

STEROID COMPOUND, AND USE THEREOF AND PREPARATION METHOD THEREFOR

FIELD OF THE INVENTION

The present invention relates to the pharmaceutical area, in particular to steroid compounds, applications and
5 preparation methods therefore.

BACKGROUND OF THE INVENTION

Neuropsychiatric diseases include anxiety, depression, schizophrenia and so on, ranking first in the total burden of
diseases in China, accounting for about 20% of the total diseases (Wang Juncheng et al., China Health Service
10 Management, 2009(5): 348-350). With the continuous social modernization, the accelerated working rhythm and the
increased living pressure, the number of patients with various neuropsychiatric diseases has increased dramatically,
and the progression of symptoms has accelerated significantly, making it more urgent to develop, research and
produce new drugs for the treatment of these diseases. Meanwhile, clinical data suggests that many patients are
troubled by a variety of neuropsychiatric disorders at the same time. The high comorbidity of neuropsychiatric
15 diseases makes clinical treatment face greater challenges.

In animals, GABA (gamma-aminobutyric acid) γ -aminobutyric acid) only exists in nerve tissues and immunological
studies have shown that the highest concentration of GABA is found in the substantia nigra. GABA is an important
inhibitory neurotransmitter that has been studied in depth. It participates in a variety of metabolic activities and has
20 high physiological. The GABA system is the main inhibitory signal pathway of the brain and central nervous system,
which is of great significance for the function of the central nervous system. GABA(A) receptor (GABA_AR) is an ionic
receptor and ligand-gated ion channel. Its endogenous ligand γ -aminobutyric acid (GABA), the main inhibitory
neurotransmitter is in the central nervous system. After the GABA(A) receptor activation, extracellular Cl^- is
selectively allowed to pass through the pores of the GABA receptors. Cl^- flows out of the neuron cells when the internal
25 potential is less than the resting potential, and flows into the neuron cells when the internal potential is greater than the
resting potential (i.e., -75 mv). This can successfully reduce the chance of action potentials, and inhibit
neurotransmission. Anxiety and depression have extremely high comorbidities and are considered to have
overlapping and co-occurring phenomena. Anxiety and depression are also important emotional symptoms of
schizophrenia. Research results confirm that GABAergic system and GABA(A) receptor play an important role in the
30 etiology and pathology of these three diseases, indicating that a pathophysiological process related to the GABAergic
system may be one of the decisive factors for all three diseases. The GABAergic system and GABA(A) receptor have
been shown to be involved in the pathological processes of anxiety, depression and schizophrenia at the molecular
level, pre-clinical and clinical level, and the GABA(A) receptor has long been used as an important drug target in the
treatment for these diseases.

35 It was confirmed by clinical trials that in addition to binding to GABA itself, forming receptor complex (GRC) to change
brain excitability, the GABA(A) receptor can bind to other small molecule compounds, such as barbiturates (trade
name Secobarbital Sodium), benzodiazepine (trade name diazepam), etc. These drugs bind to specific allosteric sites
on the GABA(A) receptor, respectively, and produce their therapeutic effects. Furthermore, studies have shown that
40 there are unique binding sites on the GABA(A) receptor for steroid compounds (Lan, NC et al., Neurochem. Res. 16:
347-356 (1991)).

Neuroactive steroids can occur endogenously. The most potent endogenous neuroactive steroids are 3α -
-hydroxy-5-reduced pregnan-20-one and 3α -21-dihydroxy-5-reduced pregnan-20-one, metabolites of hormonal
45 steroids progesterone and deoxycorticosterone, respectively. The ability of these steroid metabolites to alter brain

