0.5 μL of 10 mM DBCO-biotin

(Click Chemistry Tools, cat. # A105) and 2 μL of 0.5 M EDTA pH 8 and then the solution was incubated at 65 °C for 2 h. The RNA was ethanol precipitated by adding 35 μL of water, 2 uL of 5 mg/mL glycogen, 10 μL 3 M NaOAc pH 5, and 250 μL ethanol, incubating at -80 °C for 1 h, centrifuging at 20000xg for 30 min, washing the pellet twice with 500 μL of 70% ethanol, and then air drying the pellet.

Streptavidin Bead Capture

[00424] The RNA pellet was redissolved in 50 μL of 1X bind/wash buffer (20 mM TrisHCl pH 7, 100 mM KCl, 0.1% Tween-20). A 50 μL aliquot of MyOne Streptavidin C1 Dynabead slurry (Thermo) was captured on a magnet, washed twice with 50 μL of 1X bind/wash buffer, and then resuspended in 50 μL of the crosslinked RNA sample. The bead suspension was rotated at room temperature for 30 min and then washed twice with 100 μL of 1X bind/wash solution.

On-bead Dephosphorylation

[00425] The magnetic beads with captured RNA were resuspended in a 50 μL solution containing 1X FastAP buffer and 0.08 U/μL FastAP enzyme (Thermo) and the slurry was incubated at 37 °C for 15 min with 1200 rpm agitation. A 150 μL solution of 1X PNK buffer, 1 mM DTT, and 0.25 U/μL T4 PNK (New England Biolabs) was then added to the mixture and the slurry was incubated at 37 °C for 20 min with 1200 rpm agitation. The beads were then washed three times with 400 μL of 1X bind/wash solution.

First Linker Ligation

[00426] The beads were washed twice with 400 μL of linker wash buffer (50 mM TrisHCl pH 8, 5 mM $MgCl_2$) and then resuspended in a 27.5 μL ligation reaction mixture containing 2 μM of the PEARLv3_1st_linker oligo, 1X RNA Ligase 1 buffer, 1 mM ATP, 2.9% DMSO, 16% PEG8000, and 2.7 U/μL RNA Ligase 1 (New England Biolabs, cat # M0437). The slurry was incubated at 22 °C for 75 min with 1200 rpm agitation and then the beads were washed twice with 100 μL of 1X bind/wash buffer.

Reverse Transcription

[00427] The beads were washed twice with 200 μL of 1X first-strand buffer (Thermo) and then resuspended in a solution containing 14.75 μL water, 1.25 μL of 10 mM dNTPs, and 0.25 μL of 10 μM PEARLv3_RT oligo. The slurry was heated to 65 °C for 5 min, chilled on ice, and then a

solution containing 5 μL of 5X first-strand buffer (Thermo), 1.25 μL of 100 mM DTT, 1.25 μL of RNaseOUT (Thermo), and 1.25 μL of SuperScript III (Thermo) was added. The slurry was mixed, incubated at 50 °C for 50 min, heated to 85 °C for 5 min, and then chilled on ice.

[00428] To elute the cDNA, the 2.5 μL of 2.5 M sodium hydroxide was added to the slurry and it was heated to 95 °C for 5 min, chilled on ice, and then 3.6 μL of a 1.74 M solution of acetic acid was added to quench the pH. The supernatant was removed from the beads, brought to a final volume of 50 μL with nuclease-free water, and then purified using Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 15 μL of nuclease-free water.

Second Adapter Ligation

[00429] A 14 μL aliquot of the AMPure-eluted cDNA was brought to 20 μL reaction mixture with a final concentration of 1X CircLigase buffer, 80 nM PEARLv3_2nd_adapter oligo, 50 μM ATP, 2.5 mM $MnCl_2$, and 5 U/μL CircLigase ssDNA ligase (EpiCentre) and incubated at 60 °C for 2 h and then 80 °C for 10 min. The ligated cDNA was purified using Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 25 μL of nuclease-free water.

Polymerase Chain Reaction

[00430] The adaptor-ligated cDNA was PCR amplified using Q5 high-fidelity DNA polymerase (New England Biolabs) to install the Illumina adaptor sequences. A 5 μL aliquot of the adaptor-ligated cDNA was brought to a final volume of 50 μL and final concentration of 0.2 μM PEARLv3_for_pri primer, 0.2 μM PEARLv3_rev_pri primer, and 1X Q5 master mix. The polymerase chain reaction was carried out with heating to 98 °C for 30 seconds; 5 cycles of 98 °C for 10 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds; 15 cycles of 98 °C for 10 seconds, and 72 °C for 30 seconds; 72 °C for 2 min. A different Illumina barcode was installed in each PCR product via the forward primer. PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter) using the standard protocol from the manufacturer and eluted in 30 μL of nuclease-free water.

Next Generation Sequencing

[00431] The concentrations of different PCR products were measured using the Denovix dsDNA fluorescence quantitation kit (Denovix) and multiplexed at equal concentrations with a

20% PhiX spike-in. Sequencing was performed on an Illumina MiSeq with 150 bp paired-end reads using the standard manufacturer protocol.

Capture of Aptamer 21 from PolyA+ RNA Extract

Isolation of PolyA+ RNA from Cells

[00432] Total RNA was extracted from a pellet of $5x10^6$ HepG2 cells using the ReliaPrep mini-prep kit (Promega) according to the manufacturer's standard protocol. The polyA+ RNA fraction was isolated from 50 µL sample of total RNA at 454 ng/µL using the magnetic mRNA isolation kit (New England Biolabs, cat # S1550) according to the manufacturer's standard protocol using 450 µL of lysis buffer and 100 µL of beads.

Generating Crosslinked and Click-Biotinylated RNA

[00433] A sample of folded aptamer 21 RNA was spiked into a sample of polyA+ RNA extract at a final concentration of 1 nM aptamer 21 and 3 ng/µL polyA+ RNA. A 50 µL sample of this RNA mixture was then added to a 0.5 µL aliquot of 100 µM ARK-816 or ARK-2059 in DMSO or a DMSO control and the mixture was incubated for 20 min at 37 °C. The solution was then diluted with 50 µL of 1X folding buffer and then irradiated with long wave UV light (~365 nm) for 30 min in a UV crosslinker (Fisher Scientific). To this solution was added 1 µL of 5 mM DBCO-biotin (Click Chemistry Tools, cat. # A105) and 1 µL of 0.5 M EDTA pH 8 and then the solution was incubated at 65 °C for 2 h. The RNA was ethanol precipitated by adding 35 µL of water, 2 uL of 5 mg/mL glycogen, 10 µL 3 M NaOAc pH 5, and 250 µL ethanol, incubating at -80 °C for 1 h, centrifuging at 20,000g for 30 min, washing the pellet twice with 500 µL of 70% ethanol, and then air drying the pellet.

Fragment RNA

[00434] RNA samples were fragmented using the Ambion fragmentation kit (Thermo). RNA pellets were resuspended in 50 µL of 1X fragmentation buffer, incubated at 70 °C for 15 min, and then quenched by the addition of 5 µL of stop solution. The sample was then ethanol precipitated by adding 50 µL water, 10 µL 3 M NaOAc pH 5, 2 µL 5 mg/mL glycogen and 280 µL ethanol, incubating for 1 h at -80 °C, centrifuging at 20,000g for 30 min, washing the pellet twice with 500 µL of 70% ethanol, and then air drying the pellet.

Avidin Bead Enrichment and Sequencing Library Preparation

[00435] Avidin bead capture, dephosphorylation, first linker ligation, reverse transcription, second adapter ligation, and polymerase chain reaction were performed as per the "Capture of Aptamer 21 with Click-Biotinylated Probes" section above.

Size Selection of Sequencing Library

[00436] To remove primer dimer and size select the sequencing library, the PCR product was run on a 3% agarose gel with 1X SYBR-gold. The region from 275 to 400 bp was cut out and the DNA was extracted using the Zymoclean Gel DNA Recovery Kit (Zymo Research) according to the manufacturer's suggested protocol and eluted into 12 μL of nuclease-free water.

Next Generation Sequencing

[00437] Sequencing was performed on a single Illumina HiSeq 4000 lane with 150 bp paired-end reads using the standard manufacturer protocol.

PEARL-seq Informatics

Genome references

[00438] All analyses were referenced to the GRCh38 human genome assembly and GENCODE release 28 transcript annotations, with Aptamer 21 added (AGGGGTAGGCCAGGCAGCCAACTAGCGAGAGCTTAAATCTCTGAGCCCGAGAGGG TTCAGTGCTGCTTATGTGGACGGCTTGAT; SEQ. ID:25).

Read stitching

[00439] To ensure that only high-quality, concordant read pairs were used to define mutations and reverse transcriptase stalls, reads were first stitched with Paired-End reAd mergeR (PEAR) (https://dx.doi.org/10.1093%2Fbioinformatics%2Fbtt593), requiring a minimum assembled length of at least 10 nucleotides (nt).

Single-transcript read alignment (SHAPEware)

[00440] For all SHAPEware analysis, reads were adapter-trimmed aligned as previously described (https://bitbucket.org/arrakistx/shapeware/src) using Trimmomatic (http://dx.doi.org/10.1093/bioinformatics/btu170) and bwa-mem (https://arxiv.org/abs/1303.3997), respectively.

SHAPE reactivity calculation (SHAPEware)

[00441] Mutations (substitutions, insertions, and deletions) were tabulated using bam-readcount (https://github.com/genome/bam-readcount). Mutations were then filtered to retain only those that

can be unambiguously linked to a given transcript position (ambiguous mutations could arise from multiple positions). SHAPE reactivities were defined based upon the excess of mutations in SHAPE reagent-treated sample compared to untreated sample, normalized to an unfolded control: $Reactivity = \frac{Mut_{treated} - Mut_{untreated}}{Mut_{denatured}}$. Reactivities were then further normalized within each transcript as described in Deigan *et al.* (PNAS January 6, 2009, 106 (1) 97-102).

