RNAI AGENT TARGETING INHBE

The present disclosure claims priority to Chinese Patent Application No. CN202410263661.7 filed on Mar. 8, 2024 and Chinese Patent Application No. CN202410594262.9 filed on May 14, 2024.

TECHNICAL FIELD

The present disclosure pertains to the field of biomedicine, and particularly relates to an siRNA targeting the inhibin βE (INHBE) gene, a conjugate, a composition, and pharmaceutical use thereof.

BACKGROUND

The inhibin βE (INHBE) gene, one member of the TGF-β (transforming growth factor-β) superfamily proteins, is involved in the regulation of various cellular processes, including cell proliferation, apoptosis, immune responses, and hormone secretion. Inhibins are responsible for inhibiting the secretion of follicle-stimulating hormone from the pituitary gland, and may be involved in the regulation of many different functions, such as hypothalamic and pituitary hormone secretion, gonadal hormone secretion, germ cell development and maturation, red blood cell differentiation, insulin secretion, nerve cell survival, embryo axial development, or bone growth. In addition, INHBE may be up-regulated under endoplasmic reticulum stress conditions, and thus the protein may inhibit the proliferation and growth of cells in the pancreas and the liver. Body fat distribution is an important determination criterion for cardiovascular and metabolic diseases. A specific one among various body fat distributions is apt to give rise to serious cardiovascular or metabolic diseases, and is characterized by the distribution of more fat on the waist and/or the accumulation of less fat in the hip, resulting in a relatively high waist-hip ratio (WHR). Metabolic diseases caused by abnormal body fat distribution include: type 2 diabetes, hyperlipidemia, dyslipidemia (elevated or changed circulation levels of low-density lipoprotein cholesterol LDL-C, triglyceride, very low-density lipoprotein cholesterol VLDL-C, apolipoprotein B, or other lipid fractions), obesity (particularly abdominal obesity), lipodystrophy, insulin resistance, nonalcoholic steatohepatitis, hypertension, hyperglycemia, metabolic syndrome, coronary artery disease, other atherosclerotic disorders, and the like.

At present, methods for treating metabolic diseases mainly include lifestyle modifications, dietary adjustments, physical exercise, and administration of lipid-lowering drugs (e.g., statins). However, these methods have relatively poor effects, and the administration of lipid-lowering drugs may bring some side effects. RNA interference (RNAi; siRNA) is an effective approach to silence gene expression, which can specifically degrade mRNAs of target genes (e.g., INHBE) associated with metabolic diseases through a post-transcriptional regulatory mechanism, thereby

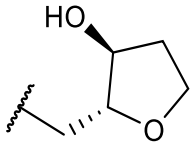
effectively inhibiting the expression of the target genes and achieving the purpose of effectively treating such diseases.

SUMMARY

The present disclosure provides an RNAi agent targeting INHBE.

In some embodiments, the present disclosure provides an RNAi agent comprising a sense strand and an antisense strand that form a double-stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing from the nucleotide sequence set forth in any one of SEQ ID NO: 1 to SEQ ID NO: 9 by no more than 3 nucleotides, and the antisense strand comprises at least 15 contiguous nucleotides differing from the nucleotide sequence set forth in any one of SEQ ID NO: 10 to SEQ ID NO: 18 by no more than 3 nucleotides, wherein the sense strand comprises at least one reverse abasic nucleoside and/or the antisense strand comprises at least one nucleoside with thermally destabilizing modification.

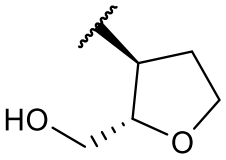
Unless otherwise specified, in the context of the present disclosure, the nucleoside in the reverse abasic nucleotide has a structure represented by formula (3-1), formula (3-2), or formula (3-3), wherein, when the reverse abasic nucleoside is the first nucleoside at the 5' end of the sequence, the nucleoside has a structure represented by formula (3-1); when the reverse abasic nucleoside is the first nucleoside at the 3' end, the nucleoside has a structure represented by formula (3-2); when the reverse abasic nucleoside is located in the middle of the sequence, the nucleoside has a structure represented by formula (3-3):



bond towards 3' end of oligonucleotide

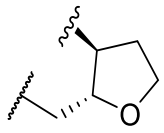
formula (3-1)

bond towards 5' end of oligonucleotide



formula (3-2);

bond towards 5' end of oligonucleotide



bond towards 3' end of oligonucleotide

formula (3-3).

In some embodiments, the sense strand comprises two reverse abasic nucleosides, and the reverse abasic nucleosides are the first nucleoside of the 5' end and the first nucleoside of the 3' end of the sense strand.

In some embodiments, the term “differing by no more than 3 nucleotides” means that the difference may be 0, 1, 2, or 3 nucleotides.

In some embodiments, the term “at least 15 contiguous nucleotides” refers to at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides; preferably, at least 17, 18, 19, 20, or 21 contiguous nucleotides.

In some embodiments, the antisense strand is at least partially complementary to a target sequence to mediate RNA interference. In some embodiments, there are no more than 5, 4, 3, 2, or 1 mismatches between the antisense strand and the target sequence. In some embodiments, the antisense strand is fully reverse complementary to the target sequence.

In some embodiments, the sense strand is at least partially reverse complementary to the antisense strand to form a double-stranded region. In some embodiments, there are no more than 5, 4, 3, 2, or 1 mismatches between the sense strand and the antisense strand. In some embodiments, the sense strand is fully reverse complementary to the antisense strand.

In some embodiments, the RNAi agent of the present disclosure comprises one or two blunt ends.

In some embodiments, the sense strand and/or the antisense strand of the RNAi agent of the present disclosure each independently comprise(s) 1 or 2 unpaired nucleotides. In some embodiments, the 3' end of the antisense strand comprises an overhang formed by unpaired nucleotides.

In some embodiments, the sense strand and the antisense strand each independently consist of 16 to 35, 16 to 34, 17 to 34, 17 to 33, 18 to 33, 18 to 32, 18 to 31, 18 to 30, 18 to 29, 18 to 28, 18 to 27, 18 to 26, 18 to 25, 18 to 24, 18 to 23, 19 to 25, 19 to 24, or 19 to 23 nucleotides. In some embodiments, the sense strand and the antisense strand each independently consist of 18, 19, 20, 21, 22, or 23 nucleotides.

In some embodiments, the sense strand and the antisense strand are identical or different in length; the sense strand is 19-23 nucleotides in length, and the antisense strand is 19-26 nucleotides in length. Therefore, the length ratio of the sense strand to the antisense strand of the RNAi agent provided by the present disclosure may be 19/19, 19/20, 19/21,

19/22, 19/23, 19/24, 19/25, 19/26, 20/19, 20/20, 20/21, 20/22, 20/23, 20/24, 20/25, 20/26, 21/19, 21/20, 21/21, 21/22, 21/23, 21/24, 21/25, 21/26, 22/19, 22/20, 22/21, 22/22, 22/23, 22/24, 22/25, 22/26, 23/19, 23/20, 23/21, 23/22, 23/23, 23/24, 23/25, or 23/26. In some embodiments, the length ratio of the sense strand to the antisense strand of the RNAi agent is 19/19, 19/21, 21/21, 21/23, 23/23, or 23/25. In some specific embodiments, the length ratio of the sense strand to the antisense strand is 19/21, 21/21, or 21/23.

In some embodiments, the sense strand comprises the nucleotide sequence set forth in any one of SEQ ID NO: 1 to SEQ ID NO: 9; and/or the antisense strand comprises the nucleotide sequence set forth in any one of SEQ ID NO: 10 to SEQ ID NO: 18.

In some embodiments, the sense strand is the nucleotide sequence set forth in any one of SEQ ID NO: 1 to SEQ ID NO: 9; and/or the antisense strand is the nucleotide sequence set forth in any one of SEQ ID NO: 10 to SEQ ID NO: 18.

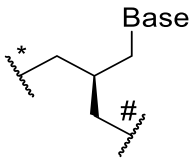
In some embodiments, at least one nucleotide in the sense strand and/or the antisense strand is a modified nucleotide. In some embodiments, all nucleotides in the sense strand and/or the antisense strand are modified nucleotides.

In some embodiments, the sense strand comprises three contiguous 2'-fluoro-modified nucleotides. In some embodiments, in the direction from the 5' end to the 3' end, the nucleotides at positions 7, 8, and 9 are 2'-fluoro-modified nucleotides, and when one or more nucleosides of the 5' end of the sense strand are reverse abasic nucleosides, the nucleosides at positions 7, 8, and 9 mean that positions 7, 8, and 9 are counted starting from the first nucleoside that is not a reverse abasic nucleoside as position 1; for example, for a sense strand set forth in SEQ ID NO: 35: IBsUmsCmAmUmCmAmGfCfUfUmUmGmCmUmAmCmUmGmsUmsIB, the count starts from the second nucleoside, Um, as position 1. The nucleotides at the remaining positions are non-2'-fluoro-modified nucleotides, and preferably, the non-2'-fluoro-modified nucleotides are 2'-methoxy-modified nucleotides.

In some embodiments, the antisense strand comprises at least 5 2'-fluoro-modified nucleotides, for example, may comprise 5, 6, 7, 8, 9, or 10 2'-fluoro-modified nucleotides. In some embodiments, in the direction from the 5' end to the 3' end, each of the nucleotides at positions 2, 6, 12, 14, and 16 is independently a 2'-fluoro-modified nucleotide. In some embodiments, the antisense strand comprises 5 2'-fluoro-modified nucleotides; each of the nucleotides at positions 2, 6, 12, 14, and 16 of the antisense strand is independently a 2'-fluoro-modified nucleotide, and the nucleotides at the remaining positions are non-2'-fluoro-modified nucleotides. In some embodiments, the antisense strand comprises 8 2'-fluoro-modified nucleotides; each of the nucleotides at positions 2, 4, 6, 10, 12, 14, 16, and 18 of the antisense strand is independently a 2'-fluoro-modified nucleotide, and the nucleotides at the remaining positions are non-2'-fluoro-modified nucleotides. In some embodiments, the nucleotides at positions 2, 4, 6, 10, 12, 14, 16, and 18 of the antisense strand are 2'-fluoro-modified nucleotides, the

nucleoside in the nucleotide at position 7 is a nucleoside with thermally destabilizing modification, and the nucleotides at the remaining positions are 2'-methoxy-modified nucleotides.

In some embodiments, the non-fluoro-modified nucleotides are 2'-methoxy-modified nucleotides or nucleotides with thermally destabilizing modification. The nucleotide with thermally destabilizing modification is capable of reducing the melting temperature of a nucleic acid duplex compared with an unmodified nucleic acid duplex. In some embodiments, the nucleotide with thermally destabilizing modification has a structure as shown in WO2022028462A1. In some embodiments, the nucleoside in the nucleotide with thermally destabilizing modification has a structure represented by formula (1):



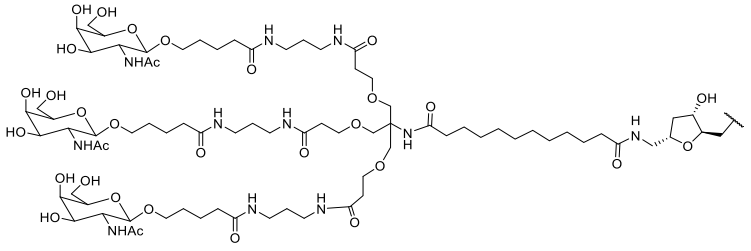
formula (1),

wherein Base represents a base; in the direction from the 5' end to the 3' end of the sequence, "*" represents a site where the nucleoside is linked to a nucleotide adjacent to its 5' end, and "#" represents a site where the nucleoside is linked to a nucleotide adjacent to its 3' end.

In some embodiments, in the antisense strand, other than the 2'-fluoro-modified nucleotides, the nucleotides at the remaining positions are all 2'-methoxy-modified nucleotides; or the nucleoside in the nucleotide at position 7 of the antisense strand is a nucleoside with thermally destabilizing modification, and the nucleotides at the remaining positions are 2'-methoxy-modified nucleotides.

In some embodiments, at least one phosphodiester group in the sense strand and/or the antisense strand is a phosphodiester group with a modification group. The modification group causes the RNAi agent to have increased stability in a biological sample or environment. In some embodiments, the sense strand and/or the antisense strand comprise(s) a plurality of phosphodiester groups with modification groups. In some embodiments, the sense strand and the antisense strand each comprise a plurality of phosphodiester groups with modification groups; preferably, the sense strand and the antisense strand each independently comprise at least 2 phosphodiester groups with modification groups. In some embodiments, the sense strand comprises 3 phosphodiester groups with modification groups, and the antisense strand comprises 4 phosphodiester groups with modification groups. In some embodiments, the phosphodiester group with a modification group is present at one or more of the following positions selected from the group consisting of: between any two adjacent nucleotides of the 1st to the 4th nucleotides of the 5' end and/or 3' end of the sense strand, and/or between any two adjacent nucleotides of the 1st to the 4th nucleotides of

the 5' end and/or 3' end of the antisense strand. For example, the phosphodiester group with a modification group may be located between the 1st and 2nd nucleotides of the 5' end of the sense strand;
between the 2nd and 3rd nucleotides of the 5' end of the sense strand;
between the 1st and 2nd nucleotides of the 3' end of the sense strand;
between the 2nd and 3rd nucleotides of the 3' end of the sense strand;
between the 1st and 2nd nucleotides of the 5' end of the antisense strand;
between the 2nd and 3rd nucleotides of the 5' end of the antisense strand;
between the 1st and 2nd nucleotides of the 3' end of the antisense strand; and
between the 2nd and 3rd nucleotides of the 3' end of the antisense strand.
In some embodiments, the phosphodiester group with a modification group is a phosphorothioate diester group.
In some embodiments, the sense strand is selected from the group consisting of or comprises the nucleotide sequence set forth in any one of SEQ ID NO: 19 to SEQ ID NO: 45.
In some embodiments, the antisense strand is selected from the group consisting of or comprises the nucleotide sequence set forth in any one of SEQ ID NO: 74 to SEQ ID NO: 100.
In some embodiments, the RNAi agent of the present disclosure further comprises one or more delivery groups. In some embodiments, the one or more delivery groups are linked to the sense strand. Preferably, the RNAi agent of the present disclosure comprises one delivery group.
In some embodiments, the delivery group comprises at least one targeting ligand targeting the liver. In some embodiments, the targeting ligand binds to an asialoglycoprotein receptor (ASGPR). In some embodiments, the targeting ligand comprises a galactose cluster or a galactose derivative cluster, wherein the galactose derivative is selected from the group consisting of N-acetyl-galactosamine, N-trifluoroacetylgalactosamine, N-propionylgalactosamine, N-n-butyrylgalactosamine, and N-isobutyrylgalactosamine; preferably, the targeting ligand is a N-acetylgalactosamine (GalNAc) ligand. In some embodiments, the delivery group may specifically be a nucleic acid ligand represented by formula (I) in the PCT application WO2023274395A.
In some embodiments, the delivery group has a structure represented by formula (2):



formula (2).