Transcriptome-wide read alignment (PEARL-seq)

[00442] Stitched reads were then processed with Cutadapt (http://dx.doi.org/10.14806/ej.17.1.200) with at least 5-nts of overlap and a maximum of 20% mismatches to simultaneously 1) define a unique 6-nt unique molecular identifier (UMI) in the correct adapter context (ATATAGGN_6AGATCGG) (SEQ. ID:35) and 2) trim away adapters to allow more precise definition of fragment termini. UMIs were appended to read names, and reads were mapped the against the genome and transcriptome references (plus Aptamer 21) using the STAR aligner (http://dx.doi.org/10.1093/bioinformatics/bts635) with at most 10% mismatches. Groups of reads with identical or near-identical UMIs were then collapsed using the Directional Adjacency methods of UMI-tools (http://dx.doi.org/10.1101/gr.209601.116) with default parameters. As a result, PCR duplicates were removed from the data to avoid potential artifacts that might introduce bias or variance.

Definition of RT stall sites

[00443] Sites of interaction between probe and transcripts were defined by reverse transcriptase (RT) stalling sites. RT stalling sites were defined based upon the mapped position of the 5' end of a trimmed read. For short defined transcripts such as Aptamer 21, high-confidence RT stall sites were defined from reads with 3' ends mapping to the precise transcript end. The frequency of high-confidence stall sites are plotted for each position in Aptamer 21.

Peakcalling (PEARL-seq)

[00444] True sites of small molecule-transcript interaction were defined based upon significant enrichment of stall sites in a PEARL-seq probe compared to a warhead-only control. These peaks are expected to be quite narrow based upon the single-nucleotide resolution of RT stall sites. Thus, we developed a peakcalling method optimized to detect these narrow peaks and thus identify sites enriched for RT sites in probe when compared to a warhead-only control. Briefly, uniquely mapping reads were trimmed to retain their 5' most nucleotide only. These 5' ends were then

counted across 10-nt bins throughout the whole genome using Deeptools (http://dx.doi.org/10.1093/nar/gkw257) combined with custom scripts. Each bin with detectable read 5' ends was tested for significant enrichment with the Empirical Analysis of Digital Gene Expression Data in R (edgeR) pipeline (https://doi.org/doi:10.18129/B9.bioc.edgeR), using Benjamini-Hochberg multiple hypothesis correction. Peaks with both positive enrichment in probe over control and an FDR < 0.01 were considered to contain probe-induced RT stalling events corresponding to probe-transcript interactions.

Example 7: Surface Plasmon Resonance Assay for Aptamer 21

[00445] Surface plasmon resonance (SPR) may be used to screen ligands and hook and click constructs for binding to a target RNA of interest. SPR is especially useful for monitoring biomolecular interactions in real time. Typically, target species and unrelated control are immobilized to a sensor chip, then analytes (compounds/fragments) are flowed over the surface. Binding of the compound to target species results in increase of SPR signal (association phase). Washing away bound compound with buffer results in a decrease of SPR signal (dissociation phase). Fitting of sensorgrams recorded at different compound concentrations is performed to an appropriate interaction model. The method allows extraction of kinetic parameters (k_a, k_d → K_D). Requirements/limitations include that the k_a / k_d values be in reasonable ranges; and the target size must not be too large (< 100 kDa). It is an excellent method to screen fragments and profile or validate hits. BC4000 may be used for primary screening (up to 4,000 data pts/week). Biacore T200 is suitable for hit profiling and validation.

[00446] Aptamer evolution for codeine binding has been achieved and the aptamer's binding constant determined by use of SPR. Win, N. M. *et al., Nucleic Acids Research* **2006**, *34(19)*, 5670–5682. See, e.g., Chang, A. L. *et al., Anal. Chem.* **2014**, *86*, 3273-3278.

[00447] In the PEARL-seq context, SPR allows monitoring binding of "hooks" to DNA/RNA aptamers. The target species is immobilized to sensor chip, analytes (i.e. hooks) are flowed over surface (association phase), DNA/RNA aptamer is flowed over surface (plateau phase), competitor compound is washed over surface (dissociation phase), thus yielding binding data.

Sensor Chip Surface Preparation

[00448] Experiments were performed on a MASS-2 (Sierra Sensors) at 25°C. A high capacity amine chip was equilibrated with PBS. The chip was conditioned with alternating injections of 10 mM HCl and 10 mM NaOH in 1 M NaCl. The carboxymethylated dextran of the surface of the amine chip was activated for 7 minutes at a flow rate of 10 µL/min using a 1:1 volume ratio of 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Sierra) and 0.1 M N-hydroxysuccinimide (Sierra). Neutravidin (70 µg/mL, Thermofisher) was injected over the activated surface for 10 minutes at a flow rate of 10 µl/min. Excess activated groups were blocked by an injection of 1 M ethanolamine, pH 8.5 (Sierra) for 7 minutes at a flow rate of 10 µl/min. A final injection of 10 mM NaOH in 1 M NaCl for 7 minutes at 10 µl/min was used to remove any unbound material to the chip. The immobilization reaction typically yielded approximately 12,000 resonance units (RU) of neutravidin. At least one spot in a channel was not injected with RNA and was used as a background control. Unrelated RNA was also used as a background control.

RNA Preparation

[00449] A 1 µM sample of biotin-aptamer 21 was prepared in RNAse free water, heated to 95 °C for 3 minutes, cooled on ice for 4 minutes, diluted with an equal volume 2X folding buffer (40 mM Tris-HCl, pH 8.0, 6 mM $MgCl_2$, 200 mM KCl), and incubated at 37 °C for 30 minutes. The final concentration of RNA was 0.5 µM.

RNA Surface Capture

[00450] The MASS-2 was primed 2 times with 20 mM Tris-HCl, pH 8.0, 3 mM $MgCl_2$, 100 mM KCl (Capture Buffer). RNA was injected over the neutravidin surface for 7 minutes at a flow rate of 10 µl/min. Capture levels typically reached approximately 3500-4500 of resonance units of aptamer 21.

Compound Testing

[00451] Compounds were diluted 2-fold in 100% DMSO at a starting concentration of 500 µM. Dilutions of 1:100, compound to buffer resulted in compound solutions in 20 mM Tris-HCl, pH 8.0, 3 mM $MgCl_2$, 100 mM KCl, 1% DMSO.

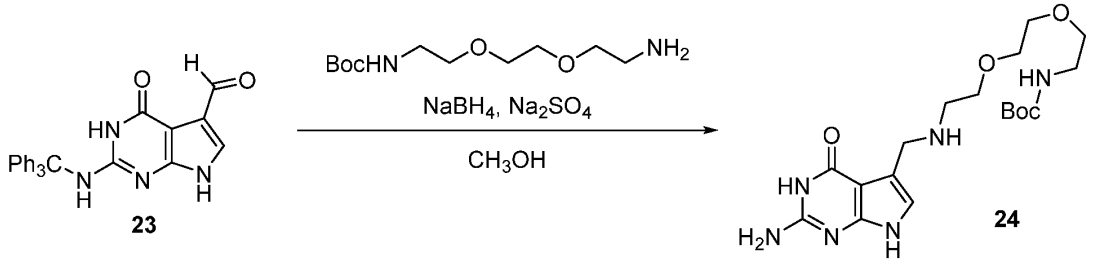
[00452] The MASS-2 was primed 2 times with 20 mM Tris-HCl, pH 8.0, 3 mM $MgCl_2$, 100 mM KCl, 1% DMSO (running buffer). Prior to testing compounds 3 injections of running buffer were used to equilibrate the sensor surface. Injections of DMSO correction solutions ranging from 0.6 %-1.8 % DMSO were injected over the surface for 30 µl at a flow rate of 30 µl/min to generate

a DMSO correction curve for data analysis. Compound (120 µL) was injected over the RNA surface at a flow rate of 10 µl/min with a dissociation time of 240 sec.

Data Analysis

[00453] Data processing and analysis were done using Sierra Analyzer Software (Sierra Sensors). A double-referencing method was performed to process all datasets and a DMSO correction curve was applied to account for any differences in bulk refractive index changes between samples and running buffer. Double-referenced data were fit to a 1:1 binding model for kinetic analysis. The calculated K_D was ~0.8 nM under these conditions.

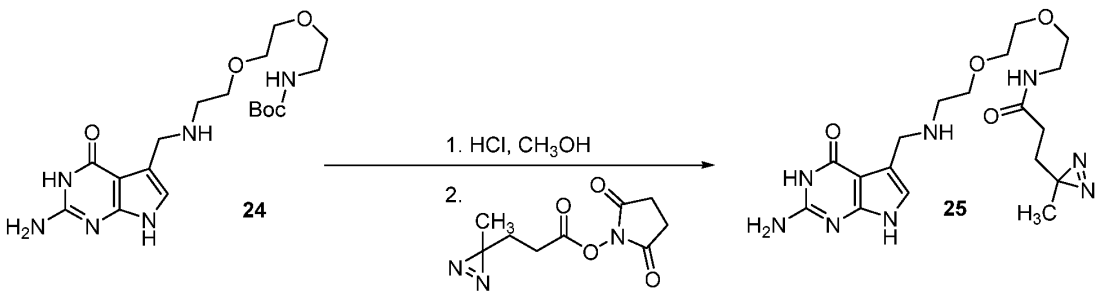
Example 8: Preparation of PreQ$_1$ RNA Photoprobe



[00454] Use of the PreQ$_1$ riboswitch and preparation of small molecule ligands for it are described in Roth, A. *et al., Nature Structural & Molecular Biology* **2007**, *14(4)*, 308-317, which is hereby incorporated by reference.

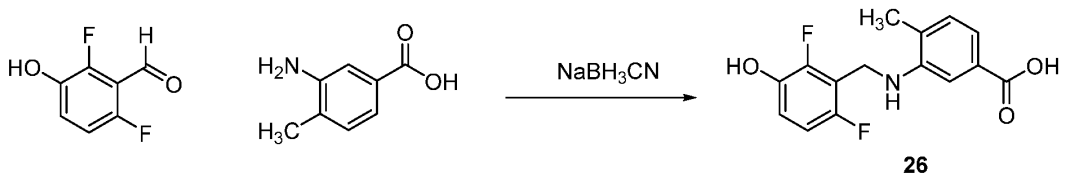
[00455] Compound **24**: To a suspension of **23** (500 mg, 1.19 mmol, 1 eq,) and tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (354 mg, 1.43 mmol, 1.2 eq.) in methanol (14.0 mL) was added sodium sulfate (14 mg, 0.09 mmol, 0.08 eq.) at room temperature. The resulting suspension was stirred at room temperature for 2 h. Sodium borohydride (135 mg, 3.57 mmol, 3.0 eq.) was then added in small portions to the reaction mixture, then stirring at room temperature was maintained for and additional 4 h. The reaction mixture was diluted with ethyl acetate (250 mL), then was washed with sequential 50 mL portions of water (thee times) and sat. aq. NaCl solution. The product solution was dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The obtained crude material was purified by reverse phase chromatography using C_{18}-silica gel (42 – 45% CH_3CN in 10 mM NH_4HCO_3 in water) to afford **24** as an off-white solid (302 mg, 39% yield). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.61 (s, 1 H), 10.32 (br s,1 H), 7.38 (s, 1 H), 7.31 – 7.28 (m, 12 H),

7.24 – 7.19 (m, 3 H), 6.82 (t, J = 5.6 Hz, 1 H), 6.31 (d, J = 2 Hz, 1 H), 3.56 (s, 2 H), 3.46 – 3.35 (m, 11 H), 3.08 – 3.03 (m, 2 H), 1.36 (s, 9 H). MS (ESI-MS): m/z calc. for $C_{37}H_{44}N_6O_5[MH]^+$ 653.34; found 653.14.

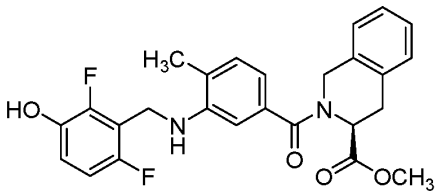


[00456] Compound **25** (**I-23**): A stirring solution of **24** (21 mg, 32 µmol, 1 eq.) in CH_3OH (1.0 mL) at room temperature was treated dropwise with a 1.25 M CH_3OH solution of HCl 583 µL, 0.73 mmol, 23 eq.). The resulting mixture was allowed to stir at room temperature for 24 h, then the precipitated solids were collected by filtration. The resulting crude solid was triturated with sequential 10 mL portions of pentane (twice) and diethyl ether (twice) to afford the intermediate primary amine as a white solid. The primary amine (10 mg, 32 µmol, 1 eq.) was resuspended in DMF (1.0 mL), then was treated with *N*,*N*-diisopropylethylamine (22 µL, 0.13 mmol, 4.0 eq.), and 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3*H*-diazirin-3-yl)propanoate (7.6 mg, 34 µmol, 1.05 eq.). The resulting mixture was maintained at room temperature for 16 h, then was immediately loaded onto a C_{18}-silica gel column, eluting with 0 – 70% CH_3CN in water containing 0.1% formic acid). Fractions containing the desired product were combined and partially evaporated under reduced pressure to remove CH_3CN, then were frozen and lyophilized to afford the desired product **I-23** as a white solid (formate salt, 9 mg, 60% yield).

Example 9: Synthesis of Additional Exemplary Photoprobe Compounds

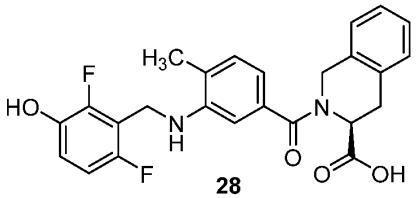


[00457] Compound **26**: To a solution of 3-amino-4-methylbenzoic acid (4.00 g, 26.5 mmol, 1 eq.) in acetic acid (80 mL) was added 2,6-difluoro-3-hydroxybenzaldehyde (5.01 g, 31.7 mmol, 1.2 eq.) at 0 °C. The reaction was slowly warmed to room temperature and stirred at room temperature for 3 h. The reaction mixture was again cooled to 0 °C. $NaCNBH_3$ (3.32 g, 52.9 mmol, 2.0 eq.) was added, in small portions. The reaction mixture was allowed to warm to room temperature, then was stirred at room temperature for 16 h. The resulting reaction mixture was poured into ice-cold water (500 mL). The resulting precipitate was collected by filtration and washed with water (3 x 25 mL). The obtained solid was dried under high vacuum to afford **26** as a white solid (6.00 g, 62% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (br s, 1H), 9.74 (s, 1 H), 7.22 (d, J = 1.2 Hz, 1 H), 7.13 (dd, J = 7.6, 1.6 Hz, 1 H), 7.05 (d, J = 8.0 Hz, 1 H), 6.86 - 6.83 (m, 2H), 5.38 (t, J = 5.6 Hz, 1 H), 4.36 (d, J = 5.2 Hz, 2 H), 2.11 (s, 3H). MS (ESI-MS): m/z calcd for $C_{15}H_{13}F_2NO_3^+$ = 294.09, found 294.16.



27

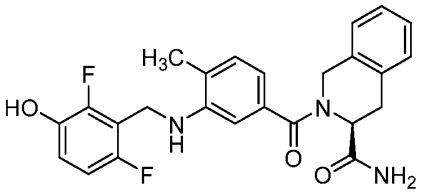
[00458] Compound **27**: Compound **27** was synthesized according to General Procedure A from **26** (2.00 g, 6.82 mmol) and methyl (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate hydrochloride in DMF (20 mL) at room temperature. Following stirring at room temperature for 3 h, the reaction mixture was diluted with water and the resulting solids collected by filtration. The crude product was purified by flash column chromatography over silica gel (40% EtOAc/hexanes) to afford **27** as a light pink solid (1.60 g, 34% yield). MS (ESI-MS): m/z calcd for $C_{26}H_{24}F_2N_2O_4^+$ = 467.17, found 467.22.



28

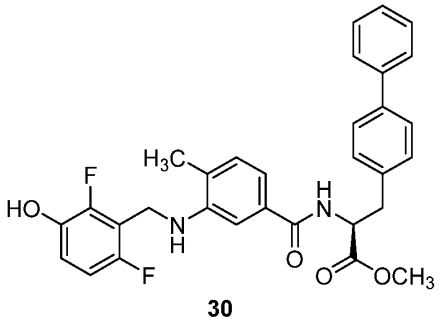
[00459] Compound **28**: To a stirred solution of **27** (1.60 g, 3.93 mmol, 1 eq.) in THF : MeOH : Water (4 : 2 : 1, 11.2 mL) was added $LiOH•H_2O$ (0.43 g, 10.3 mmol, 3.0 eq.) at room temperature.

The reaction mixture was stirred at room temperature for 3 h, then was evaporated under vacuum. The obtained crude material was diluted with water and washed with diethyl ether. The aqueous layer was separated, acidified using 1N HCl and extracted with ethyl acetate (4 x 100 mL). The combined organic layer was washed with brine solution (150 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The obtained crude material was purified by column chromatography over silica gel (7 % MeOH/DCM) to afford **28** as an off-white solid (1.55 g, 100% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (br s, 1 H), 9.76 (s, 1 H), 7.26 - 7.13 (m, 3 H), 7.07 - 7.03 (m, 1 H), 6.96 - 6.78 (m, 3 H), 6.63 (d, J = 8.8 Hz, 1 H), 6.58 - 6.52 (m, 1 H), 5.39 - 5.38 (m, 1 H), 5.13 - 4.42 (m, 3 H), 4.32 - 4.30 (m, 2 H), 3.18 - 3.11 (m, 2 H), 2.10 (d, J = 8.4 Hz, 3 H). MS (ESI-MS): m/z calcd for $C_{25}H_{22}F_2N_2O_4^+$ = 453.16, found 453.27.



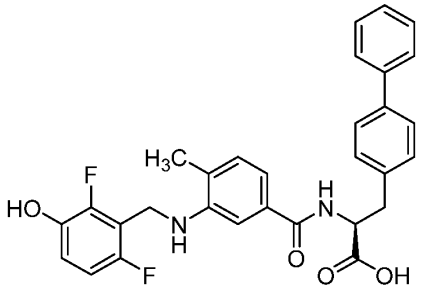
29

[00460] Compound **29**: Compound **29** (ARK-852) was synthesized according to General Procedure A from **26** (100 mg, 351 μmol) and (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide in DMF (2 mL) at room temperature. The resulting dark mixture was allowed to stir at room temperature for 60 minutes, then was purified by reverse-phase flash column chromatography over C_{18} silica gel (C_{18}-silica gel, eluting with 0-100% acetonitrile in water containing 0.1% formic acid) to afford **29** as an off-white solid (formate salt, 21 mg, 12% yield). MS (ESI-MS): t_R = 1.54 min; m/z calcd for $C_{30}H_{27}F_2N_3O_3^+$ = 498.1, found 520.1 ([M+Na]$^+$).



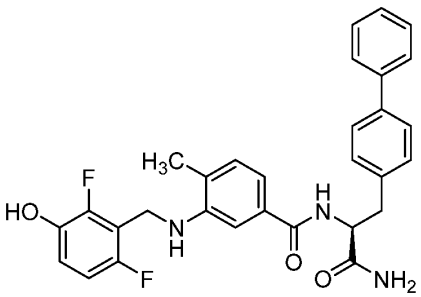
30

[00461] Compound **30**: Compound **30** was prepared by General Procedure A from **26** (3.00 g, 10.2 mmol) and (*S*)-4-phenylphenylalanine methyl ester hydrochloride. After stirring at room temperature for 4 h, the reaction mixture was poured into ice-cold water (250 mL). The resulting solids were collected by filtration and purified by column chromatography over silica gel (30% EtOAc/hexanes) to afford **30** as a white solid (4.20 g, 77% yield). MS (ESI-MS): m/z calcd for $C_{31}H_{28}F_2N_2O_4^+$ = 531.04, found 531.27.



31

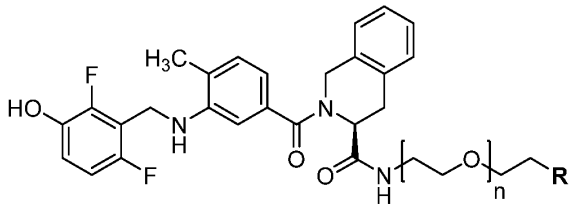
[00462] Compound **31**: Compound **31** was prepared analogously to **28** above from **30** (4.20 g, 7.91 mmol). The crude product was purified by column chromatography over silica gel (7% MeOH in CH_2Cl_2) to afford **31** as a white solid (1.70 g, 42% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (br s, 1 H), 9.76 (s, 1 H), 8.42 (s, 1 H), 7.60 - 7.54 (m, 4 H), 7.40- 7.33 (m, 5 H), 7.08 - 7.01 (m, 3 H), 6.86 - 6.84 (m, 2 H), 5.21 - 5.19 (m, 1 H), 4.56 (s, 1 H), 4.33 (s, 2 H), 3.18 - 3.10 (m, 2 H), 2.07 (s, 3 H). MS (ESI-MS): m/z calcd for $C_{30}H_{26}F_2N_2O_4^+$ 517.19, found 517.07.