In some embodiments, the delivery group is directly linked to the nucleoside in the first nucleotide at the 3' end of the sense strand by a phosphodiester group or a phosphorothioate diester group.

In some embodiments, in the RNAi agent of the present disclosure, the sense strand comprises or is selected from the group consisting of the nucleotide sequence set forth in any one of SEQ ID NO: 46 to SEQ ID NO: 73, and the antisense strand comprises or is selected from the group consisting of the nucleotide sequence set forth in any one of SEQ ID NO: 101 to SEQ ID NO: 128.

In some embodiments, the RNAi agent is selected from the group consisting of any one of the RNAi agents shown in Table 2b. In some embodiments, the RNAi agent is selected from the group consisting of TJR102314, TJR102316, TJR102317, TJR102318, TJR102323, TJR102526, TJR102153, TJR102705, TJR102701, TJR102702, TJR102703, TJR102704, TJR102706, TJR102707, TJR102938, TJR102939, TJR102940, TJR102942, TJR102943, TJR102944, TJR102969, TJR102970, and TJR102971.

In some embodiments, the RNAi agent is selected from the group consisting of the following structures or pharmaceutically acceptable salts thereof:

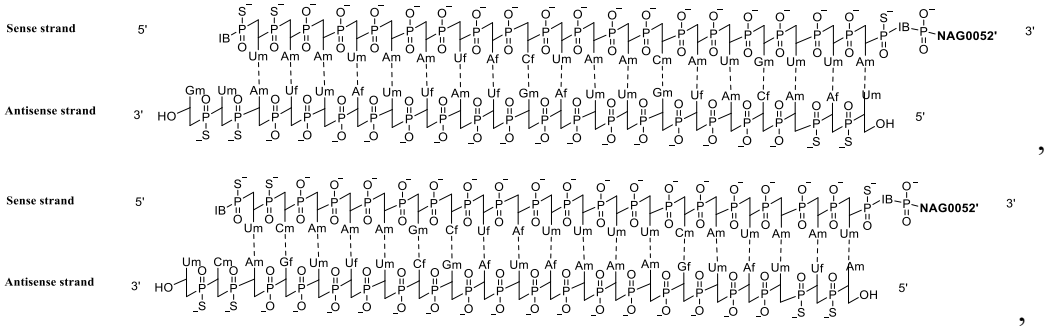


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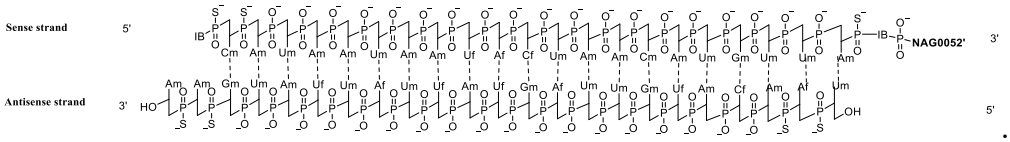
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In some embodiments, the RNAi agent is selected from the group consisting of the following structures:

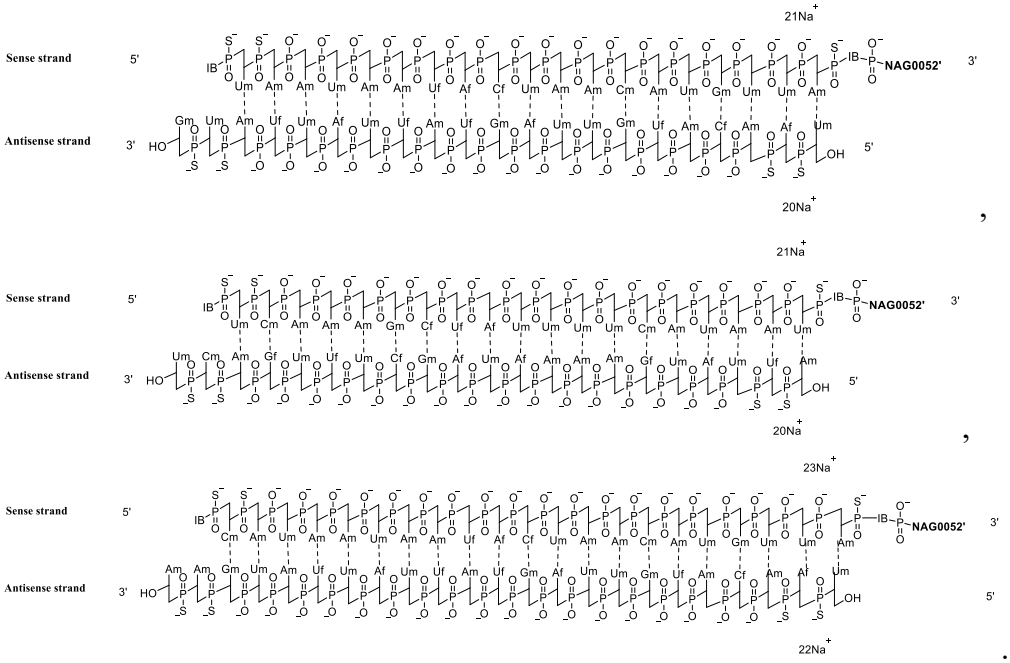


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In some embodiments, the pharmaceutically acceptable salt of the RNAi agent is selected from the group consisting of the following structures:



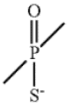
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In the structural formulas described above, the direction from the 5' end to the 3' end in the sense strand is a direction from left to right, and the direction from the 3' end to the 5' end in the antisense strand is a direction from left to right; IB represents a reverse abasic nucleoside; m means that the nucleoside adjacent to the letter m on the left side is a 2'-methoxy-modified nucleoside; f means that the nucleoside adjacent to the letter f on the left side is a 2'-fluoro-modified nucleoside; NAG0052' represents a delivery group NAG0052'.

The structures of the reverse abasic nucleoside, the 2'-methoxy-modified nucleoside, the 2'-fluoro-modified nucleoside, and the delivery group NAG0052' are all as shown in Table 1 below.

 represents an anionic form of a phosphorothioate diester group,  represents an anionic form of a phosphodiester group,  represents an ionic form of a phosphorothioate diester group,  represents an ionic form of a

phosphodiester group,  represents a phosphorothioate diester group, and  represents a phosphodiester group. The structures of the ionic form of the phosphorothioate diester group, the ionic form of the phosphodiester group, the phosphorothioate diester group, and the phosphodiester group are shown in Table 1 below.

In another aspect, the present disclosure provides a pharmaceutical composition comprising the RNAi agent described in the present disclosure, and one or more pharmaceutically acceptable carriers, for example, but not limited to, excipients, vehicles, carriers, diluents, and/or delivery systems (e.g., delivery polymers). Various drug delivery systems are known and can be used for the RNAi agent of the present disclosure, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the RNAi agent, receptor-mediated endocytosis, and construction of a nucleic acid as part of a retroviral vector or other vectors.

In some embodiments, the pharmaceutical composition may further comprise a pharmaceutically acceptable auxiliary material and/or adjuvant, and the auxiliary material may be one or more of the various formulations or compounds conventionally used in the art. For example, the pharmaceutically acceptable auxiliary material may include at least one of a pH buffer, a protective agent, and an osmotic pressure regulator. In some embodiments, a unit dose of the pharmaceutical composition is 0.001 mg-1000 mg.

In certain embodiments, the pharmaceutical composition comprises 0.01%-99.99% of the aforementioned RNAi agent or the pharmaceutically acceptable salt thereof or the isotopically substituted form thereof based on the total weight of the composition. In certain embodiments, the pharmaceutical composition comprises 0.1%-99.9% of the aforementioned RNAi agent or the pharmaceutically acceptable salt thereof or the isotopically substituted form thereof. In certain embodiments, the pharmaceutical composition comprises 0.5%-99.5% of the aforementioned RNAi agent or the pharmaceutically acceptable salt thereof or the isotopically substituted form thereof. In certain embodiments, the pharmaceutical composition comprises 1%-99% of the aforementioned RNAi agent or the pharmaceutically acceptable salt thereof or the isotopically substituted form thereof. In certain embodiments, the pharmaceutical composition comprises 2%-98% of the aforementioned RNAi agent or the pharmaceutically acceptable salt thereof or the isotopically substituted form thereof.

In certain embodiments, the pharmaceutical composition comprises 0.01%-99.99% of the pharmaceutically acceptable carrier based on the total weight of the composition. In certain embodiments, the pharmaceutical composition comprises 0.1%-99.9% of the pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition comprises 0.5%-99.5% of the pharmaceutically acceptable carrier. In

certain embodiments, the pharmaceutical composition comprises 1%-99% of the pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition comprises 2%-98% of the pharmaceutically acceptable carrier.

In some embodiments, when the RNAi agent or the pharmaceutical composition of the present disclosure is in contact with a target gene-expressing cell, the RNAi agent or the pharmaceutical composition of the present disclosure inhibits the expression of the target gene by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, as measured by, for example, psiCHECK activity screening and luciferase reporter gene assay, other methods such as PCR or branched DNA (bDNA)-based methods, or protein-based methods such as immunofluorescence assay, e.g., western blot or flow cytometry.

In some embodiments, when the RNAi agent or the pharmaceutical composition of the present disclosure is in contact with a target gene-expressing cell, the RNAi agent or the pharmaceutical composition of the present disclosure results in a remaining expression percentage of the target gene mRNA of no greater than 99%, no greater than 95%, no greater than 90%, no greater than 85%, no greater than 80%, no greater than 75%, no greater than 70%, no greater than 65%, no greater than 60%, no greater than 55%, no greater than 50%, no greater than 45%, no greater than 40%, no greater than 35%, no greater than 30%, no greater than 25%, no greater than 20%, no greater than 15%, or no greater than 10%, as measured by, for example, psiCHECK activity screening and luciferase reporter gene assay, other methods such as PCR or branched DNA (bDNA)-based methods, or protein-based methods such as immunofluorescence assay, e.g., western blot or flow cytometry.

In some embodiments, when the RNAi agent or the pharmaceutical composition of the present disclosure is in contact with a target gene-expressing cell, the RNAi agent, while retaining on-target activity, reduces off-target activity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%, as measured by, for example, psiCHECK activity screening and luciferase reporter gene assay, other methods such as PCR or branched DNA (bDNA)-based methods, or protein-based methods such as immunofluorescence assay, e.g., western blot or flow cytometry.

In some embodiments, when the RNAi agent or the pharmaceutical composition of the present disclosure is in contact with a target gene-expressing cell, the RNAi agent, while reducing on-target activity by no more than 20%, at most 19%, at most 15%, at most 10%, at most 5%, or more than 1%, reduces off-target activity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%, as measured by, for example,

psiCHECK activity screening and luciferase reporter gene assay, other methods such as PCR or branched DNA (bDNA)-based methods, or protein-based methods such as immunofluorescence assay, e.g., western blot or flow cytometry.

In some embodiments, when the RNAi agent or the pharmaceutical composition of the present disclosure is in contact with a target gene-expressing cell, the RNAi agent, while increasing on-target activity by at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, or at least 80%, reduces off-target activity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%, as measured by, for example, psiCHECK activity screening and luciferase reporter gene assay, other methods such as PCR or branched DNA (bDNA)-based methods, or protein-based methods such as immunofluorescence assay, e.g., western blot or flow cytometry.

In another aspect, the present disclosure provides a method for reducing INHBE expression, comprising administering to a subject an effective amount or effective dose of the RNAi agent and/or the pharmaceutical composition of the present disclosure.

In another aspect, the present disclosure provides a method for treating and/or preventing a disease associated with INHBE gene expression in a subject, comprising administering to the subject an effective amount or effective dose of the RNAi agent and/or the pharmaceutical composition of the present disclosure. In some embodiments, the associated disease is a metabolic disorder. In some embodiments, the metabolic disorder is one or more selected from the group consisting of metabolic syndrome (MS), a cardiovascular disease, obesity, hepatitis, and a kidney disease.

In another aspect, the present disclosure provides use of the RNAi agent and/or the pharmaceutical composition of the present disclosure in the manufacture of a medicament for treating and/or preventing an associated disease. In some embodiments, the associated disease is a disease associated with INHBE gene expression. In some embodiments, the associated disease is a metabolic disorder. In some embodiments, the metabolic disorder is one or more selected from the group consisting of metabolic syndrome (MS), a cardiovascular disease, obesity, hepatitis, and a kidney disease.

In another aspect, the present disclosure provides use of the RNAi agent and/or the pharmaceutical composition of the present disclosure in the manufacture of a medicament for inhibiting INHBE expression.

In another aspect, the present disclosure discloses a method for delivering an RNAi agent inhibiting INHBE expression and/or replication *in vivo*, and the method comprises administering to a subject an effective amount or effective dose of the RNAi agent and/or the pharmaceutical composition of the present disclosure.

The RNAi agent or the pharmaceutical composition and the methods disclosed herein can reduce the level of a target mRNA in a cell, a cell population, a cell population, a

tissue, or a subject, comprising administering to the subject a therapeutically effective amount of the RNAi agent or the pharmaceutical composition described in the present disclosure. The RNAi agent is linked to the delivery group, thereby inhibiting the expression of the target mRNA in the subject.

In some embodiments, the subject has been identified as having pathological up-regulation of the target gene in the targeted cell or tissue prior to administration of the RNAi agent and/or the pharmaceutical composition of the present disclosure.

The subject described in the present disclosure refers to a subject having (or suspected to have or susceptible to) a disease or disorder that would benefit from reduction or inhibition of target mRNA expression.

The RNAi agent and/or the pharmaceutical composition of the present disclosure may be delivered by topical administration (e.g., direct injection, implantation, or topical administration), systemic administration, or through subcutaneous, intravenous, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal, and intrathecal), intramuscular, transdermal, airway (aerosol), nasal, oral, rectal, or topical (including buccal and sublingual) administration and any other suitable mode of administration commonly used in the art.

In an optional embodiment, the pharmaceutical composition provided by the present disclosure may be administered by injection, e.g., intravenous, intramuscular, intradermal, subcutaneous, intraduodenal, or intraperitoneal injection.

In an optional embodiment, after the targeting ligand is linked to the RNAi agent to form a conjugate, the RNAi agent in the form of the conjugate can be packaged in a kit.

In another aspect, the present disclosure provides a cell comprising the RNAi agent of the present disclosure. The cell cannot develop into an animal or plant individual.

In another aspect, the present disclosure provides a kit comprising the RNAi agent and/or the pharmaceutical composition of the present disclosure. In an optional embodiment, after the delivery group is linked to the RNAi agent to form a conjugate, the conjugate can be packaged in a kit.

The present disclosure further provides a method for silencing an mRNA of a target gene in a cell, and the method comprises a step of introducing into the cell the RNAi agent and/or the pharmaceutical composition of the present disclosure.

The present disclosure further provides a method for silencing a target gene or mRNA of the target gene in a cell *in vivo* or *in vitro*, and the method comprises a step of introducing into the cell the RNAi agent and/or the pharmaceutical composition of the present disclosure.

The present disclosure further provides a method for inhibiting the expression of a target gene or mRNA of the target gene, and the method comprises administering to a subject in need thereof an effective amount or effective dose of the RNAi agent and/or the pharmaceutical composition according to the present disclosure.

In some embodiments, the effective amount or effective dose of the RNAi agent and/or

the pharmaceutical composition is about 0.001 mg/kg body weight to about 200 mg/kg body weight, about 0.01 mg/kg body weight to about 100 mg/kg body weight, or about 0.5 mg/kg body weight to about 50 mg/kg body weight.

In some embodiments, the target gene is the INHBE gene, and the target mRNA is an mRNA expressed by the target gene.

The present disclosure further provides a method for preparing an RNAi agent, comprising: synthesizing the RNAi agent described in the present disclosure.

The pharmaceutically acceptable salts of the compounds described in the present disclosure are selected from the group consisting of inorganic salts and organic salts. The compounds of the present disclosure can react with acidic or basic substances to form corresponding salts. In the context of the present disclosure, the compound includes the RNAi agent of the present disclosure. In some embodiments, the pharmaceutically acceptable salt is a sodium salt.

In another aspect, where the configuration is not specified, the compounds of the present disclosure may have particular geometric or stereoisomeric forms. The present disclosure contemplates all such compounds, including cis and trans isomers, (-)- and (+)-enantiomers, (R)- and (S)-enantiomers, diastereomers, (D)-isomer, (L)-isomer, and racemic mixtures and other mixtures thereof, such as enantiomerically or diastereomerically enriched mixtures, all of which fall within the scope of the present disclosure. Additional asymmetric carbon atoms may be present in substituents such as an alkyl group. All such isomers and mixtures thereof are included within the scope of the present disclosure.

In addition, when the configuration is not specified, the compounds and intermediates of the present disclosure can also be present in different tautomeric forms, and all such forms fall within the scope of the present disclosure. The term “tautomer” or “tautomeric form” refers to structural isomers of different energies that can interconvert via a low-energy barrier.

The compounds of the present disclosure may be asymmetric, for example, having one or more stereoisomers. Unless otherwise specified, all stereoisomers are included, for example, enantiomers and diastereomers. The compounds of the present disclosure containing asymmetric carbon atoms may be separated in an optically active pure form or in a racemic form. The optically active pure form may be isolated from a racemic mixture or synthesized using chiral starting materials or chiral reagents.

Optically active (R)- and (S)-isomers, and D- and L-isomers may be prepared by chiral synthesis, chiral reagents, or other conventional techniques. If one enantiomer of a certain compound of the present disclosure is desired, it may be prepared by asymmetric synthesis or derivatization with a chiral auxiliary, wherein the resulting mixture of diastereomers is separated, and the auxiliary group is cleaved to provide the pure desired enantiomer. Alternatively, when the molecule contains a basic functional group (e.g., amino) or an acidic functional group (e.g., carboxyl), salts of diastereomers are

formed with an appropriate optically active acid or base, diastereomeric resolution is then performed by conventional methods well-known in the art, and pure enantiomers are then recovered. In addition, the separation of enantiomers and diastereomers is generally accomplished by chromatography using a chiral stationary phase, optionally in combination with chemical derivatization (e.g., carbamate formation from amines).

The present disclosure also includes some isotopically labeled compounds of the present disclosure that are identical to those recited herein but have one or more atoms replaced with an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into the compounds of the present disclosure include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, iodine, and chlorine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, ^{123}I, ^{125}I, and ^{36}Cl.

Where the configurations are not specified, in the chemical structures of the present disclosure, the bond "" indicates an unspecified configuration; that is, if chiral isomers exist in the chemical structures, the bond "" may be "" or "", or includes both the configurations "" and "" simultaneously. Although all of the structural formulas of the present disclosure are drawn as certain isomeric forms for the sake of simplicity, the present disclosure may include all isomers, such as tautomers, rotamers, geometric isomers, diastereomers, racemates, and enantiomers. In the chemical structures of the compounds of the present disclosure, the bond "" does not specify a configuration; that is, the configuration of the bond "" may be an E configuration or a Z configuration, or includes both the E configuration and the Z configuration simultaneously.

WO2023138663A1, WO2022028462A1, WO2023274395A1, WO2023274395A, and WO2024179573A1 are incorporated in the present disclosure by reference in their entirety.

Terms and Definitions

In order to facilitate the understanding of the present disclosure, some technical and scientific terms are specifically defined below. Unless otherwise specifically defined herein, all other technical and scientific terms used herein have the meanings generally understood by those of ordinary skill in the art to which the present disclosure pertains.

As used herein, "RNAi agent" (also referred to as "RNAi trigger agent") comprises an RNA or RNA-like (e.g., chemically modified RNA) oligonucleotide molecule that is capable of degrading or inhibiting (e.g., degrading or inhibiting under appropriate conditions) translation of a messenger RNA (mRNA) transcript of a target mRNA in a sequence-specific manner. The RNAi agent used herein may act through an RNA interference mechanism (i.e., inducing RNA interference through interaction with the RNA interference pathway machinery (RNA-induced silencing complex or RISC) of mammalian cells) or through any alternative mechanism or pathway. Although the term

RNAi agent as used herein is believed to act primarily through an RNA interference mechanism, the disclosed RNAi agent is not bound or limited by any particular pathway or mechanism of action. The RNAi agent disclosed herein consists of a sense strand and an antisense strand, and includes, but is not limited to, short (or small) interfering RNAs (siRNAs). The antisense strand of the RNAi agent described herein is at least partially complementary to the targeted mRNA. The RNAi agent may comprise one or more modified nucleotides and/or one or more non-phosphodiester linkages.

Unless otherwise specified, in the context of the present disclosure, the terms "inhibin βE" and "INHBE" are used interchangeably in the present disclosure. INHBE includes, but is not limited to, human INHBE, cynomolgus monkey INHBE, mouse INHBE, and rat INHBE, and their amino acids, complete coding sequences, and mRNA sequences are readily accessible using publicly available databases, e.g., GenBank, UniProt, OMIM, and the Macaca genome project website.

The term "INHBE" also refers to naturally occurring DNA sequence variations of the INHBE gene, such as a single nucleotide polymorphism (SNP) in the INHBE gene. Exemplary SNPs may be found in the dbSNP database.

The term "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of INHBE, including an mRNA that is a product of RNA processing of a primary transcription product. The targeted portion in the target sequence should be long enough to serve as a substrate for iRNA-directed cleavage. In one embodiment, the target sequence is within the protein-coding region of INHBE. As used herein, in the context of RNA-mediated gene silencing, the sense strand (also referred to as SS or SS strand) refers to a strand comprising a sequence identical or substantially identical to a target mRNA sequence; the antisense strand (also referred to as AS or AS strand) refers to a strand comprising a sequence complementary or substantially complementary to a target mRNA sequence.

In the context describing the sense strand of the RNAi agent described herein, the term "a sequence of at least 15 contiguous nucleotides differing from the nucleotide sequence set forth in any one of SEQ ID NO: 1 to SEQ ID NO: 9 by no more than 3 nucleotides" is intended to mean that the sense strand of the RNAi agent described herein comprises at least 15 contiguous nucleotides of the nucleotide sequence set forth in any one of SEQ ID NO: 1 to SEQ ID NO: 9, or a sequence differing from the sequence of at least 15 contiguous nucleotides of the nucleotide sequence set forth in any one of SEQ ID NO: 1 to SEQ ID NO: 9 by no more than 3 nucleotides (optionally by no more than 2 nucleotides; optionally by one nucleotide). Other similar descriptions in the context of the present disclosure should also be understood similarly. The term "differing" described herein does not include the case of nucleotides containing different modifications; that is, nucleotides containing identical bases but different modifications do not belong to the nucleotides with the difference of the present disclosure. Other similar descriptions of the sense strand and/or the antisense strand in the context of the

present disclosure should also be understood in the same way.

In the present disclosure, the “5' region”, “5' end”, and “5' terminus” of the sense or antisense strand are used interchangeably. For example, the nucleotides at positions 2 to 8 of the 5' region of the antisense strand may also be replaced with the nucleotides at positions 2 to 8 of the 5' end of the antisense strand. Likewise, the “3' region”, “3' terminus”, and “3' end” of the sense or antisense strand are also used interchangeably.

Unless otherwise specified, in the context of the present disclosure, “G”, “C”, “A”, “T”, and “U” represent nucleotides, and comprise the bases of guanine, cytosine, adenine, thymidine, and uracil, respectively. It is well-known to those skilled in the art that the mutual replacement between bases T and U does not significantly affect the properties of the RNAi agent sequence. In the sequences of the present disclosure, U can be arbitrarily replaced with T, and the sequences after the replacement also fall within the protection scope of the present disclosure. In the sequences of the present disclosure, for the same nucleic acid strand, the direction from the 5' end to the 3' end is a direction from the left to the right; the lowercase letter m means that the nucleoside adjacent to the letter m on the left side is a 2'-methoxy-modified nucleoside; the lowercase letter f means that the nucleoside adjacent to the letter f on the left side is a 2'-fluoro-modified nucleoside; the lowercase letter s means that the two nucleosides adjacent to the letter s are linked by a phosphorothioate diester group. Unless otherwise specified, two nucleosides are linked by a phosphodiester group; hmpNA means that a nucleoside in parentheses adjacent thereto on the right side is a nucleoside having an hmpNA modification; IB represents a reverse abasic nucleoside. Unless otherwise specified, the “RNAi agent”, “nucleotide”, “compound”, “chemical modification”, “oligonucleotide”, “double-stranded RNAi inhibitor molecule”, “siRNA”, “dsRNA”, “nucleic acid”, and “RNAi” of the present disclosure can each independently be present in the form of a salt or mixed salt or in a non-salt form (e.g., a free acid or free base). When present in the form of a salt or mixed salt, it may be a pharmaceutically acceptable salt.

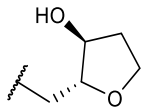
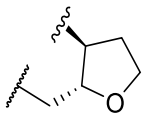
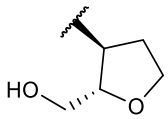
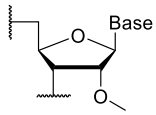
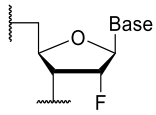
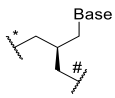
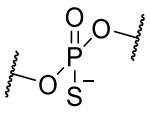
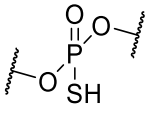
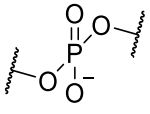
The term “pharmaceutically acceptable salt” includes pharmaceutically acceptable acid addition salts and pharmaceutically acceptable base addition salts. When it is present in the form of a salt, part of the groups may be ionized to form anions/cations; for example, phosphodiester groups and phosphorothioate diester groups may be present in the form of anions. Unless otherwise specified, the structures in the form of a salt corresponding to the following structures also fall within the protection scope of the present disclosure.