32

[00463] Compound **32**: Compound **32** (ARK-850) was synthesized according to General Procedure A from **26** (100 mg, 351 μmol) and (*S*)-4-phenylphenylalaninamide in DMF (2 mL) at room temperature. The resulting dark mixture was allowed to stir at room temperature for 30 minutes, then was partitioned between sat. aq. $NaHCO_3$ solution (30 mL) and ethyl acetate (30

mL). The aqueous phase was extracted with 30 mL EtOAc, then the combined extracts were washed with 30 mL water and 30 mL sat. aq. NaCl solution. The product solution was dried over $MgSO_4$, filtered, and concentrated to afford the crude product as a brown oil. The crude product was purified by reverse-phase flash column chromatography over C_{18} silica gel (0-100% CH_3CN in water containing 0.1% formic acid). Fractions containing **32** were combined and partially evaporated to remove CH_3CN, then the resulting suspension was partitioned between sat. aq. $NaHCO_3$ solution (30 mL) and EtOAc (30 mL). The aqueous phase was extracted with 30 mL EtOAc, then the combined extracts were washed with 30 mL water and 30 mL sat. aq. NaCl solution. The product solution was dried over $MgSO_4$, filtered, and concentrated to afford **32** as an off-white foam (62 mg, 40% yield). MS (ESI-MS): t_R = 1.67 min; m/z calcd for $C_{30}H_{27}F_2N_3O_3^+$ = 516.1, found 499.1 ($[M + H - NH_3]^+$.



33: n = 1, R = CO_2tBu
34: n = 2, R = CO_2tBu
35: n = 4, R = CO_2tBu
36: n = 1; R = NHBoc
37: n = 2; R = NHBoc
38: n = 4, R = NHBoc

[00464] Compound **33**: Compound **33** was synthesized according to General Procedure A from **28** (30 mg, 66 μmol) and NH_2-PEG_1-$CO_2{}^t$Bu. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **33** as a colorless film (40 mg, 98% yield). MS (ESI-MS): m/z calcd for $C_{34}H_{40}F_2N_3O_6^+$ = 624.3, found 624.3.

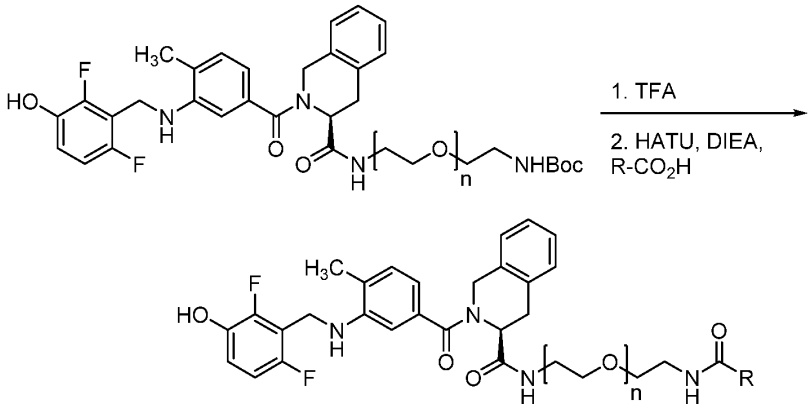
[00465] Compound **34**: Compound **34** was synthesized according to General Procedure A from **28** (30 mg, 66 μmol) and NH_2-PEG_2-$CO_2{}^t$Bu. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **34** as a colorless film (41 mg, 93% yield). MS (ESI-MS): m/z calcd for $C_{36}H_{44}F_2N_3O_7^+$ = 668.3, found 668.3.

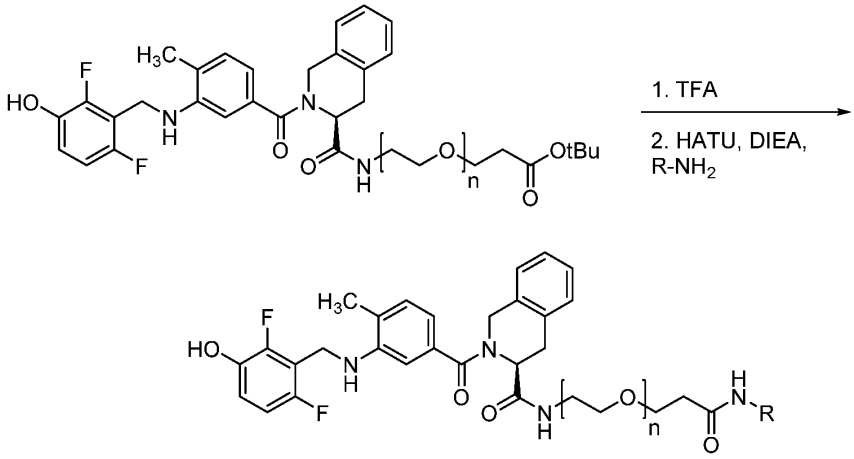
[00466] Compound **35**: Compound **35** was synthesized according to General Procedure A from **28** (30 mg, 66 μmol) and NH_2-PEG_4-$CO_2{}^tBu$. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **35** as a colorless film (47 mg, 94% yield). MS (ESI-MS): m/z calcd for $C_{40}H_{52}F_2N_3O_9^+$ = 756.4, found 756.4.

[00467] Compound **36**: Compound **36** was synthesized according to General Procedure A from **28** (30 mg, 66 μmol) and NH_2-PEG_1-NHBoc. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **36** as a colorless film (35 mg, 82% yield). MS (ESI-MS): m/z calcd for $C_{34}H_{41}F_2N_4O_6^+$ = 639.3, found 639.3.

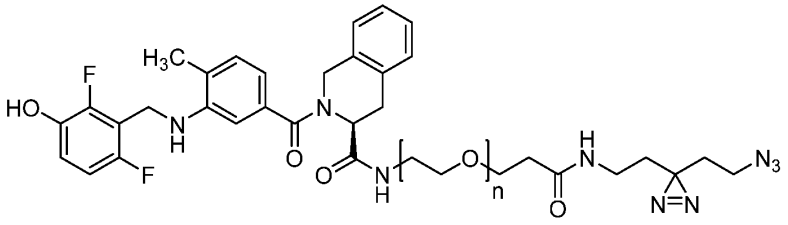
[00468] Compound **37**: Compound **37** was synthesized according to General Procedure A from **28** (100 mg, 221 μmol) and NH_2-PEG_2-NHBoc. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **37** as a colorless film (126 mg, 84% yield). MS (ESI-MS): m/z calcd for $C_{36}H_{45}F_2N_4O_7^+$ = 683.3, found 683.3.

[00469] Compound **38**: Compound **33** was synthesized according to General Procedure A from **28** (30 mg, 66 μmol) and NH_2-PEG_4-NHBoc. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **38** as a colorless film (37 mg, 72% yield). MS (ESI-MS): m/z calcd for $C_{40}H_{53}F_2N_4O_9^+$ = 771.4, found 771.4.





[00470] General Procedure C – Synthesis of Photoprobes from Boc- or *tert*-butyl ester-Protected Ligands: The Boc- or *tert*-butyl ester-protected ligand (1 eq.) was treated with neat trifluoroacetic acid (2 mL). The mixture was allowed to stir at room temperature for 5 minutes, then was concentrated to dryness under reduced pressure. The residue was then resuspended in DMF (2 mL), then was treated with DIEA (10 eq.), HATU (2.0 eq.) and the photoreactive warhead (as an free amine, amine hydrochloride, or carboxylic acid). The resulting mixtures were stirred at room temperature until LC-MS analysis indicated that the reaction was complete, then the photoprobes were purified by reverse-phase flash column chromatography. Fractions containing the desired products were combined and concentrated to remove CH_3CN, then were frozen and lyophilized to afford the final photoprobes as white solids.



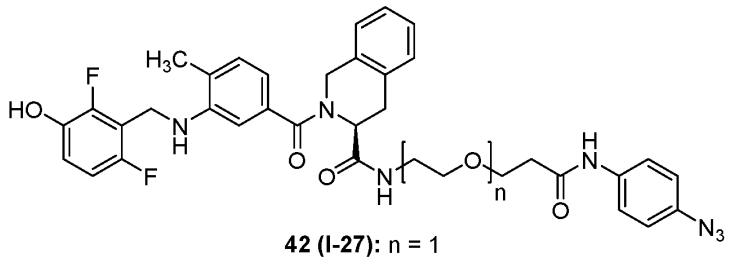
39 (I-24): n = 1
40 (I-25): n = 2
41 (I-26): n = 4

[00471] Compound **39**: Compound **39** (**I-24**) was synthesized according to General Procedure C from **33** (33 mg, 53 μmol) and 2-(3-(2-azidoethyl)-3*H*-diazirin-3-yl)ethan-1-amine. See Pan, S.; Jang, S.; Wang, D.; Liew, S.; Li, Z.; Lee, J.; Yao, S. Q. *Angew. Chem. Int. Ed.*, **2017**, *39*, 11816 – 11821. After stirring at room temperature for 1 h, the mixture was purified by flash column

chromatography over C_{18} silica gel to afford **39** as a white solid (17 mg, 46% yield). MS (ESI-MS): m/z calcd for $C_{35}H_{40}F_2N_9O_5^+$ = 704.3, found 704.3.

[00472] Compound **40**: Compound **40** (**I-25**) was synthesized according to General Procedure C from **34** (22 mg, 33 μmol) and 2-(3-(2-azidoethyl)-3*H*-diazirin-3-yl)ethan-1-amine. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **40** as a white solid (15 mg, 62% yield). MS (ESI-MS): m/z calcd for $C_{37}H_{44}F_2N_9O_6^+$ = 748.3, found 748.3.

[00473] Compound **41**: Compound **41** (**I-26**) was synthesized according to General Procedure C from **35** (32 mg, 43 μmol) and 2-(3-(2-azidoethyl)-3*H*-diazirin-3-yl)ethan-1-amine. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **41** as a white solid (16 mg, 45% yield). MS (ESI-MS): m/z calcd for $C_{41}H_{52}F_2N_9O_8^+$ = 836.4, found 836.4.