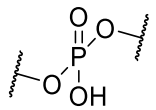
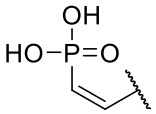
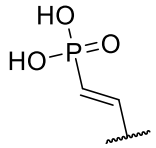
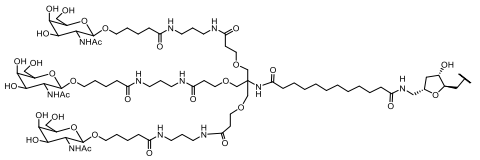
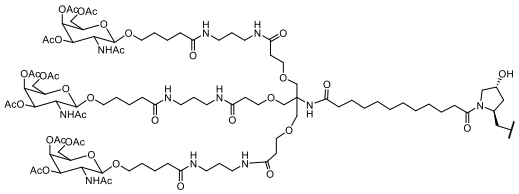
Unless otherwise specified, the 3' position of the first nucleotide of the 3' end of each strand is hydroxy; the 5' position of the first nucleotide of the 5' end of each strand is hydroxy.

The modification and linking groups described above separately have the structures shown in Table 1 below, where Base represents a base at the corresponding position:

Table 1

Structure	Name

 bond towards 3' end of oligonucleotide ;	Reverse abasic nucleoside when located at the 5' end of the sequence
bond towards 5' end of oligonucleotide  bond towards 3' end of oligonucleotide	Reverse abasic nucleoside when located in the middle of the sequence
bond towards 5' end of oligonucleotide 	Reverse abasic nucleoside when located at the 3' end of the sequence
	2'-Methoxy-modified nucleoside
	2'-Fluoro-modified nucleoside
 In the direction from the 5' end to the 3' end of the sequence, "*" represents a site where the nucleoside is linked to a nucleotide adjacent to its 5' end, and "#" represents a site where the nucleoside is linked to a nucleotide adjacent to its 3' end	hmpNA-modified nucleoside
	Anion form of phosphorothioate diester group
	Phosphorothioate diester group
	Anion form of phosphodiester group

	Phosphodiester group
	*Z*-Vinylphosphoric acid group
	*E*-Vinylphosphoric acid group
	-NAG0052’
	-L96’

As used herein, the terms “complementary” and “reverse complementary” are used interchangeably and have the meaning well-known to those skilled in the art; that is, in a double-stranded nucleic acid molecule, the bases of one strand are paired with the bases of the other strand in a complementary manner. In DNA, the purine base adenine is always paired with the pyrimidine base thymine (or uracil in RNA), and the purine base guanine is always paired with the pyrimidine base cytosine. Each base pair comprises a purine and a pyrimidine. When adenines of one strand are always paired with thymines (or uracils) of another strand and guanines are always paired with cytosines, the two strands are considered complementary to each other, and the sequences of the strands can be deduced from the sequences of their complementary strands. Accordingly, “mismatch” in the art means that in a double-stranded nucleic acid, the bases at the corresponding positions are not paired in a complementary manner. As used herein, the term “inhibit” is used interchangeably with “decrease”, “silence”, “down-regulate”, “repress”, and other similar terms, and includes any level of inhibition. Inhibition can be assessed in terms of a decrease in the absolute or relative level of one or more of these variables relative to a control level. The control level can be any type of control level used in the art, such as a pre-dose baseline level or a level determined from an untreated or control (e.g., buffer-only control or inert agent control) treated subject, cell, or sample. For example, the remaining mRNA expression level can be used to characterize the degree of inhibition of target gene expression by the RNAi

agent; for example, the remaining expression level of mRNA is not greater than 99%, not greater than 95%, not greater than 90%, not greater than 85%, not greater than 80%, not greater than 75%, not greater than 70%, not greater than 65%, not greater than 60%, not greater than 55%, not greater than 50%, not greater than 45%, not greater than 40%, not greater than 35%, not greater than 30%, not greater than 25%, not greater than 20%, not greater than 15%, or not greater than 10%. The inhibition rate of target gene expression can be measured using Dual-Glo® Luciferase Assay System: the Firefly chemiluminescence value (Fir) and the Renilla chemiluminescence value (Ren) are each read, and the relative value Ratio = Ren/Fir is calculated; in the present disclosure, the ratio of the remaining mRNA expression level (or residual activity%) = Ratio (RNAi agent-treated group)/Ratio (RNAi agent-free control group), and the inhibition rate (%) = 100% - remaining mRNA expression level (%).

The term "pharmaceutically acceptable salt" includes pharmaceutically acceptable acid addition salts and pharmaceutically acceptable base addition salts.

"Pharmaceutically acceptable acid addition salt" refers to salts that are capable of retaining the biological effectiveness of free bases without having any undesirable effects and that are formed with inorganic or organic acids. Inorganic acid salts include, but are not limited to, hydrochlorides, hydrobromides, sulfates, nitrates, phosphates, etc.; organic acid salts include, but are not limited to, formates, acetates, 2,2-dichloroacetates, trifluoroacetates, propionates, caproates, caprylates, caprates, undecenates, glycolates, gluconates, lactates, sebacates, adipates, glutarates, malonates, oxalates, maleates, succinates, fumarates, tartrates, citrates, palmitates, stearates, oleates, cinnamates, laurates, malates, glutamates, pyroglutamates, aspartates, benzoates, mesylates, benzenesulfonates, p-toluenesulfonates, alginates, ascorbates, salicylates, 4-aminosalicylates, napadisylates, etc. These salts can be prepared using methods known in the art.

"Pharmaceutically acceptable base addition salt" refers to salts that are capable of retaining the biological effectiveness of free acids without having any undesirable effects and that are formed with inorganic bases or organic bases. Salts derived from inorganic bases include, but are not limited to, sodium salts, potassium salts, lithium salts, ammonium salts, calcium salts, magnesium salts, iron salts, zinc salts, copper salts, manganese salts, aluminum salts, etc. The inorganic salts in some embodiments are ammonium salts, sodium salts, potassium salts, calcium salts, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of the following: primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, diethanolamine, triethanolamine, dimethylethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, choline, betaine, ethylenediamine, glucosamine,

methylglucamine, theobromine, purine, piperazine, piperidine, N-ethylpiperidine, polyamine resins, etc. The organic bases in some embodiments include isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline, and caffeine. These salts can be prepared using methods known in the art.

"Effective amount" or "effective dose" refers to the amount of an RNAi agent, a compound, or a pharmaceutical composition necessary to obtain any one or more beneficial or desired therapeutic results. For prophylactic use, the beneficial or desired results include elimination or reduction of risk, reduction of severity, or delay of the onset of a disorder, including the biochemistry, histology, and/or behavioral symptoms of the disorder, complications thereof, and intermediate pathological phenotypes that appear during the progression of the disorder. For therapeutic applications, the beneficial or desired results include clinical results, such as reducing the incidence of various disorders related to the target gene, target mRNA, or target protein of the present disclosure or ameliorating one or more symptoms of the disorders, reducing the dose of other agents required to treat the disorders, enhancing the therapeutic effect of another agent, and/or delaying the progression of disorders related to the target gene, target mRNA, or target protein of the present disclosure in patients.

As used herein, "patient", "subject", and "individual" are used interchangeably and include human or non-human animals, e.g., mammals, e.g., humans or monkeys.

The RNAi agent provided by the present disclosure can be obtained using a preparation method conventional in the art (e.g., solid-phase synthesis and liquid-phase synthesis). Solid-phase synthesis has been commercially available as a customization service. A modified nucleotide group can be introduced into the RNAi agent described in the present disclosure using a nucleoside monomer with a corresponding modification. Methods of preparing a nucleoside monomer with a corresponding modification and introducing a modified nucleotide group into an RNAi agent are also well known to those skilled in the art.

The term "chemical modification" or "modification" includes all changes made to a nucleotide by chemical means or enzymatic activity means, such as the addition or removal of a chemical moiety, or the substitution of one chemical moiety for another.

The term "base" encompasses any known DNA and RNA bases and base analogs such as purines or pyrimidines, and also includes the natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs.

The terms "blunt" and "blunt-ended" are used interchangeably and mean that there are no unpaired nucleotides or nucleotide analogs at a given end of an RNAi agent, i.e., no nucleotide overhangs. In most cases, an RNAi agent whose ends are both blunt-ended will be double-stranded over its entire length.

The terms "about" and "approximately" mean that a numerical value is within an acceptable margin of error for the specific value determined by those of ordinary skill in the art, and the numerical value depends in part on how the value is measured or

determined (i.e., the limitations of the measurement system). For example, “about” may mean a standard deviation within 1 or greater than 1. Alternatively, “about” or “substantially comprise” may mean a range of at most 20%, e.g., a change of between 1% and 15%, between 1% and 10%, between 1% and 5%, between 0.5% and 5%, or between 0.5% and 1%. In the present disclosure, every instance where a number or numerical range is preceded by the term “about” also includes an embodiment of the given number. Unless otherwise specified, when a specific value is provided in the present application and claims, the meaning of “about” or “substantially comprise” should be assumed to be within an acceptable margin of error for that specific value.

Unless otherwise specified, “optionally” or “optional” means that the event or circumstance subsequently described may, but does not necessarily, occur, and this description includes instances where the event or circumstance occurs or does not occur. For example, “optionally, R1 and R2 are directly linked to form a ring” means that R1 and R2 being directly linked to form a ring may occur, but does not necessarily exist, and this description includes an instance where R1 and R2 are directly linked to form a ring and an instance where R1 and R2 do not form a ring.

In the chemical structural formulas of the present disclosure, “” or  or  may be linked to any group or groups in accordance with the scope of the invention described herein.

The term “link”, when referring to a relationship between two molecules, means that the two molecules are linked by a covalent bond or that the two molecules are associated via a non-covalent bond (e.g., a hydrogen bond or an ionic bond), and includes direct linkage and indirect linkage.

The term “directly linked” means that a first compound or group is linked to a second compound or group without any atom or group of atoms interposed between.

The term “indirectly linked” means that a first compound or group is linked to a second compound or group by an intermediate group, a compound, or a molecule (e.g., a linking group).

The term “substituted” means that any one or more hydrogen atoms on the specified atom (usually a carbon, oxygen, or nitrogen atom) are replaced with any group as defined herein, provided that the normal valency of the specified atom is not exceeded and that the substitution results in a stable compound. Non-limiting examples of substituents include C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, cyano, hydroxy, oxo, carboxyl, cycloalkyl, cycloalkenyl, heterocyclyl, heteroaryl, aryl, ketone, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, or halogen (e.g., F, Cl, Br, or I). When the substituent is a ketone or oxo (i.e., =O), two (2) hydrogens on the atom are replaced.

“Substituted with one or more...” refers to substitution with a single substituent or multiple substituents. In the case of substitution with multiple substituents, there may be a plurality of identical substituents, or one or a group of a plurality of different

substituents.

DETAILED DESCRIPTION

The present disclosure is further described below with reference to examples, but these examples are not intended to limit the scope of the present disclosure. In the examples of the present disclosure, experimental methods without specific conditions indicated were generally conducted under conventional conditions, such as those outlined in Antibodies: A Laboratory Manual and Molecular Cloning: A Laboratory Manual by Cold Spring Harbor Laboratory, or under conditions recommended by the manufacturers of the starting materials or commercial products. Where the specific source of a reagent is not indicated, the reagent may be obtained from any supplier of molecular biology reagents at a quality/purity level for molecular biology applications. Unless otherwise specified, all reagents used in the following examples are commercially available.

Example 1. Design of INHEBE RNAi Agents

With the human **INHBE** gene (NM_031479.5) as a target gene, the RNAi agents of the present disclosure were designed in accordance with the general rules for active siRNAs. The sequences of unmodified sense and antisense strands are shown in Table 2a, and the sequences of sense and antisense strands modified with 2'-fluoro, 2'-methoxy, etc. are detailed in Table 2b.

Table 2a. Unmodified sense and antisense strands

Double strand No.	SEQ ID NO:	SS strand (5'-3')	SEQ ID NO:	AS strand (5'-3')
TJR102661	1	UCAAAGCUAUUUUCAUAAU	10	AUUAUGAAAAUAGCUUUGACU
TJR102826	2	UAAUGGCAAUGUGGUCAAA	11	UUUGACCACAUUGCCAUUAUG
TJR102831	3	UCAUCAGCUUUGCUACUGU	12	ACAGUAGCAAAGCUGAUGACC
TJR102835	4	AGACCAAGAUGAAGUUUCA	13	UGAAACUUCAUCUUGGUCUCU
TJR102668	5	UUAAUAAAAAGGAGAAAGA	14	UCUUUCUCCUUUUUAUUAAGA
TJR102673	6	AAAAGGAGAAAGAAAAUCA	15	UGAUUUUCUUUCUCCUUUUUA

TJR102664	7	UAAUAAUACUAACAUGUUA	16	UAACAUGUUAGUAUUAUUAUG
TJR102667	8	CAUAAUAAUACUAACAUGUUA	17	UAACAUGUUAGUAUUAUUAUGAA
TJR102676	9	UAAAAAGGAGAAAGAAAAU	18	AUUUUCUUUCUCCUUUUUAUU