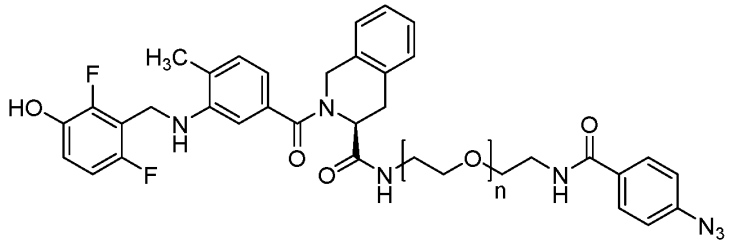
42 (I-27): n = 1
43 (I-28): n = 2
44 (I-29): n = 4

[00474] Compound **42**: Compound **42** (**I-27**) was synthesized according to General Procedure C from **33** (33 mg, 53 μmol) and 4-azidoaniline hydrochloride. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **42** as a white solid (14 mg, 39% yield). MS (ESI-MS): m/z calcd for $C_{36}H_{36}F_2N_7O_5^+$ = 684.3, found 684.3.

[00475] Compound **43**: Compound **43** (**I-28**) was synthesized according to General Procedure C from **34** (22 mg, 33 μmol) and 4-azidoaniline hydrochloride. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **43** as a white solid (11 mg, 43% yield). MS (ESI-MS): m/z calcd for $C_{38}H_{40}F_2N_7O_6^+$ = 728.4, found 728.4.

[00476] Compound **44**: Compound **44** (**I-29**) was synthesized according to General Procedure C from **35** (32 mg, 43 μmol) and 4-azidoaniline hydrochloride. After stirring at room temperature

for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **44** as a white solid (17 mg, 49% yield). MS (ESI-MS): m/z calcd for $C_{42}H_{48}F_2N_7O_8^+$ = 816.4, found 816.4.

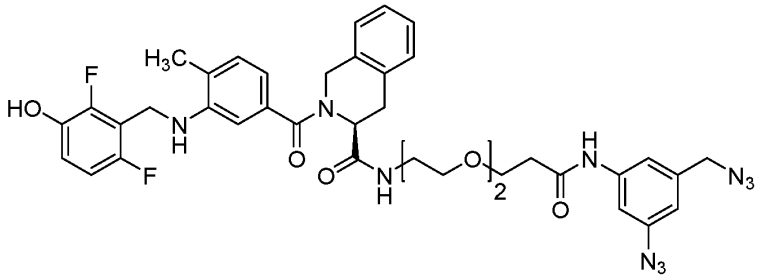


45 (I-30): n = 1
46 (I-31): n = 2
47 (I-32): n = 4

[00477] Compound **45**: Compound **45** (**I-30**) was synthesized according to General Procedure C from **36** (11 mg, 17 μmol) and 4-azidobenzoic acid. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **45** as a white solid (10 mg, 85% yield). MS (ESI-MS): m/z calcd for $C_{36}H_{36}F_2N_7O_5^+$ = 684.3, found 684.3.

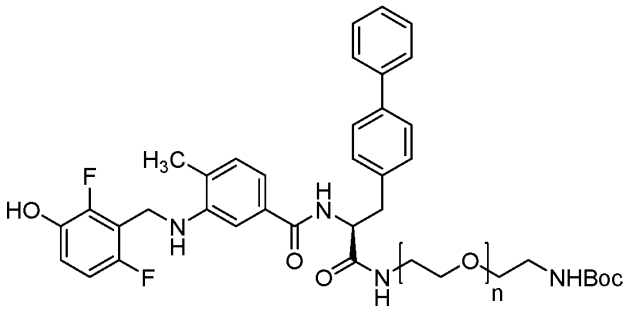
[00478] Compound **46**: Compound **46** (**I-31**) was synthesized according to General Procedure C from **37** (14 mg, 21 μmol) and 4-azidobenzoic acid. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **46** as a white solid (11 mg, 71% yield). MS (ESI-MS): m/z calcd for $C_{38}H_{40}F_2N_7O_6^+$ = 728.4, found 728.4.

[00479] Compound **47**: Compound **47** (**I-32**) was synthesized according to General Procedure C from **38** (21 mg, 27 μmol) and 4-azidobenzoic acid. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **47** as a white solid (12 mg, 54% yield). MS (ESI-MS): m/z calcd for $C_{42}H_{48}F_2N_7O_8^+$ = 816.4, found 816.4.



48
I-33

[00480] Compound **48**: Compound **48** (**I-33**) was synthesized according to General Procedure C from **37** (126 mg, 184 μmol) and 3-azido-5-(azidomethyl)benzoic acid. After stirring at room temperature for 16 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **48** as a white solid (26 mg, 18% yield). MS (ESI-MS): m/z calcd for $C_{39}H_{41}F_2N_{10}O_6^+$ = 783.4, found 783.4.

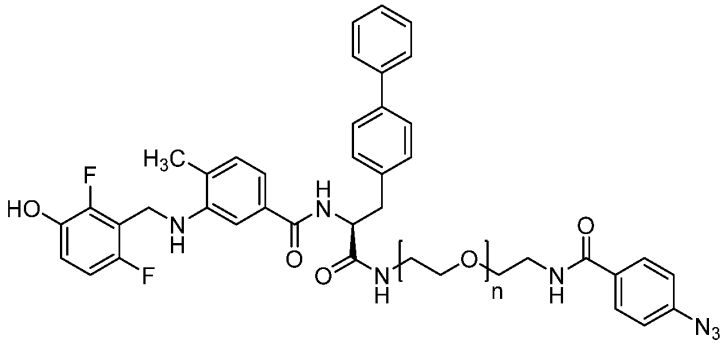


49: n = 1
50: n = 2
51: n = 4

[00481] Compound **49**: Compound **49** was synthesized according to General Procedure A from **31** (30 mg, 58 μmol) and NH_2-PEG_1-NHBoc. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **49** as a colorless film (27 mg, 65% yield). MS (ESI-MS): m/z calcd for $C_{39}H_{45}F_2N_4O_6^+$ = 703.3, found 703.3.

[00482] Compound **50**: Compound **50** was synthesized according to General Procedure A from **31** (30 mg, 58 μmol) and NH_2-PEG_2-NHBoc. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **50** as a colorless film (31 mg, 69% yield). MS (ESI-MS): m/z calcd for $C_{41}H_{49}F_2N_4O_7^+$ = 747.3, found 747.3.

[00483] Compound **51**: Compound **51** was synthesized according to General Procedure A from **31** (30 mg, 58 μmol) and NH_2-PEG_4-NHBoc. The reaction mixture was stirred at room temperature for 1 h, then was purified by column chromatography (C_{18} silica gel, eluting with 0-100% CH_3CN in water containing 0.1% formic acid) to afford **51** as a colorless film (41 mg, 84% yield). MS (ESI-MS): m/z calcd for $C_{45}H_{57}F_2N_4O_9^+$ = 835.3, found 835.3.



52 (I-34): n = 1
53 (I-35): n = 2
54 (I-36): n = 4

[00484] Compound **52**: Compound **52** (**I-34**) was synthesized according to General Procedure C from **49** (25 mg, 36 µmol) and 4-azidobenzoic acid. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **52** as a white solid (9 mg, 34% yield). MS (ESI-MS): m/z calcd for $C_{41}H_{40}F_2N_7O_5^+$ = 748.3, found 748.3.

[00485] Compound **53**: Compound **53** (**I-35**) was synthesized according to General Procedure C from **50** (30 mg, 40 µmol) and 4-azidobenzoic acid. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **53** as a white solid (6 mg, 19% yield). MS (ESI-MS): m/z calcd for $C_{43}H_{44}F_2N_7O_6^+$ = 792.3, found 792.3.

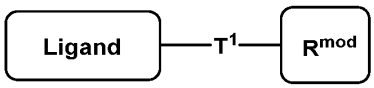
[00486] Compound **54**: Compound **54** (**I-36**) was synthesized according to General Procedure C from **51** (31 mg, 37 µmol) and 4-azidobenzoic acid. After stirring at room temperature for 1 h, the mixture was purified by flash column chromatography over C_{18} silica gel to afford **54** as a white solid (23 mg, 71% yield). MS (ESI-MS): m/z calcd for $C_{47}H_{52}F_2N_7O_8^+$ = 880.4, found 880.4.

[00487] While we have described a number of embodiments of this invention, it is apparent that our basic examples may be altered to provide other embodiments that utilize the compounds and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments that have been represented by way of example.

CLAIMS

We claim:

1. A compound of Formula **I**:



I

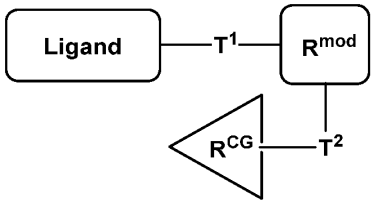
or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

T^1 is a bivalent tethering group; and

R^{mod} is a photoactivatable group.

2. A compound of Formula **II**:



II

or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

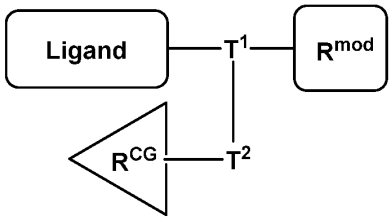
T^1 is a bivalent tethering group;

T^2 is a covalent bond or a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group.

3. A compound of Formula **III**:



III

or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

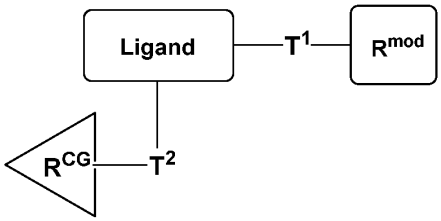
T^1 is a trivalent tethering group;

T^2 is a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group.

4. A compound of Formula **II-a**:



II-a

or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

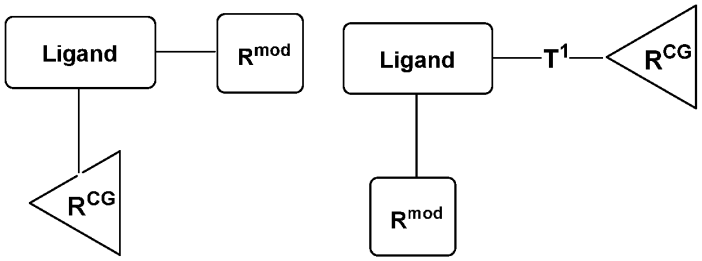
T^1 is a covalent bond or a bivalent tethering group;

T^2 is a covalent bond or a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group.

5. A compound of Formulae **II-b** or **II-c**:



II-b II-c

or a pharmaceutically acceptable salt thereof; wherein:

Ligand is a small molecule RNA binder;

T^1 is a bivalent tethering group;

R^{mod} is a photoactivatable group; and

R^{CG} is a click-ready group or a pull-down group.

6. The compound of any one of claims 1-5, wherein **Ligand** is selected from a heteroaryldihydropyrimidine (HAP), a macrolide, an alkaloid, an aminoglycoside, a tetracycline, a SMN2 ligand, a pleuromutilin, theophylline or an analogue thereof, ribocil or an analogue thereof, a substituted anthracene, a substituted triptycene, an oxazolidinone, or CPNQ or an analogue thereof; wherein **Ligand** may be optionally substituted with one or more substituents.

7. The compound of any one of claims 1-6, wherein **Ligand** is selected from an optionally substituted heteroaryldihydropyrimidine (HAP), erythromycin, azithromycin, berberine, palmatine, a paromomycin, a neomycin, a kanamycin, doxycycline, oxytetracycline, pleuromutilin, theophylline or an analogue thereof, ribocil or an analogue thereof, LMI070 (NVS-SM1), a substituted triptycene, linezolid, tedizolid, or CPNQ or an analogue thereof; wherein **Ligand** may be optionally substituted with 1, 2, 3, or 4 substituents.

8. The compound of any one of claims 1-7, wherein T^1 is selected from a C_{1-20} bivalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -

N(R)C(O)N(R)-, -S-, -SO-, -SO_2-, -SO_2N(R)-, -(R)NSO_2-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with -OCH_2CH_2-;

wherein each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

9. The compound of claim 8, wherein T^1 is selected from a C_{1-10} bivalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, or 5 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, -SO_2-, -SO_2N(R)-, -(R)NSO_2-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1, 2, 3, 4, or 5, of the methylene units of the chain are independently and optionally replaced with -OCH_2CH_2-.

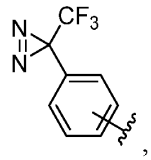
10. The compound of any one of claims 2-4, wherein T^2 is selected from a C_{1-20} bivalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, $-SO_2-$, $-SO_2N(R)-$, $-(R)NSO_2-$, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2-$;

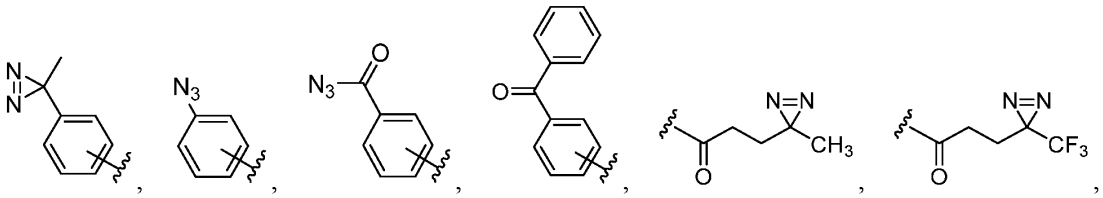
wherein each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

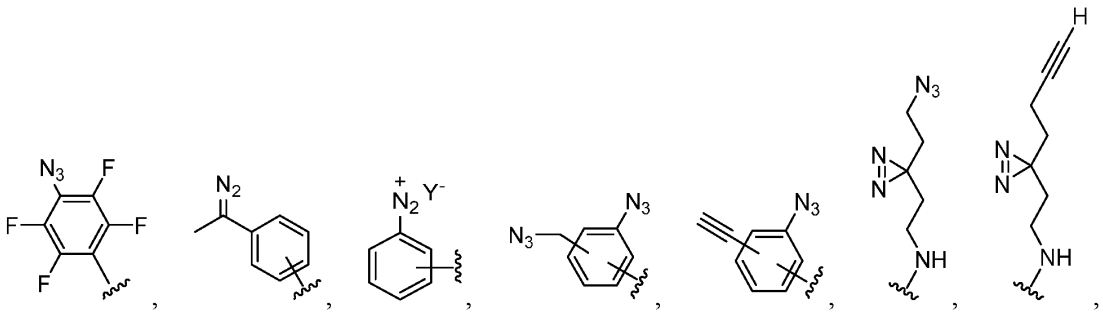
each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

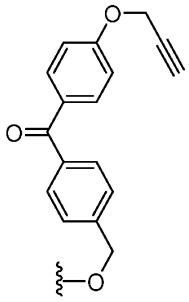
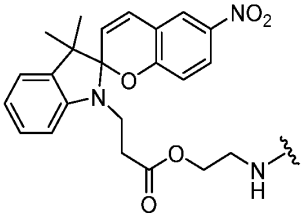
11. The compound of any one of claims 1-10, wherein R^{mod} is selected from a functional group that generates a radical, an aryl or heteroaryl carbocation, a nitrene, or a carbene intermediate upon irradiation with ultraviolet (UV) radiation; and wherein R^{mod} is capable of reacting with a target RNA to which **Ligand** binds to produce a covalent bond with the target RNA upon irradiation.

12. The compound of any one of claims 1-10, wherein R^{mod} is selected from an optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic azide or 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl azide, optionally substituted benzoyl azide or 5-8 membered heteroaroyl azide or 8-10 membered heteroaroyl azide wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic diazonium salt, optionally substituted 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl diazonium salt wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted C_{2-6} aliphatic diazo functional group, optionally substituted C_{2-6} aliphatic diazirine, or optionally substituted diphenyl or 8-10-membered diheteroaryl ketone wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted dihydropyrene, optionally substituted spirooxazine, optionally substituted anthracene, optionally substituted fulgide, or optionally substituted spiropyran.

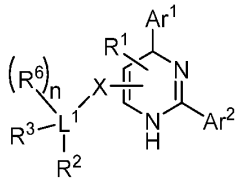
13. The compound of any one of claims 1-12, wherein R^{mod} is selected from ,

,

.


, or 
; wherein Y^- is a pharmaceutically acceptable anion.

14. A compound of Formula **X-a**:



X-a

or a tautomer or pharmaceutically acceptable salt thereof, wherein:

Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Ar^2 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, $-NR^6$-, -S-, -C(O)-, $-CO_2$- -CS-, $-C(NR^6)$-, -S(O)-, or $-S(O)_2$-;

R^1 is selected from $-C(O)R^6$, $-CO_2R$, $-C(O)NR_2$, $-C_{1-6}$ aliphatic, -CN, $-(CH_2)_{1-3}OR$, $-(CH_2)_{1-3}NHR$, $-N(R)C(O)OR^6$, $-N(R^6)C(O)R$, -OC(O)R, -OR, $-NHR^6$, or -N(R)C(O)NHR;

R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent;

R^3 is absent or is a click-ready group or a pull-down group;

each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms;

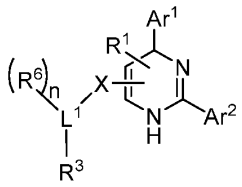
each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^1 is a C_{1-20} bivalent, trivalent, or tetravalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, $-SO_2-$, $-SO_2N(R)-$, $-(R)NSO_2-$, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2-$;

each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

n is 0 or 1.

15. A compound of Formula **X-b**:



X-b

or a tautomer or pharmaceutically acceptable salt thereof, wherein:

Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Ar^2 is an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

wherein one of Ar^1 or Ar^2 is substituted with one R^2;

X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -$C(NR^6)$-, -S(O)-, or -$S(O)_2$-;

R^1 is selected from -$C(O)R^6$, -CO_2R, -$C(O)NR_2$, -C_{1-6} aliphatic, -CN, -$(CH_2)_{1-3}OR$, -$(CH_2)_{1-3}NHR$, -$N(R)C(O)OR^6$, -$N(R^6)C(O)R$, -OC(O)R, -OR, -NHR^6, or -N(R)C(O)NHR;

R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent;

R^3 is absent or is a click-ready group or a pull-down group;

each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms;

each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered

bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

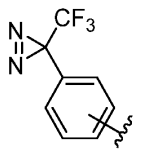
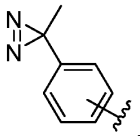
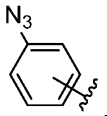
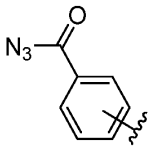
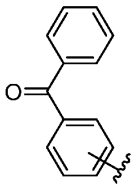
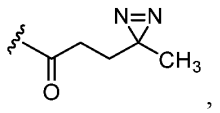
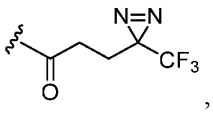
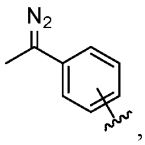
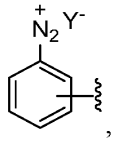
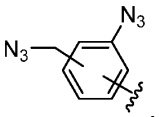
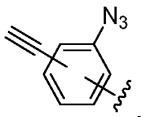
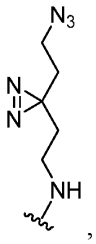
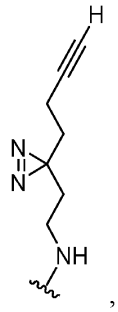
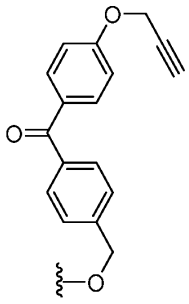
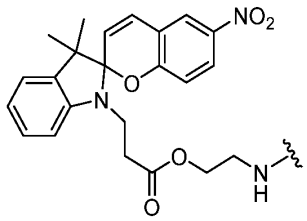
L^1 is a C_{1-20} bivalent or trivalent straight or branched hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, $-SO_2$-, $-SO_2N(R)$-, $-(R)NSO_2$-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2$-;

each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

n is 0 or 1.