Table 2b. The sequences of the RNAi agents targeting the INHBE gene

Double strand No.	SEQ ID NO	SS strand (5'-3')	SEQ ID NO:	AS strand (5'-3')
TJR103001	19	IBsUmsCmAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmUmsIB	74	AmsUfsUmAmUmGfAmAmAmAmUmAfGmCfUmUfUmGmAmsCmsUm
TJR103003	20	IBsUmsCmAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmUmsIB	75	AmsUfsUmAfUmGfAmAmAmAfUmAfGmCfUmUfUmGfAmsCmsUm
TJR103004	21	IBsUmsCmAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmUmsIB	76	AmsUfsUmAfUmGfhmpNA(A)AmAmAfUmAfGmCfUmUfUmGfAmsCmsUm
TJR103005	22	IBsUmsAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB	77	UmsAfsAmCmAmUfGmUmUmAmGmUfAmUfUmAfUmUmAmsUmsGm
TJR103007	23	IBsUmsAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB	78	UmsAfsAmCfAmUfGmUmUmAfGmUfAmUfUmAfUmUfAmsUmsGm
TJR103008	24	IBsUmsAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB	79	UmsAfsAmCfAmUfhmpNA(G)UmUmAfGmUfAmUfUmAfUmUfAmsUmsGm
TJR103009	25	IBsUmsUmAmAmUmAmAfAfAfAmGmGmAmGmAmAmAmGmAmsIB	80	UmsCfsUmUmUmCfUmCmCmUmUmUfUmUfAmUfUmAmAmsGmsAm
TJR103011	26	IBsUmsUmAmAmUmAmAfAfAfAmGmGmAmGmAmAmAmGmAmsIB	81	UmsCfsUmUfUmCfUmCmCmUfUmUfUmUfAmUfUmAfAmsGmsAm

TJR103012	27	IBsUmsUmAmAmUmAmAfAfAfAmGmGmAmGmAmAmAmGmAmsIB	82	UmsCfsUmUfUmCfhmpNA(U)CmCmUfUmUfUmUfAmUfUmAfAmsGmsAm
TJR103013	28	IBsAmsAmAmAmGmGmAfGfAfAmAmGmAmAmAmAmAmUmCmsAmsIB	83	UmsGfsAmUmUmUfUmCmUmUmUmCfUmCfCmUfUmUmUmsUmsAm
TJR103015	29	IBsAmsAmAmAmGmGmAfGfAfAmAmGmAmAmAmAmAmUmCmsAmsIB	84	UmsGfsAmUfUmUfUmCmUmUfUmCfUmCfCmUfUmUfUmsUmsAm
TJR103016	30	IBsAmsAmAmAmGmGmAfGfAfAmAmGmAmAmAmAmAmUmCmsAmsIB	85	UmsGfsAmUfUmUfhmpNA(U)CmUmUfUmCfUmCfCmUfUmUfUmsUmsAm
TJR103017	31	IBsUmsAmAmUmGmGmCfAfAfUmGmUmGmGmUmCmAmAmAmsIB	86	UmsUfsUmGmAmCfCmAmCmAmUmUfGmCfCmAfUmUmAmsUmsGm
TJR103019	32	IBsUmsAmAmUmGmGmCfAfAfUmGmUmGmGmUmCmAmAmAmsIB	87	UmsUfsUmGfAmCfCmAmCmAfUmUfGmCfCmAfUmUfAmsUmsGm
TJR103020	33	IBsUmsAmAmUmGmGmCfAfAfUmGmUmGmGmUmCmAmAmAmsIB	88	UmsUfsUmGfAmCfhmpNA(C)AmCmAfUmUfGmCfCmAfUmUfAmsUmsGm
TJR103021	34	IBsUmsCmAmUmCmAmGfCfUfUmUmGmCmUmAmCmUmGmsUmsIB	89	AmsCfsAmGmUmAfGmCmAmAmAmGfCmUfGmAfUmGmAmsCmsCm
TJR103023	35	IBsUmsCmAmUmCmAmGfCfUfUmUmGmCmUmAmCmUmGmsUmsIB	90	AmsCfsAmGfUmAfGmCmAmAfAmGfCmUfGmAfUmGfAmsCmsCm
TJR103024	36	IBsUmsCmAmUmCmAmGfCfUfUmUmGmCmUmAmCmUmGmsUmsIB	91	AmsCfsAmGfUmAfhmpNA(G)CmAmAfAmGfCmUfGmAfUmGfAmsCmsCm

TJR103025	37	IBsAmsGmAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmsAmsIB	92	UmsGfsAmAmAmCfUmUmCmAmUmCfUmUfGmGfUmCmUmsCmsUm
TJR103027	38	IBsAmsGmAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmsAmsIB	93	UmsGfsAmAfAmCfUmUmCmAfUmCfUmUfGmGfUmCfUmsCmsUm
TJR103028	39	IBsAmsGmAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmsAmsIB	94	UmsGfsAmAfAmCfhmpNA(U)UmCmAfUmCfUmUfGmGfUmCfUmsCmsUm
TJR103029	40	IBsCmsAmUmAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB	95	UmsAfsAmCmAmUfGmUmUmAmGmUfAmUfUmAfUmUmAmUmGmsAmsAm
TJR103031	41	IBsCmsAmUmAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB	96	UmsAfsAmCfAmUfGmUmUmAfGmUfAmUfUmAfUmUfAmUmGmsAmsAm
TJR103032	42	IBsCmsAmUmAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB	97	UmsAfsAmCfAmUfhmpNA(G)UmUmAfGmUfAmUfUmAfUmUfAmUmGmsAmsAm
TJR103033	43	IBsUmsAmAmAmAmAmGfGfAfGmAmAmAmGmAmAmAmAmUmsIB	98	AmsUfsUmUmUmCfUmUmUmCmUmCfCmUfUmUfUmUmAmsUmsUm
TJR103035	44	IBsUmsAmAmAmAmAmGfGfAfGmAmAmAmGmAmAmAmAmUmsIB	99	AmsUfsUmUfUmCfUmUmUmCfUmCfCmUfUmUfUmUfAmsUmsUm
TJR103036	45	IBsUmsAmAmAmAmAmGfGfAfGmAmAmAmGmAmAmAmAmUmsIB	100	AmsUfsUmUfUmCfhmpNA(U)UmUmCfUmCfCmUfUmUfUmUfAmsUmsUm
TJR102938	46	IBsUmsAmAmUmGmGmCfAfAfUmGmUmGmGmUmCmAmAmAmsIB	101	UmsUfsUmGmAmCfCmAmCmAmUmUfGmCfCmAfUmUmAmsUmsGm

		-NAG0052’		
TJR102939	47	IBsUmsCmAmUmCmAmGfCfUfUmUmGmCmUmAmCmUmGmUmsIB-NAG0052’	102	AmsCfsAmGmUmAfGmCmAmAmAmGfCmUfGmAfUmGmAmsCmsCm
TJR102940	48	IBsAmsGmAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmAmsIB-NAG0052’	103	UmsGfsAmAmAmCfUmUmCmAmUmCfUmUfGmGfUmCmUmsCmsUm
TJR102707	49	IBsUmsUmAmAmUmAmAfAfAfAmGmGmAmGmAmAmAmGmAmsIB-NAG0052’	104	UmsCfsUmUmUmCfUmCmCmUmUmUfUmUfAmUfUmAmAmsGmsAm
TJR102703	50	IBsAmsAmAmAmGmGmAfGfAfAmAmGmAmAmAmAmUmCmAmsIB-NAG0052’	105	UmsGfsAmUmUmUfUmCmUmUmUmCfUmCfCmUfUmUmUmsUmsAm
TJR102153	51	IBsUmsAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB-NAG0052’	106	UmsAfsAmCmAmUfGmUmUmAmGmUfAmUfUmAfUmUmAmsUmsGm
TJR102526	52	IBsUmsCmAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmUmsIB-NAG0052’	107	AmsUfsUmAmUmGfAmAmAmAmUmAfGmCfUmUfUmGmAmsCmsUm
TJR102941	53	IBsCmsAmUmAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB-NAG0052’	108	UmsAfsAmCmAmUfGmUmUmAmGmUfAmUfUmAfUmUmAmUmGmsAmsAm
TJR102706	54	IBsUmsAmAmAmAmAmGfGfAfGmAmAmAmGmAmAmAmAmUmsIB-NAG0052’	109	AmsUfsUmUmUmCfUmUmUmCmUmCfCmUfUmUfUmUmAmsUmsUm
TJR102942	55	IBsUmsAmAmUmGmGmCfAfAfUmGmUmGm	110	UmsUfsUmGfAmCfCmAmCmAfUmUfGmCfCmAfUmUfAmsUmsGm

		GmUmCmAmAmAmsIB-NAG0052’		
TJR102943	56	IBsUmsCmAmUmCmAmGfCfUfUmUmGmCmUmAmCmUmGmUmsIB-NAG0052’	111	AmsCfsAmGfUmAfGmCmAmAfAmGfCmUfGmAfUmGfAmsCmsCm
TJR102944	57	IBsAmsGmAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmAmsIB-NAG0052’	112	UmsGfsAmAfAmCfUmUmCmAfUmCfUmUfGmGfUmCfUmsCmsUm
TJR102945	58	IBsUmsUmAmAmUmAmAfAfAfAmGmGmAmGmAmAmAmGmAmsIB-NAG0052’	113	UmsCfsUmUfUmCfUmCmCmUfUmUfUmUfAmUfUmAfAmsGmsAm
TJR102701	59	IBsAmsAmAmAmGmGmAfGfAfAmAmGmAmAmAmAmUmCmAmsIB-NAG0052’	114	UmsGfsAmUfUmUfUmCmUmUfUmCfUmCfCmUfUmUfUmsUmsAm
TJR102314	60	IBsUmsAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB-NAG0052’	115	UmsAfsAmCfAmUfGmUmUmAfGmUfAmUfUmAfUmUfAmsUmsGm
TJR102316	61	IBsUmsCmAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmUmsIB-NAG0052’	116	AmsUfsUmAfUmGfAmAmAmAfUmAfGmCfUmUfUmGfAmsCmsUm
TJR102318	62	IBsCmsAmUmAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB-NAG0052’	117	UmsAfsAmCfAmUfGmUmUmAfGmUfAmUfUmAfUmUfAmUmGmsAmsAm
TJR102704	63	IBsUmsAmAmAmAmAmGfGfAfGmAmAmAmGmAmAmAmAmUmsIB-NAG0052’	118	AmsUfsUmUfUmCfUmUmUmCfUmCfCmUfUmUfUmUfAmsUmsUm

TJR102969	64	IBsUmsAmAmUmGmGmCfAfAfUmGmUmGmGmUmCmAmAmAmsIB-NAG0052’	119	UmsUfsUmGfAmCfhmpNA(C)AmCmAfUmUfGmCfCmAfUmUfAmsUmsGm
TJR102970	65	IBsUmsCmAmUmCmAmGfCfUfUmUmGmCmUmAmCmUmGmUmsIB-NAG0052’	120	AmsCfsAmGfUmAfhmpNA(G)CmAmAfAmGfCmUfGmAfUmGfAmsCmsCm
TJR102971	66	IBsAmsGmAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmAmsIB-NAG0052’	121	UmsGfsAmAfAmCfhmpNA(U)UmCmAfUmCfUmUfGmGfUmCfUmsCmsUm
TJR102972	67	IBsUmsUmAmAmUmAmAfAfAfAfAmGmGmAmGmAmAmAmGmAmsIB-NAG0052’	122	UmsCfsUmUfUmCfhmpNA(U)CmCmUfUmUfUmUfAmUfUmAfAmsGmsAm
TJR102702	68	IBsAmsAmAmAmGmGmAfGfAfAfAmAmGmAmAmAmAmUmCmAmsIB-NAG0052’	123	UmsGfsAmUfUmUfhmpNA(U)CmUmUfUmCfUmCfCmUfUmUfUmsUmsAm
TJR102315	69	IBsUmsAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB-NAG0052’	124	UmsAfsAmCfAmUfhmpNA(G)UmUmAfGmUfAmUfUmAfUmUfAmsUmsGm
TJR102317	70	IBsUmsCmAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmUmsIB-NAG0052’	125	AmsUfsUmAfUmGfhmpNA(A)AmAmAfUmAfGmCfUmUfUmGfAmsCmsUm
TJR102319	71	IBsCmsAmUmAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAmsIB-NAG0052’	126	UmsAfsAmCfAmUfhmpNA(G)UmUmAfGmUfAmUfUmAfUmUfAmUmGmsAmsAm
TJR102705	72	IBsUmsAmAmAmAmAmGfGfAfGmAmAmAmGmAmAmAmAmUmsIB-NAG0052’	127	AmsUfsUmUfUmCfhmpNA(U)UmUmCfUmCfCmUfUmUfUmUfAmsUmsUm

TJR102323	73	UmsCmsAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmsUm-NAG0052’	128	AmsUfsUmAfUmGfhmpNA(A)AmAmAfUmAfGmCfUmUfUmGfAmsCmsUm

Table 3. The sequences of the RNAi agents targeting the INHBE gene

Double strand No.	SEQ ID NO:	SS strand (5'-3')	SEQ ID NO:	AS strand (5'-3')
TJR100817	129	UmsCmsAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmUm	164	AmsUfsUmAmUmGfAmAmAmAmUmAfGmCfUmUfUmGmAmsCmsUm
TJR100820	130	CmsUmsAmUmUmUmUfCfAfUmAmAmUmAmAmUmAmCmUm	165	AmsGfsUmAmUmUfAmUmUmAmUmGfAmAfAmAfUmAmGmsCmsUm
TJR102322	131	UmsCmsAmAmAmGmCfUfAfUmUmUmUmCmAmUmAmAmsUm-NAG0052’	166	AmsUfsUmAfUmGfAmAmAmAfUmAfGmCfUmUfUmGfAmsCmsUm
TJR102928	132	UmsCmsAmAmAfGmCfUfAfUmUmUmUmCmAmUmAmAmUm	167	AmsUfsUmAmUmGfAmAmAmAmUmAmGmCfUmUfUmGmAmsCmsUm
TJR102930	133	GmsUmsCmCmAfCmAfAfAfGmUmCmAmAmAmGmCmUmAmUmUm	168	AmsAfsUmAmGmCfUmUmUmGmAmCmUmUfUmGfUmGmGmAmCmsAmsCm
TJR102665	134	AUACUAACAUGUUAUUUGA	169	UCAAAUAACAUGUUAGUAUUA
TJR102666	135	CUAACAUGUUAUUUGCCUUUU	170	AAAAGGCAAAUAACAUGUUAGUA
TJR100532	136	UmsAmsAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAm	171	UmsAfsAmCmAmUfGmUmUmAmGmUfAmUfUmAfUmUmAmsUmsGm
TJR100533	137	AmsUmsAmCmUmAmAfCfAfUmGmUmUmAmUmUmUmGmAm	172	UmsCfsAmAmAmUfAmAmCmAmUmGfUmUfAmGfUmAmUmsUmsAm