16. The compound of claim 14 or 15, wherein R^2 is selected from an optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic azide or 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl azide, optionally substituted benzoyl azide or 5-8 membered heteroaroyl azide or 8-10 membered heteroaroyl azide wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted phenyl or 8-10 membered bicyclic aromatic carbocyclic diazonium salt, optionally substituted 5-8 membered heteroaryl or 8-10 membered bicyclic heteroaryl diazonium salt wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or oxygen, optionally substituted C_{2-6} aliphatic diazo functional group, optionally substituted C_{2-6} aliphatic diazirine, or optionally substituted diphenyl or 8-10-membered diheteroaryl ketone wherein 1-3 atoms of the ring atoms are selected from nitrogen, sulfur, or

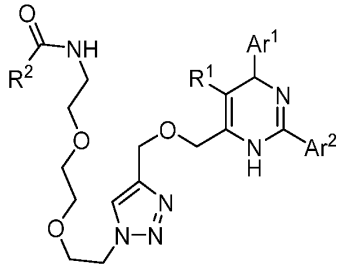
oxygen, optionally substituted dihydropyrene, optionally substituted spirooxazine, optionally substituted anthracene, optionally substituted fulgide, optionally substituted spiropyran, optionally substituted α-pyrone or optionally substituted pyrimidone.

17. The compound of any one of claims 14-16, wherein R^2 is selected from , , , , , , , , , , , , , , , ; wherein Y^- is a pharmaceutically acceptable anion.

18. The compound of any one of claims 14-17, wherein R^3 is an azide, an alkyne, 4-dibenzocyclooctynol (DIBO) a *gem*-difluorinated cyclooctyne (DIFO or DFO),

biarylazacyclooctynone (BARAC), bicyclononyne (BCN), a strained cyclooctyne, an oxime, oxanorbornadiene, or biotin.

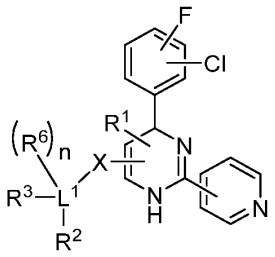
19. The compound of any one of claims 14-17, wherein the compound is of Formula **XIII**:



XIII

or a tautomer or pharmaceutically acceptable salt thereof.

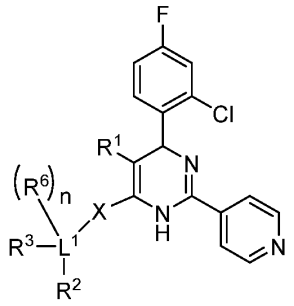
20. The compound of any one of claims 14 or 16-18, wherein the compound is of Formula **XIV**:



XIV

or a tautomer or pharmaceutically acceptable salt thereof, wherein each of X, L^1, R^1, R^2, R^3, R^6, R, -Cy-, and n is as defined above and described in embodiments herein, both singly and in combination.

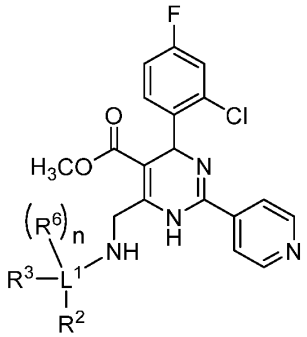
21. The compound of any one of claims 14 or 16-18, wherein the compound is of Formula **XV**:



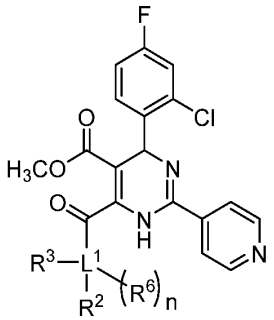
XV

or a tautomer or pharmaceutically acceptable salt thereof.

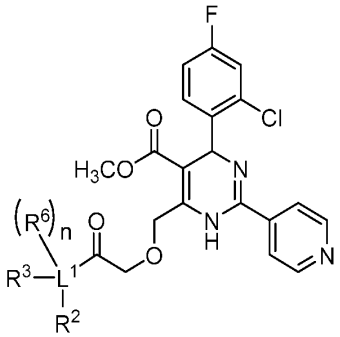
22. The compound of any one of claims 14 or 16-18, wherein the compound is of Formulae **XVI**, **XVII**, **XVIII**, or **XIX**:



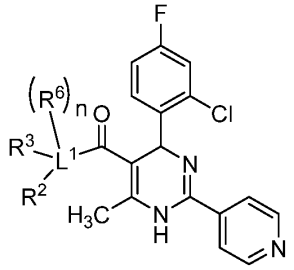
XVI



XVII



XVIII

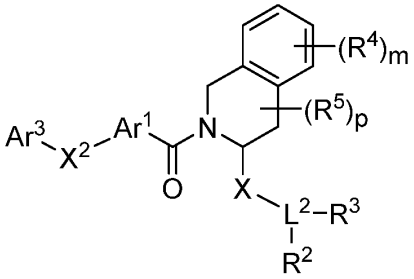


XIX

or a tautomer or pharmaceutically acceptable salt thereof.

23. A compound selected from one of those in Table 5, or a tautomer or pharmaceutically acceptable salt thereof.

24. A compound of Formula **XXV**:



XXV

or a pharmaceutically acceptable salt thereof, wherein:

Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Ar^3 is an optionally substituted phenyl, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-12 membered bicyclic aromatic ring, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -C(NR^6)-, -S(O)-, or -$S(O)_2$-;

X^2 is is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -C(NR^6)-, -S(O)-, or -$S(O)_2$-;

R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent;

R^3 is absent or is a click-ready group or a pull-down group;

each R^4 is independently R, halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2R$, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, -$OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, -$N(R)C(O)NR_2$, -

$N(R)S(O)_2R$, or $-N(R)S(O)_2NR_2$; or two instances of R^4 may be taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring;

each R^5 is independently R, halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, $-OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, $-N(R)C(O)NR_2$, $-N(R)S(O)_2R$, or $-N(R)S(O)_2NR_2$; or two instances of R^5 may be taken together to form =O or =S;

each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms;

each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^2 is a C_{1-20} bivalent or trivalent, straight or branched, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, $-SO_2-$, $-SO_2N(R)-$, $-(R)NSO_2-$, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with $-OCH_2CH_2-$;

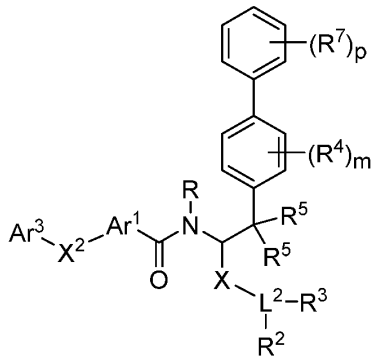
each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10

membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

m is 0, 1, 2, 3, or 4; and

p is 0, 1, 2, 3, or 4.

25. A compound of Formula **XXVI**:



XXVI

or a pharmaceutically acceptable salt thereof, wherein:

Ar^1 is an optionally substituted phenyl or optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Ar^3 is an optionally substituted phenyl, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-12 membered bicyclic aromatic ring, or an optionally substituted 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

X is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -C(NR^6)-, -S(O)-, or -$S(O)_2$-;

X^2 is is selected from a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -C(NR^6)-, -S(O)-, or -$S(O)_2$-;

R^2 is a photoactivatable group that optionally comprises a click-ready group if R^3 is absent;

R^3 is absent or is a click-ready group or a pull-down group;

each R^4 is independently R, halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2R$, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, -$OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, -$N(R)C(O)NR_2$, -$N(R)S(O)_2R$, or -$N(R)S(O)_2NR_2$; or two instances of R^4 may be taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring;

each R^5 is independently R, halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2R$, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, -$OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, -$N(R)C(O)NR_2$, -$N(R)S(O)_2R$, or -$N(R)S(O)_2NR_2$; or two instances of R^5 may be taken together to form =O or =S;

each R^6 is independently hydrogen or C_{1-6} alkyl optionally substituted with 1, 2, 3, 4, 5, or 6 deuterium or halogen atoms;

each R^7 is independently R, halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2R$, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -C(O)N(R)OR, -OC(O)R, -$OC(O)NR_2$, -N(R)C(O)OR, -N(R)C(O)R, -$N(R)C(O)NR_2$, -$N(R)S(O)_2R$, or -$N(R)S(O)_2NR_2$; or two instances of R^4 may be taken together with the atoms to which they are attached to form a C_{4-8} partially unsaturated carbocyclic ring;

each R is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^2 is a C_{1-20} bivalent or trivalent, straight or branched, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 methylene units of the chain are independently and optionally replaced with a natural or non-natural amino acid, -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -OC(O)N(R)-, -(R)NC(O)O-, -N(R)C(O)N(R)-, -S-, -SO-, -SO_2-, -SO_2N(R)-, -(R)NSO_2-, -C(S)-, -C(S)O-, -OC(S)-, -C(S)N(R)-, -(R)NC(S)-, -(R)NC(S)N(R)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with -OCH_2CH_2-;

each -Cy- is independently a bivalent optionally substituted 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, optionally substituted phenylene, an optionally substituted 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an optionally substituted 8-10 membered bicyclic or bridged bicyclic saturated or partially unsaturated heterocyclic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bicyclic or bridged bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

m is 0, 1, 2, 3, or 4; and

p is 0, 1, 2, 3, or 4.

26. The compound of claim 24 or 25, wherein Ar^1 is phenyl optionally subsituted with 1, 2, 3, or 4 substituents selected from halogen, -C_{1-6} aliphatic, -CN, -OR, -NR_2, -CO_2R, -C(O)R, -SR, or -C(O)NR_2.

27. The compound of any one of claims 24-26, wherein Ar^3 is phenyl optionally subsituted with 1, 2, 3, or 4 substituents selected from halogen, -C_{1-6} aliphatic, -CN, -OR, -NR_2, -CO_2R, -C(O)R, -SR, or -C(O)NR_2.

28. The compound of any one of claims 24-27, wherein X is a bivalent C_{1-3} alkylene chain wherein 1-2 methylene units of the chain are independently and optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -C(NR^6)-, -S(O)-, or -$S(O)_2$-.