TJR101194	138	CmsAmsUmAmAmUmAmAmUfAfCfUmAmAmCmAmUmGmUmUmAm	173	UmsAfsAmCmAmUfGmUmUmAmGmUfAmUfUmAfUmUmAmUmGmsAmsAm
TJR102925	139	CmsAmsUmAmAfUmAfAfUfAmCmUmAmAmCmAmUmGmUmUmAm-L96’	174	UmsAfsAmCfAmUfGmUmUmAfGmUfAmUfUmAfUmUfAmUmGmsAmsAm
TJR102675	140	AAUAAAAAGGAGAAAGAAA	175	UUUCUUUCUCCUUUUUAUUAA
TJR100543	141	AmsAmsAmAmGmGmAfGfAfAmAmGmAmAmAmAmUmCmAm	176	UmsGfsAmUmUmUfUmCmUmUmUmCfUmCfCmUfUmUmUmsUmsAm
TJR102694	142	AmsAmsUmAmAmAmAfAfGfGmAmGmAmAmAmGmAmAmAm	177	UmsUfsUmCmUmUfUmCmUmCmCmUfUmUfUmUfAmUmUmsAmsAm
TJR102927	143	AmsAmsAmAmGfGmAfGfAfAmAGmAmAmAmAmUmCmAm-L96’	178	UmsGfsAmUfUmUfUmCmUmUfUmCfUmCfCmUfUmUfUmsUmsAm
TJR102690	144	AmsCmsUmUmUmCmUfUfAfAmUmAmAmAmAmAmGmGmAm	179	UmsCfsCmUmUmUfUmUmAmUmUmAfAmGfAmAfAmGmUmsAmsUm
TJR102691	145	UmsUmsUmCmUmUmAfAfUfAmAmAmAmAmGmGmAmGmAm	180	UmsCfsUmCmCmUfUmUmUmUmAmUfUmAfAmGfAmAmAmsGmsUm
TJR102692	146	UmsUmsCmUmUmAmAfUfAfAmAmAmAmGmGmAmGmAmAm	181	UmsUfsCmUmCmCfUmUmUmUmUmAfUmUfAmAfGmAmAmsAmsGm
TJR102926	147	UmsUmsAmAmUfAmAfAfAfAmGmGmAmGmAmAmAmGmAm-L96’	182	UmsCfsUmUfUmCfUmCmCmUfUmUfUmUfAmUfUmAfAmsGmsAm
TJR102827	148	UAAUGGCAAUGUGGUCAAG	183	CUUGACCACAUUGCCAUUA

TJR102829	149	UAAUGGCAAUGUGGUCAAA	184	UUUGACCACAUUGCCAUUA
TJR100497	150	UmsAmsAmUmGmGmCfAfAfUmGmUmGmGmUmCmAmAmAm	185	UmsUfsUmGmAmCfCmAmCmAmUmUfGmCfCmAfUmUmAmsUmsGm
TJR102843	151	UmsAmsAmUmGmGmCfAfAfUmGmUmGmGmUmCmAmAmGm	186	CmsUfsUmGmAmCfCmAmCmAmUmUfGmCfCmAfUmsUmsAm
TJR102845	152	UmsAmsAmUmGmGmCfAfAfUmGmUmGmGmUmCmAmAmAm	187	UmsUfsUmGmAmCfCmAmCmAmUmUfGmCfCmAfUmsUmsAm
TJR102836	153	AGACCAAGAUGAAGUUUCC	188	GGAAACUUCAUCUUGGUCU
TJR102838	154	AGACCAAGAUGAAGUUUCA	189	GGAAACUUCAUCUUGGUCU
TJR100500	155	AmsGmsAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmAm	190	UmsGfsAmAmAmCfUmUmCmAmUmCfUmUfGmGfUmCmUmsCmsUm
TJR102850	156	AmsGmsAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmCm	191	GmsGfsAmAmAmCfUmUmCmAmUmCfUmUfGmGfUmsCmsUm
TJR102852	157	AmsGmsAmCmCmAmAfGfAfUmGmAmAmGmUmUmUmCmAm	192	UmsGfsAmAmAmCfUmUmCmAmUmCfUmUfGmGfUmsCmsUm
TJR102832	158	GGUCAUCAGCUUUGCUACU	193	AGUAGCAAAGCUGAUGACC
TJR102833	159	UCAUCAGCUUUGCUACUGU	194	ACAGUAGCAAAGCUGAUGA
TJR102695	160	UmsAmsAmAmAmAmGfGfAfGmAmAmAmGmAmAmAmAmUm	195	AmsUfsUmUmUmCfUmUmUmCmUmCfCmUfUmUfUmUmAmsUmsUm
TJR102924	161	UmsAmsAmAmAfAmGfGfAfGmAmAmAmGmAmAmAmAmUm-L96’	196	AmsUfsUmUmUmCfUmUmUmCmUmCmCmUfUmUfUmUmAmsUmsUm

TJR102931	162	UmsAmsAmAmAfAmGfGfAfGmAmAmAmGmAmAmAmAmUm	197	AmsUfsUmUfUmCfUmUmUmCfUmCfCmUfUmUfUmUfAmsUmsUm
TJR102933	163	AmsAmsUmAmAfAmAfAfGfGmAmGmAmAmAmGmAmAmAm	198	UmsUfsUmCfUmUfUmCmUmCfCmUfUmUfUmUfAmUfUmsAmsAm
TJR102669	205	ACUUUCUUAAUAAAAAGGA	209	UCCUUUUUAUUAAGAAAGUAU
TJR102670	206	UUUCUUAAUAAAAAGGAGA	210	UCUCCUUUUUAUUAAGAAAGU
TJR102671	207	UUCUUAAUAAAAAGGAGAA	211	UUCUCCUUUUUAUUAAGAAAG
TJR100534	208	UmsUmsAmAmUmAmAfAfAfAmGmGmAmGmAmAmAmGmAm	212	UmsCfsUmUmUmCfUmCmCmUmUmUfUmUfAmUfUmAmAmsGmsAm

In Table 2a, Table 2b, and Table 3 described above, in the same nucleic acid sequence, G, C, A, and U represent nucleosides having guanine, cytosine, adenine, and uracil, respectively; in the direction from the 5' end to the 3' end (from left to right), the lowercase letter m means that the nucleoside adjacent to the letter m on the left side is a 2'-methoxy-modified nucleoside; the lowercase letter f means that the nucleoside adjacent to the letter f on the left side is a 2'-fluoro-modified nucleoside; the uppercase letter IB represents a reverse abasic nucleoside; hmpNA means that a nucleoside in parentheses adjacent thereto is a nucleoside having an hmpNA modification; IB represents a reverse abasic nucleoside; the lowercase letter s means that the two nucleosides adjacent to the letter s or a nucleoside linked thereto and the delivery group NAG0052' or L96' are linked by a phosphorothioate diester group, and unless otherwise specified, two adjacent nucleosides or the nucleoside linked thereto and the delivery group are linked by a phosphodiester group; unless otherwise specified, the 3' position of the first nucleotide of the 3' end of each strand is hydroxy; the 5' position of the first nucleotide of the 5' end of each strand is hydroxy.

The structures of the 2'-methoxy-modified nucleoside, the 2'-fluoro-modified nucleoside, the hmpNA-modified nucleoside, the phosphorothioate diester group, the phosphodiester group, NAG0052', and the reverse abasic nucleoside are shown in Table 1 above. When the RNAi agents of the present disclosure are present in the form of a salt, for example, in the form of a sodium salt, the structures of the salt forms corresponding to the structures in Table 1 also fall within the protection scope of the present disclosure; base in the table represents a base.

Example 2. Synthesis of RNAi Agents of Present Disclosure

The synthesis of RNAi agents of the present disclosure does not differ from the conventional phosphoramidite solid-phase synthesis method. The synthesis process is briefly described below: Unmodified nucleoside phosphoramidite monomers or nucleoside phosphoramidite monomers having modifications at corresponding positions in the sequence were linked one by one according to a synthesis program on a Dr. Oligo48 synthesizer (Biolytic), with a universal CPG support as a start. The nucleoside phosphoramidite monomers used in the examples of the present disclosure were purchased from Shanghai Hongene and Suzhou Genepharma. 5-Ethylthio-1H-tetrazole (ETT) was used as an activator (0.6 M in acetonitrile), a 0.22 M solution of PADS in acetonitrile and 3-methylpyridine (1:1 by volume) (Shanghai Lingjiang) was used as a sulfurizing agent, and an aqueous iodopyridine solution (Shanghai Lingjiang) was used as an oxidant.

After solid-phase synthesis was completed, the oligoribonucleotides were cleaved from the solid support by soaking in a solution of 28% ammonia water and ethanol (3:1) at 50 °C for 16 h. Centrifugation was then performed, and the supernatant was transferred to another centrifuge tube. After the supernatant was concentrated to dryness by evaporation, the residue was purified by C18 reversed-phase chromatography with 0.1 M TEAA and acetonitrile as the mobile phase. The target oligonucleotides were collected, then lyophilized, identified as the target products by LC-MS, and quantified by UV (260 nm). The single-stranded oligonucleotides obtained were complementarily paired in an equimolar ratio and annealed. The finally obtained double-stranded RNAi agents were dissolved in 1× PBS, and the concentration was adjusted to the concentration required for the experiment so that they were ready for use.

The RNAi agent of the present disclosure comprising NAG0052' was prepared according to the method described in the patent application WO2023138663A1. The RNAi agent of the present disclosure comprising the hmpNA-modified nucleotide was prepared according to the method described in the patent application WO2022028462A1. The RNAi agent comprising L96' was prepared according to the same method as described in the example of WO2014025805A1.

Example 3. psiCHECK Validation of On-Target Levels

The RNAi agents of the present disclosure were subjected to an *in vitro* molecular-level simulation of on-target activity screening in HEK293A cells using 9 concentration gradients.

The psi-CHECK plasmids used in this example were purchased from Sangon Biotech (Shanghai) Co., Ltd. Corresponding on-target sequences of the RNAi agents were constructed from the INHBE gene and inserted into the psiCHECK-2 plasmids, thereby obtaining the GSCM on-target plasmids. The plasmids contained the renilla luciferase

gene and the firefly luciferase gene. In the dual reporter gene system, the target sequence of the RNAi agents was inserted into the 3' UTR region of the renilla luciferase gene. The activity of the RNAi agents for the target sequence was reflected by measuring the renilla luciferase expression after calibration with firefly luciferase. Dual-Luciferase Reporter Assay System (Promega, E2940) was used for the measurement.

The HEK293A cells were cultured at 37 °C with 5% CO_2 in a DMEM high glucose culture medium containing 10% fetal bovine serum. 24 h prior to transfection, the HEK293A cells were seeded into a 96-well plate at a density of 8×10^3 cells per well, with each well containing 100 μL of the culture medium.

The cells were co-transfected with the RNAi agents and corresponding plasmids using Lipofectamine2000 (ThermoFisher, 11668019) according to the instructions, with 0.2 μL of Lipofectamine2000 used per well. The amount of plasmid for transfection was 20 ng per well. For on-target sequence plasmids, a total of 9 concentration points were set for the RNAi agents; the highest concentration point final concentration was 20 nM, and a 4-fold serial dilution was performed to obtain 20 nM, 5 nM, 1.25 nM, 0.3125 nM, 0.0781 nM, 0.0195 nM, 0.0049 nM, 0.0012 nM, and 0.0003 nM. 24 h after transfection, the on-target levels were determined using a Dual-Luciferase Reporter Assay System (Promega, E2940).

The experimental materials and instruments used in this example are detailed in Table 4 and Table 5. The dilution protocol used in this example is shown in Table 6. The experimental results are shown in Table 7. The results in Table 7 indicate that the RNAi agents of the present disclosure exhibited excellent pis-Check system on-target activity.

Table 4. Experimental consumables and reagents for psi-CHECK

Name	**Company**	**Cat. No./model**
HEK293A cells	Nanjing Cobioer	ATCC-Cobioer/CBP60202
Dual-Glo® Luciferase Assay System	Promega	E2940
Lipofectamine® 2000	Invitrogen	11668-019

Table 5. Experimental instruments for psi-CHECK

Experimental instrument		
Name	**Company**	**Cat. No./model**
Nanodrop	Thermo	Nanodrop One
Microplate reader	PerkinElmer	EnVision2105
Graphing software	/	Graph Prism 5

Table 6. Multi-concentration dilution protocol for samples

Final concentration of RNAi agent (nM)	Addition of water and RNAi agent
20.0000	4 μL of RNAi agent + 36 μL of H2O
5.0000	10 μL of RNAi agent + 30 μL of H2O
1.2500	10 μL of RNAi agent + 30 μL of H2O
0.3125	10 μL of RNAi agent + 30 μL of H2O
0.0781	10 μL of RNAi agent + 30 μL of H2O
0.0195	10 μL of RNAi agent + 30 μL of H2O
0.0049	10 μL of RNAi agent + 30 μL of H2O
0.0012	10 μL of RNAi agent + 30 μL of H2O
0.0003	10 μL of RNAi agent + 30 μL of H2O

Table 7. psi-CHECK on-target activity screening results

No.	Remaining percentage of target gene mRNA expression (GSCM) (mean, %)				
	20.0000 nM	5.0000 nM	1.2500 nM	0.3125 nM	0.0781 nM
TJR102314	11.61	10.22	9.99	12.38	22.71
TJR102316	9.98	10.84	9.78	9.56	15.32
TJR102317	7.95	7.38	8.18	10.00	17.64
TJR102318	9.18	8.42	8.70	12.62	28.12
TJR102323	6.09	6.62	7.87	9.36	20.97
TJR102526	8.66	8.97	9.86	9.18	12.24
TJR102153	8.98	6.99	6.59	7.64	15.52
No.	Remaining percentage of target gene mRNA expression (mean, %)				GSCM IC50 (nM)
	0.0195 nM	0.0049 nM	0.0012 nM	0.0003 nM	
TJR102314	50.80	84.75	96.36	98.11	0.0205
TJR102316	39.52	71.19	88.36	93.42	0.0122
TJR102317	43.06	75.01	92.03	102.38	0.0140
TJR102318	65.00	90.23	100.30	102.44	0.0323
TJR102323	45.75	74.43	96.80	98.24	0.0159

TJR102526	26.09	62.01	95.87	106.52	0.0072
TJR102153	38.36	71.33	91.95	91.50	0.0123

Example 4. Multi-Concentration-Point Inhibitory Activity Against INHBE in Primary Monkey Hepatocytes (PCHs)

24 h prior to transfection, PCH cells were seeded into a 96-well plate at about 30,000 cells/well, with each well containing 100 μL of the culture medium. The cells were transfected with the samples using Lipofectamine RNAi MAX (ThermoFisher, 13778150) following the instruction manual of the product, with the final concentration gradients of the samples for transfection being 20 nM, 4 nM, 0.8 nM, 0.16 nM, 0.032 nM, 0.0064 nM, and 0.00128 nM. After 24 h of treatment, total cell RNA extraction was performed using a high-throughput cell RNA extraction kit (FG0417-L/FG0418-XL, magnetic bead method), and RNA reverse transcription (Takara, RR037A) and real-time quantitative PCR (Thermo, 4444557) detection were then performed to determine the mRNA level of monkey INHBE. The mRNA level of monkey INHBE was corrected based on the GAPDH internal reference gene level. The instruments involved in this experiment are shown in Table 8.

Table 8. Experimental instruments

Experimental instrument		
Name	Company	Cat. No./model
Nanodrop	Thermo	Nanodrop One
qPCR system	ABI	7500 Fast
PCR	Life	Pro-Flex PCR system

For the real-time quantitative PCR detection, a probe Q-PCR detection experiment was used, and its primer information is shown in Table 9.

Table 9. Taqman primer information

Primer name	SEQ ID NO	Primer sequence
mkINHBE-F	199	GGTTGCATCTGACCAGTCGT
mkINHBE-R	200	GGCTGAAGTGGAGTCTGTGAC
mkINHBE-P	201	CCATTCCCTGGAGCCACACTCC
mkGAPDH-F	202	TCAAGATCGTCAGCAACGCC
mkGAPDH-R	203	ACAGTCTTCTGGGTGGCAGT
mkGAPDH-P	204	ACCAACTGCTTAGCACCCCTGGCCA

Result analysis method

After the Q-PCR detection experiment was complete, corresponding Ct values were acquired according to a threshold value automatically set by the system, and the expression of a certain gene was relatively quantified by comparing the Ct values: comparing Ct refers to calculating differences in gene expression according to the differences from the Ct value of the internal reference gene and is also referred to as 2-△△Ct, where △△Ct = [(Ct experimental group target gene - Ct experimental group internal reference) - (Ct control group target gene - Ct control group internal reference)].

Inhibition rate (%) = (1 - remaining level of target gene expression) × 100%.

The results are expressed relative to the remaining percentage of monkey INHBE mRNA expression in cells treated with the RNAi agents. The data in Table 10 demonstrate that the RNAi agents of the present disclosure exhibited high levels of on-target inhibitory activity against the INHBE gene in PCH cells.

Table 10. The inhibitory activity of RNAi agents against INHBE in PCH cells

Sample No.	Remaining percentage of target gene mRNA expression (PCH) (mean, %)							PCH IC_{50} (nM)
	20	4	0.8	0.16	0.032	0.0064	0.00128	
TJR102705	6.86	7.14	5.96	6.64	17.75	45.22	102.14	0.0055
TJR102701	24.47	19.61	14.13	14.76	18.78	37.88	64.06	0.0043
TJR102702	29.12	16.65	12.09	9.53	15.44	41.43	92.98	0.0062
TJR102703	26.88	19.54	17.09	11.80	12.79	30.60	80.14	0.0054
TJR102704	24.39	17.00	14.88	12.75	19.70	52.88	111.09	0.0068
TJR102706	8.26	11.47	7.04	5.83	10.19	34.87	79.06	0.0042
TJR102707	13.46	16.66	14.76	18.39	40.16	77.97	79.13	0.0244

Example 5. Single-Point Inhibitory Activity Against INHBE in Primary Monkey Hepatocytes (PCHs)

24 h prior to transfection, PCH cells were seeded into a 96-well plate at about 30,000 cells/well, with each well containing 100 μL of a culture medium. The cells were transfected with the samples using Lipofectamine RNAi MAX (ThermoFisher, 13778150) following the instruction manual of the product, with the gradient final concentration of the samples for transfection being 4 nM. After 24 h of treatment, total cell RNA extraction was performed using a high-throughput cell RNA extraction kit (FG0417-L/FG0418-XL, magnetic bead method), and RNA reverse transcription (Takara, RR037A) and real-time quantitative PCR (Thermo, 4444557) detection were then performed to determine the mRNA level of monkey INHBE. The mRNA level of

monkey INHBE was corrected based on the GAPDH internal reference gene level. The instruments, primers, calculation methods, and the like were referred to those in Example 4. The data in Table 11 demonstrate that the RNAi agents of the present disclosure exhibited high levels of on-target inhibitory activity against the INHBE gene in PCH cells.

Table 11. The inhibitory activity of RNAi agents against INHBE in PCH cells

Sample No.	Remaining INHBE mRNA in PCH cells (%)
TJR102938	16.75
TJR102939	15.66
TJR102940	14.84
TJR102942	17.39
TJR102943	23.96
TJR102944	21.57
TJR102969	22.37
TJR102970	18.38
TJR102971	22.69

Example 6: Efficacy Study of RNAi Agent in AAV-Constructed Hepatic hINHBE Obese (DIO) Mouse Model

DIO male mice (purchased from Jiangsu GemPharmatech Co., Ltd. and subsidiary corporations thereof) in this example were 4-5 weeks old and weighed 30-32 g.

After entering the animal facility, all mice were subjected to acclimation for 5 days and then switched to a diet containing 60% HFD. After 6 weeks of age, 15 mice were selected based on the body weight of the mice and injected intravenously with an empty vector (2 × 10E11 vg/mouse), and the remaining mice were injected intravenously with AAV-hINHBE (2 × 10E11 vg/mouse). A liver biopsy was performed 2 weeks after injection of the empty vector and AAV, and hINHBE expression was detected by qPCR. One week after liver biopsy, the mice were fasted for 5-6 h. Blood samples (80-100 μL) of all mice were collected and centrifuged to obtain plasma samples, which were subjected to 2-fold dilution and then tested for liver function (ALT and AST) and four blood lipid measurements (TC, TG, HDL-C, and LDL-C). Subsequently, the mice were grouped according to qPCR, liver function detection results, and body weight levels as grouping indexes, with 12 mice in each of group 1, group 2, and group 3, and 4 mice at each time point. The day of grouping was defined as D-1. The mice in group 1 were empty mice, and the mice in group 2 and group 3 were AAV mice.

The administration was started on D0. The mice in group 1 and group 2 were administered PBS, and the mice in group 3 were administered the RNAi agent

TJR102316 of the present disclosure. The administration dose was 3 mg/kg, the administration volume was 5 μL/g, the mode of administration was subcutaneous administration, and the frequency of administration was a single administration.

The efficacy period was 4 weeks. Four mice were selected from each group on D7, D14, and D28 as endpoints: (1) The mice were fasted for 5-6 h on the day of the endpoint, blood was collected from the mice, and part of the blood was centrifuged to obtain plasma, which was subjected to 2-fold dilution and then tested for liver function (ALT and AST) and four blood lipid measurements (TC, TG, HDL-C, and LDL-C); (2) liver tissues were collected from the mice and weighed, part of the liver tissues was snap frozen in liquid nitrogen and then subjected to WB (Flag) detection, and another part of the liver tissues was stored in RNALater for subsequent qPCR detection of hINHBE expression. In the context of the present disclosure, "hINHBE" refers to human INHBE. Table 12 shows the expression levels of hINHBE mRNA in the livers of different groups on D7, D14, and D28. The data in Table 12 demonstrate that the hINHBE-mRNA level was significantly reduced in the mice administered the RNAi agent TJR102316 of the present disclosure as compared to group 2, indicating that the RNAi agent of the present disclosure is capable of effectively inhibiting hINHBE-mRNA expression in the mice.

Table 12. hINHBE-mRNA levels in mice

(mean hINHBE mRNA expression level in the liver, normalized on the basis of group 2)

Group	Administration	D7	D14	D28
Group 1 (WT mice)	PBS	0	0	0
Group 2 (AAV mice)	PBS	1.03 ± 0.26	1.01 ± 0.08	1.03 ± 0.28
Group 3 (AAV mice)	TJR102316	0.37 ± 0.06**	0.43 ± 0.16***	0.41 ± 0.12**

For the t-test, * indicates $p < 0.05$ as compared to group 2, ** indicates $p < 0.01$ as compared to group 2, * indicates $p < 0.001$ as compared to group 2, and **** indicates $p < 0.0001$ as compared to group 2.**

Table 13 shows the WB (Flag) detection of tag protein expression levels in the livers of different groups on D7, D14, and D28. The data in Table 13 demonstrate that the tag protein level was significantly reduced in the mice administered the RNAi agent TJR102316 of the present disclosure as compared to group 2, indicating that the RNAi agent of the present disclosure is capable of effectively inhibiting INHBE gene expression in the mice.

Table 13

Group	Administration	D7	D14	D28

Group 1 (WT mice)	PBS	0.02 ± 0.02	0.04 ± 0.01	0.03 ± 0.01
Group 2 (AAV mice)	PBS	1.00 ± 0.20	1.00 ± 0.29	1.00 ± 0.47
Group 3 (AAV mice)	TJR102316	0.07 ± 0.03****	0.19 ± 0.17***	0.17 ± 0.08**

For the One-way ANOVA test, * indicates p < 0.05 as compared to group 2, ** indicates p < 0.01 as compared to group 2, * indicates p < 0.001 as compared to group 2, and **** indicates p < 0.0001 as compared to group 2.**

Example 7. Pharmacodynamic Evaluation of RNAi Agent in High-Fat-Fed hINHBE Mouse Obesity Model

The DIO-hINHBE transgenic homozygous male mice used in this example were purchased from Cyagen (Suzhou) Biosciences Co., Ltd.

Humanized hINHBE transgenic mice were used and fed with 60% HFD (Research Diet D12492) diet to construct an obese mouse (DIO) model. Before the experiment, the mice were randomly divided into 5 groups according to body weight and body fat ratio (Lean or Fat%). Group 1 was the control group, and the vehicle control group was administered normal saline. Group 2 to group 5 were administered various doses (0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg) of the RNAi agent TJR102316 of the present disclosure, respectively. The mode of administration for each group was subcutaneous administration, and the RNAi agent TJR102316 of the present disclosure was administered at a frequency of once every 3 weeks for an efficacy period of 6 weeks. The day of grouping was defined as D-1, and the administration period was from D0 to D42.

The test results are expressed as means. The comparison between two groups of samples was performed using the independent sample T-Test, the multiple-group univariate comparison was performed using the One-way ANOVA test, and the multiple-group multivariate comparison was performed using the Two-way ANOVA test. The data analysis software was Graphpad prism 9. **** indicates $p < 0.0001$; *** indicates $p < 0.001$; ** indicates $p < 0.01$; * indicates $p < 0.05$, and $p < 0.05$ indicates a significant difference.

The body weight change rate and the food intake change of mice in different groups are recorded in Table 14 and Table 15, respectively. In combination with the experimental results in Table 14 and Table 15, it can be seen that the RNAi agent TJR102316 of the present application not only exhibited a good weight loss effect at a low dose, but also exhibited no significant effect on the food intake of mice at each dose, indicating that the RNAi agent TJR102316 of the present disclosure can have a good weight loss effect while not affecting the food intake of mice.

Table 14. Body weight change rates of mice (compared to group 1)

Group	Administration dose	D15	D21	D30	D39
2	TJR102316 (0.3 mg/Kg)	-1.77%	-3.47%	-4.40%	-5.20%
3	TJR102316 (1 mg/Kg)	-4.14%	-5.16%	-6.54%	-7.26%
4	TJR102316 (3 mg/Kg)	-5.07%	-5.72%	-8.56%	-7.83%
5	TJR102316 (10 mg/Kg)	-8.76%	-9.50%	-13.90%	-13.91%

Table 15. Food intake changes of mice (g)

Group	Administration dose	D0	D15	D21	D30	D39
1	Normal saline	2.5	15.5	20.5	28.5	37.3
2	TJR102316 (0.3 mg/Kg)	2.4	15.2	20.4	28.3	36.4
3	TJR102316 (1 mg/Kg)	2.3	14.0	18.8	26.3	34.1
4	TJR102316 (3 mg/Kg)	2.6	14.5	19.7	26.9	36.2
5	TJR102316 (10 mg/Kg)	2.5	13.7	19.1	26.2	35.5

Table 16 shows the LDL-C level, mesenteric fat (MAT) mass, liver triglyceride level, and serum β-hydroxybutyrate level in mice on day 42.