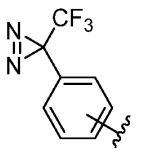
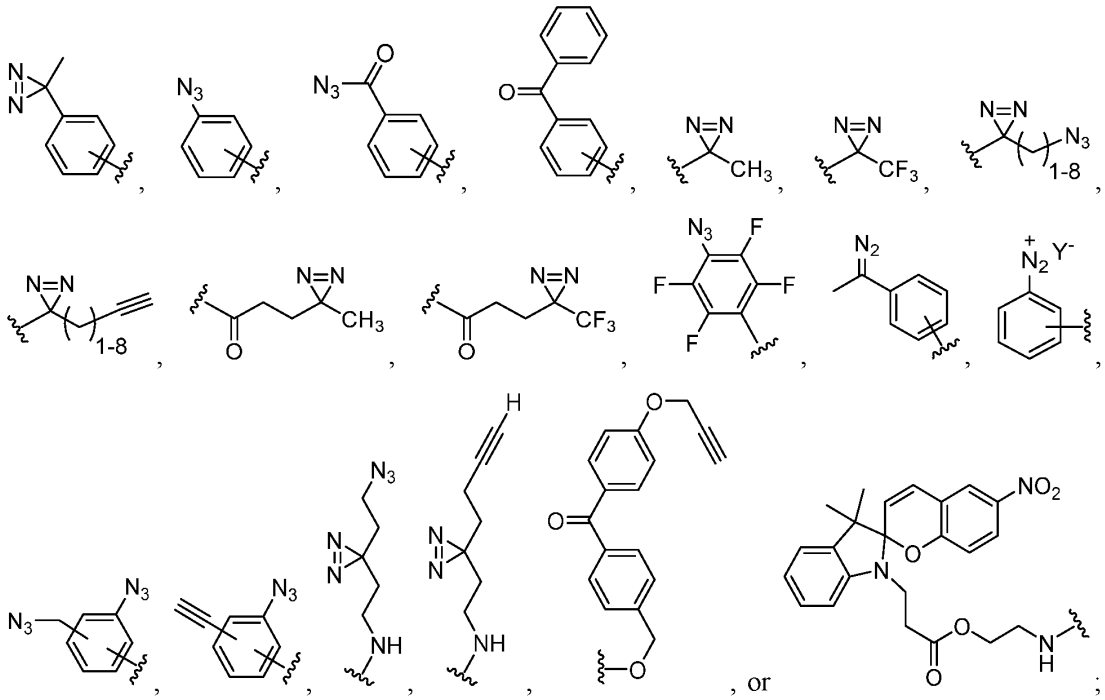
29. The compound of any one of claims 24-28, wherein X^2 is is a bivalent C_{1-2} alkylene chain wherein one methylene unit of the chain is optionally replaced with -O-, -NR^6-, -S-, -C(O)-, -CO_2- -CS-, -C(NR^6)-, -S(O)-, or -$S(O)_2$-.

30. The compound of any one of claims 24-29, wherein R^4 is hydrogen, C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2$R, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -OC(O)R, or -N(R)C(O)R.

31. The compound of any one of claims 24-30, wherein R^5 is hydrogen, C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, -NO_2, -OR, -SR, -NR_2, -$S(O)_2$R, -$S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, -$C(O)NR_2$, -OC(O)R, or -N(R)C(O)R.

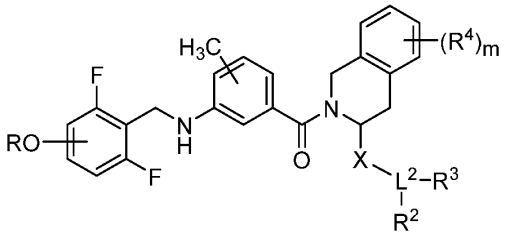
32. The compound of any one of claims 24-31, wherein L^2 is a C_{1-20} bivalent or trivalent, straight or branched, optionally substituted hydrocarbon chain wherein 1, 2, 3, 4, or 5 methylene

units of the chain are independently and optionally replaced with -O-, -C(O)-, -C(O)O-, -OC(O)-, -N(R)-, -C(O)N(R)-, -(R)NC(O)-, -S-, -SO-, -SO_2-, -C(S)-, or -Cy-; and 1-20 of the methylene units of the chain are independently and optionally replaced with -OCH_2CH_2-.

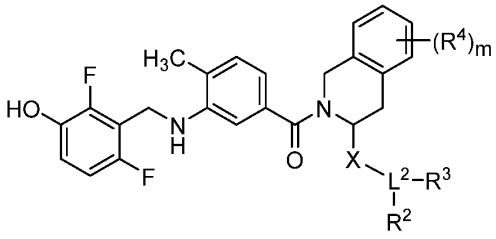
33. The compound of any one of claims 24-32, wherein R^2 is selected from , ; wherein Y^- is a pharmaceutically acceptable anion.

34. The compound of any one of claims 24-33, wherein R^3 is a C_{1-6} alkyl azide, C_{1-6} alkyne, or a hapten.

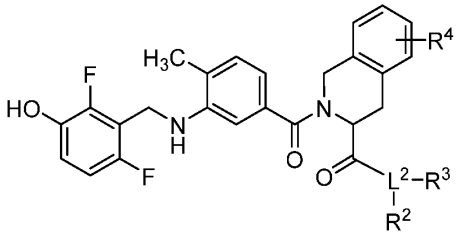
35. The compound of claim 24, wherein the compound is of Formulae **XXIX**, **XXX**, **XXXI**, **XXXII**, or **XXXIII**:



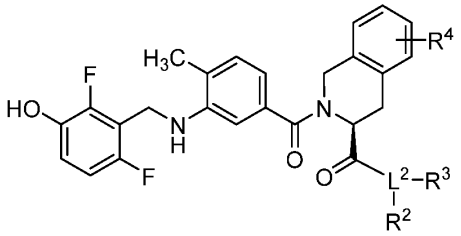
XXIX



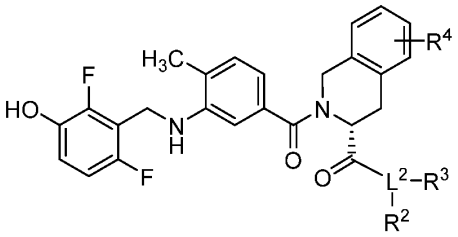
XXX



XXXI



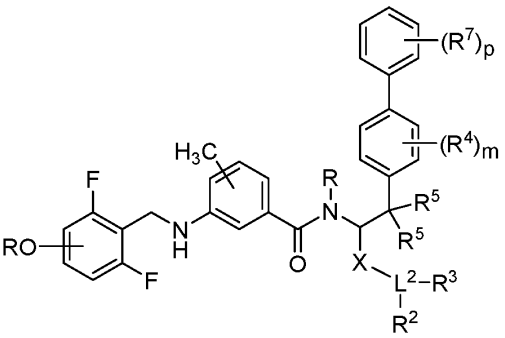
XXXII



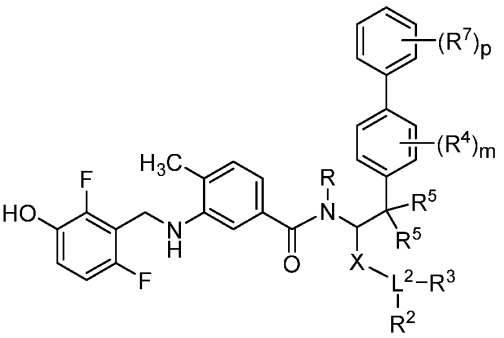
XXXIII

or a pharmaceutically acceptable salt thereof.

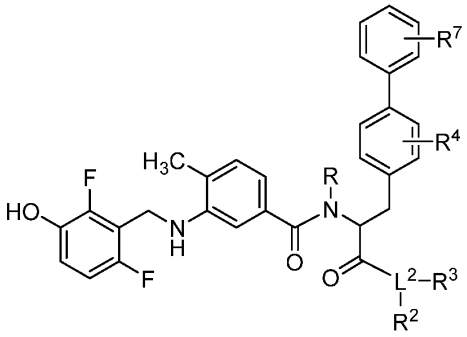
36. The compound of claim 25, wherein the compound is of Formulae **XXXVIII**, **XXXIX**, **XL**, **XLI**, or **XLII**:



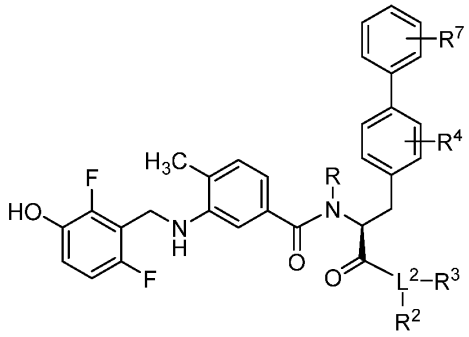
XXXVIII



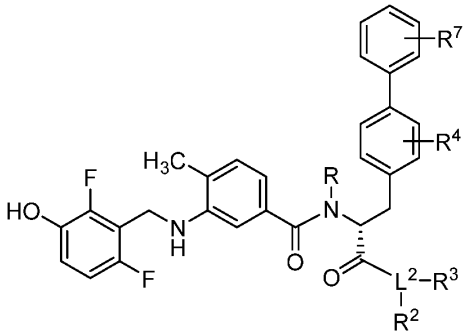
XXXIX



XL



XLI



XLII

or a pharmaceutically acceptable salt thereof.

37. The compound of claim 25, wherein R^7 is hydrogen, C_{1-6} aliphatic, a 3-8 membered saturated or partially unsaturated monocyclic carbocyclic ring, phenyl, an 8-10 membered bicyclic aromatic carbocyclic ring, a 4-8 membered saturated or partially unsaturated monocyclic heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur; a 5-6 membered monocyclic heteroaromatic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; an 8-10 membered bicyclic heteroaromatic ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; halogen, -CN, $-NO_2$, -OR, -SR, $-NR_2$, $-S(O)_2R$, $-S(O)_2NR_2$, -S(O)R, -C(O)R, -C(O)OR, $-C(O)NR_2$, -OC(O)R, or -N(R)C(O)R.

38. An RNA conjugate, comprising a target RNA and a compound of any one of claims 1-37, wherein R^{mod} or R^2 forms a covalent bond to the target RNA.

39. A method of determining the three-dimentional structure, binding site of a ligand of interest, or accessibility of a nucleotide in a target nucleic acid, comprising: contacting the target nucleic acid with a compound of any one of claims 1-37 or a pharmaceutically acceptable salt thereof; irradiating the compound; determining whether covalent modification of a nucleotide of the nucleic acid has occurred; and optionally deriving the pattern of nucleotide modification, the three-dimentional structure, ligand binding site, or other structural information about the nucleic acid.

40. A method of preparing a nucleic acid conjugate, comprising: contacting a target nucleic acid with a compound of any one of claims 1-37 or a pharmaceutically acceptable salt thereof; irradiating the compound; and optionally isolating the resulting nucleic acid conjugate by an affinity assay, pull-down method.