From the results in Table 16, it can be seen that the RNAi agent TJR102316 of the present application could effectively reduce the LDL-C level, mesenteric fat mass, and liver triglyceride in serum.

In addition, β-hydroxybutyrate in the mice administered the RNAi agent TJR102316 of the present application exhibited an increasing trend, indicating that the RNAi agent of the present application can promote liver lipid β-oxidation and use β-hydroxybutyrate as energy supply for the body.

Table 16. LDL-C content, mesenteric fat mass, liver triglyceride level, and β-hydroxybutyrate content in serum of mice on D42

Group	Administration dose	LDL-C (mmol/L)	MAT (g)	Liver triglyceride (μmol/g)	β-Hydroxybutyrate (μmol/L)
1	Normal saline	3.25	1.30	260.16	387.4
2	TJR102316 (0.3 mg/Kg)	3.36	0.81****	243.21	559.8*
3	TJR102316 (1 mg/Kg)	3.14	0.75****	252.23	728.2****

4	TJR102316 (3 mg/Kg)	2.61	0.71****	223.33	691.4****
5	TJR10231 (10 mg/Kg)	2.25*	0.66****	161.16**	675.8****

Example 8. psiCHECK Validation of On-Target Levels

The RNAi agents in Table 3 were subjected to an *in vitro* molecular-level simulation of on-target activity screening in HEK293A cells using 9 concentration gradients.

The psi-CHECK plasmids used in this example were purchased from Sangon Biotech (Shanghai) Co., Ltd. Corresponding on-target sequences of the RNAi agents were constructed from the INHBE gene and inserted into the psiCHECK-2 plasmids, thereby obtaining the GSCM on-target plasmids. The plasmids contained the renilla luciferase gene and the firefly luciferase gene. In the dual reporter gene system, the target sequence of the RNAi agents was inserted into the 3' UTR region of the renilla luciferase gene. The activity of the siRNAs for the target sequence was reflected by measuring the renilla luciferase expression after calibration with firefly luciferase. Dual-Luciferase Reporter Assay System (Promega, E2940) was used for the measurement.

The HEK293A cells were cultured at 37 °C with 5% CO_2 in a DMEM high glucose culture medium containing 10% fetal bovine serum. 24 h prior to transfection, the HEK293A cells were seeded into a 96-well plate at a density of 8×10^3 cells per well, with each well containing 100 μL of the culture medium.

The cells were co-transfected with the siRNAs and the corresponding plasmids using Lipofectamine2000 (ThermoFisher, 11668019) according to the instructions. 0.2 μL of Lipofectamine2000 was used for each well. The amount of plasmid for transfection was 20 ng per well. For the on-target sequence plasmids, a total of 9 concentration points were set up for the siRNAs, with the final concentration of the highest concentration point being 20 nM. A 4-fold serial dilution was performed, resulting in 20 nM, 5 nM, 1.25 nM, 0.3125 nM, 0.0781 nM, 0.0195 nM, 0.0049 nM, 0.0012 nM, and 0.0003 nM. 24 h after transfection, the on-target levels were determined using a Dual-Luciferase Reporter Assay System (Promega, E2940).

The experimental materials and instruments used in this example are detailed in Table 17 and Table 18. The dilution protocol used in this example is shown in Table 19. The experimental results are shown in Table 20, Table 21, Table 22, and Table 23.

Table 17. Experimental consumables and reagents for psi-CHECK

Name	Company	Cat. No./model
HEK293A cells	Nanjing Cobioer	ATCC-Cobioer/CBP60202

Dual-Glo® Luciferase Assay System	Promega	E2940
Lipofectamine® 2000	Invitrogen	11668-019

Table 18. Experimental instruments for psi-CHECK

Experimental instrument		
Name	**Company**	**Cat. No./model**
Nanodrop	Thermo	Nanodrop One
Microplate reader	PerkinElmer	EnVision2105
Graphing software	/	Graph Prism 5

Table 19. Multi-concentration dilution protocol for samples

Final concentration of RNAi agent (nM)	Addition of water and RNAi agent
20.0000	4 μL of RNAi agent + 36 μL of H2O
5.0000	10 μL of RNAi agent + 30 μL of H2O
1.2500	10 μL of RNAi agent + 30 μL of H2O
0.3125	10 μL of RNAi agent + 30 μL of H2O
0.0781	10 μL of RNAi agent + 30 μL of H2O
0.0195	10 μL of RNAi agent + 30 μL of H2O
0.0049	10 μL of RNAi agent + 30 μL of H2O
0.0012	10 μL of RNAi agent + 30 μL of H2O
0.0003	10 μL of RNAi agent + 30 μL of H2O

Table 20. On-target activity screening results of RNAi agents

Double strand code	GSCM IC_{50} (nM)
TJR100817	0.0064
TJR100820	0.4806
TJR100533	0.0461
TJR100532	0.0120

Table 21. On-target activity screening results of RNAi agents

Double strand code	GSCM IC_{50} (nM)

TJR102664	0.0107
TJR102665	0.0391
TJR102666	0.0421
TJR102667	0.0093
TJR102668	0.0680
TJR102669	>20
TJR102670	>20
TJR102671	>20
TJR102673	0.0147
TJR102675	>20
TJR102661	0.0262

Table 22. On-target activity screening results of RNAi agents

No.	GSCM IC_{50} (nM)
TJR102826	0.0592
TJR102827	0.3732
TJR102829	0.0618
TJR102831	0.0128
TJR102832	0.1881
TJR102833	0.1401
TJR102835	0.0403
TJR102836	0.4017
TJR102838	0.1451

Table 23. On-target activity screening results of RNAi agents

No.	GSCM IC_{50} (nM)
TJR102928	0.0112
TJR102930	0.2464

TJR102931	0.0317
TJR102933	0.1220
TJR102322	0.0107
TJR100497	0.0291
TJR102843	0.2453
TJR102845	0.0339
TJR100500	0.0603
TJR102850	0.8513
TJR102852	0.1766

Example 9. psiCHECK Validation of On-Target Levels

RNAi agents were subjected to an *in vitro* molecular-level simulation of on-target activity screening in HEK293A cells using 2 concentration gradients. The experimental process, experimental materials and instruments used, dilution process, and result analysis method were the same as those in Example 8.

Table 24. Inhibitory effect of RNAi agents on INHBE

No.	Remaining percentage of target gene mRNA expression (GSCM) (mean, %)	
	0.31 nM	0.08 nM
TJR100817	10.04	12.91
TJR100820	51.21	85.92
TJR100532	9.52	19.98
TJR100533	21.03	62.60
TJR101194	10.58	22.15
TJR100534	20.99	40.91
TJR102690	105.91	111.34
TJR102691	49.22	78.22
TJR102692	58.21	78.49
TJR100543	12.73	28.61
TJR102694	43.19	71.41
TJR102695	15.91	25.01

TJR102924	17.68	38.54
TJR102925	25.81	59.61
TJR102926	29.65	57.18
TJR102927	17.68	38.54

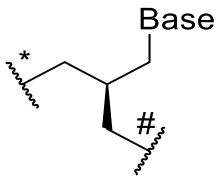
CLAIMS

1. An RNAi agent, comprising a sense strand and an antisense strand that form a double-stranded region, wherein
the sense strand comprises at least 15 contiguous nucleotides differing from the nucleotide sequence set forth in any one of SEQ ID NO: 1 to SEQ ID NO: 9 by no more than 3 nucleotides;
the antisense strand comprises at least 15 contiguous nucleotides differing from the nucleotide sequence set forth in any one of SEQ ID NO: 10 to SEQ ID NO: 18 by no more than 3 nucleotides,
wherein
the sense strand comprises at least one reverse abasic nucleoside; and/or
the antisense strand comprises at least one nucleoside with thermally destabilizing modification.

2. The RNAi agent according to claim 1, wherein:
the sense strand comprises two reverse abasic nucleosides, and the reverse abasic nucleosides are the first nucleoside of the 5' end and the first nucleoside of the 3' end of the sense strand.

3. The RNAi agent according to claim 1 or 2, wherein:
in the direction from the 5' end to the 3' end, each of the nucleotides at positions 2, 6, 12, 14, and 16 of the antisense strand is independently a 2'-fluoro-modified nucleotide, and the nucleotides at the remaining positions are non-2'-fluoro-modified nucleotides; or each of the nucleotides at positions 2, 4, 6, 10, 12, 14, 16, and 18 of the antisense strand is independently a 2'-fluoro-modified nucleotide, and the nucleotides at the remaining positions are non-2'-fluoro-modified nucleotides.

4. The RNAi agent according to claim 3, wherein:
the nucleotides at the remaining positions of the antisense strand are all 2'-methoxy-modified nucleotides; or
the nucleoside in the nucleotide at position 7 of the antisense strand is a nucleoside with thermally destabilizing modification, and the nucleotides at the remaining positions are 2'-methoxy-modified nucleotides;
preferably, the nucleoside with thermally destabilizing modification has a structure represented by formula (1):



formula (1);

wherein Base represents a base; in the direction from the 5' end to the 3' end of the sequence, "*" represents a site where the nucleoside is linked to a nucleotide adjacent to its 5' end, and "#" represents a site where the nucleoside is linked to a nucleotide adjacent to its 3' end.

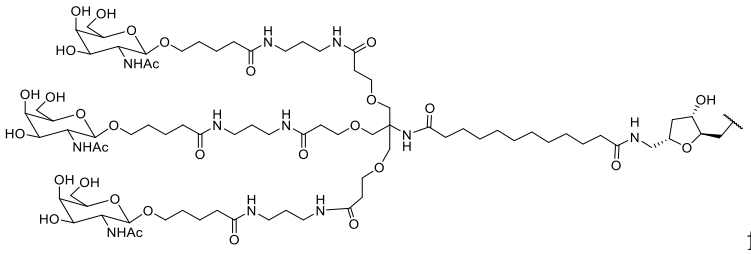
5. The RNAi agent according to any one of claims 1-4, wherein:
at least one phosphodiester group in the sense strand and/or the antisense strand is a phosphodiester group with a modification group;
preferably, the phosphodiester group with a modification group is a phosphorothioate diester group;
the phosphodiester group with a modification group is present in at least one position selected from the group consisting of the following positions:
between the 1st and 2nd nucleotides of the 5' end of the sense strand;
between the 2nd and 3rd nucleotides of the 5' end of the sense strand;
between the 1st and 2nd nucleotides of the 3' end of the sense strand;
between the 2nd and 3rd nucleotides of the 3' end of the sense strand;
between the 1st and 2nd nucleotides of the 5' end of the antisense strand;
between the 2nd and 3rd nucleotides of the 5' end of the antisense strand;
between the 1st and 2nd nucleotides of the 3' end of the antisense strand; and
between the 2nd and 3rd nucleotides of the 3' end of the antisense strand;
preferably, the sense strand and the antisense strand each independently comprise at least 2 phosphodiester groups with modification groups.

6. The RNAi agent according to any one of claims 1-5, wherein in the direction from the 5' end to the 3' end, the nucleotides at positions 7, 8, and 9 of the sense strand are 2'-fluoro-modified nucleotides, and the nucleotides at the remaining positions are non-2'-fluoro-modified nucleotides.

7. The RNAi agent according to any one of claims 1-6, wherein
the sense strand comprises the nucleotide sequence set forth in any one of SEQ ID NO: 19 to SEQ ID NO: 45;
the antisense strand comprises the nucleotide sequence set forth in any one of SEQ ID NO: 74 to SEQ ID NO: 100.

8. The RNAi agent according to any one of claims 1-7, wherein the RNAi agent further comprises one or more delivery groups, and the delivery group is linked to the sense strand and/or the antisense strand.

9. The RNAi agent according to claim 8, wherein the delivery group has a structure represented by formula (2):



formula (2).

10. The RNAi agent according to claim 9, wherein:
the sense strand comprises the nucleotide sequence set forth in any one of SEQ ID NO: 46 to SEQ ID NO: 73; and/or
the antisense strand comprises the nucleotide sequence set forth in any one of SEQ ID NO: 101 to SEQ ID NO: 128.

11. The RNAi agent according to any one of claims 1-10, wherein the RNAi agent is selected from the group consisting of TJR102314, TJR102316, TJR102317, TJR102318, TJR102323, TJR102526, TJR102153, TJR102705, TJR102701, TJR102702, TJR102703, TJR102704, TJR102706, TJR102707, TJR102938, TJR102939, TJR102940, TJR102942, TJR102943, TJR102944, TJR102969, TJR102970, and TJR102971.

12. A pharmaceutical composition, comprising:
the RNAi agent according to any one of claims 1-11, and
a pharmaceutically acceptable carrier.

13. A kit, comprising:
the RNAi agent according to any one of claims 1-11; and/or
the pharmaceutical composition according to claim 12.

14. A method for reducing inhibin βE (INHBE) expression, comprising administering to a subject an effective amount or effective dose of the RNAi agent according to any one of claims 1-11 and/or the pharmaceutical composition according to claim 12.

15. A method for treating and/or preventing a metabolic disorder in a subject, comprising administering to the subject an effective amount or effective dose of the RNAi agent according to any one of claims 1-11 and/or the pharmaceutical composition according to claim 12, wherein
the metabolic disorder is selected from the group consisting of: metabolic syndrome, a cardiovascular disease, obesity, hepatitis, and a kidney disease.

16. A method for delivering an RNAi agent inhibiting INHBE expression and/or

replication *in vivo*, comprising administering to a subject the RNAi agent according to any one of claims 1-11 and/or the pharmaceutical composition according to claim 12.

17. A method for preparing an RNAi agent, comprising:
synthesizing the RNAi agent according to any one of claims 1-11 or the pharmaceutical composition of claim 12.