excitability was recognized in 1986 (Majewska, M. D. et al., Science 232: 1004-1007 (1986); Harrison, N. L. et al., J. Pharmacol. Exp. Ther. 241:346-353 (1987)). The ovarian hormone progesterone and its metabolites have been demonstrated to have profound effects on brain excitability (Backstrom, T. et al, Acta Obstet. Gynecol. Scand. Suppl. 130: 19-24 (1985); Pfaff, D.W and McEwen, B. S., Science 219:808-814 (1983); Gyermek et al, J Med Chem. 11 : 117
5 (1968); Lambert, J. et al, Trends Pharmacol. Sci. 8:224-227 (1987)). The levels of progesterone and its metabolites vary with the phases of the menstrual cycle. It has been well documented that the levels of progesterone and its metabolites decrease before menstruation begins. The monthly recurrence of certain physical symptoms prior to the onset of menstruation has also been well documented. These symptoms, which have become associated with premenstrual syndrome (PMS), include stress (tension), anxiety, and migraine headaches (Dalton, K., Premenstrual
10 Syndrome and Progesterone Therapy, 2nd edition, Chicago Yearbook, Chicago (1984)). Subjects with PMS have symptoms that recur once a month and are present pre-menstruation and absent post-menstruation.

Other studies have found that a reduction in progesterone is associated with an increase in seizure frequency in female patients with epilepsy (Laidlaw, J., Lancet, 1235-1237 (1956)). A more direct correlation has been observed
15 with a reduction in progesterone metabolites (Roszczewska et al, J. Neurol. Neurosurg. Psych. 49:47-51 (1986)). In addition, for subjects with primary generalized petit mal epilepsy, the temporal incidence of seizures has been correlated with the incidence of the symptoms of premenstrual syndrome (Backstrom, T. et al, J. Psychosom. Obstet. Gynaecol. 2:8-20 (1983)). The steroid deoxycorticosterone has been found to be effective in treating subjects with epileptic spells correlated with their menstrual cycles (Aird, RB. and Gordan, G, J. Amer. Med. Soc. 145:715-719
20 (1951)).

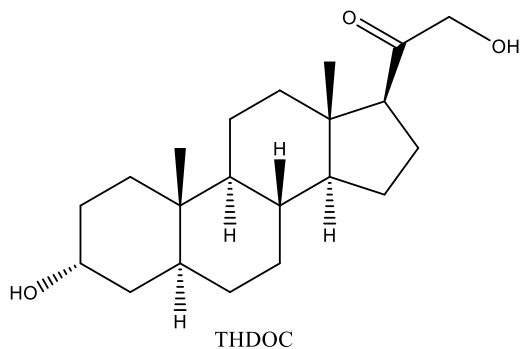
A syndrome also related to low progesterone levels is postpartum depression (PPD). Immediately after birth, progesterone levels decrease dramatically leading to the onset of PPD. The symptoms of PPD range from mild depression to psychosis requiring hospitalization. PPD is also associated with severe anxiety and irritability. PPD is
25 not amenable to treatment by classic antidepressants, and women experiencing PPD show an increased incidence of PMS (Dalton, K., Premenstrual Syndrome and Progesterone Therapy, 2nd edition, Chicago Yearbook, Chicago (1984)). Collectively, these observations imply a crucial role for progesterone and deoxycorticosterone and more specifically their metabolites in the homeostatic regulation of brain excitability, which is manifested as an increase in seizure activity or symptoms associated with catamenial epilepsy, PMS, and PPD.

Many studies are based on GABA(A) receptors in order to obtain drugs that can effectively treat related diseases. CN103958540A, CN10533928A, etc. have disclosed a series of neurological steroid compounds for the treatment of neuropsychiatric diseases. GABA(A): a subtype of γ -aminobutyric acid receptor (GABA R), GABA R has three subtypes, including GABA (A) , GABA (B) and GABA (C). GABA (A) is a chloride ion channel.

The GABA (A) is composed of five subunits (polypeptide chain) to form a heteropentameric structure. Among the five subunits, two pairs are the same, so a specific GABA(A) receptor contains three types of subunits, commonly known as α , β and γ . Each subunit has several different subtypes, such as α has α_1 , α_2 , α_3 , α_4 , α_5 , α_6 , β has β_1 , β_2 , β_3 , γ has γ_1 , γ_2 , γ_3 , γ_4 . Furthermore, there are subunits such as δ , ϵ , ρ_{1-3} , θ , π , etc., which can form pentamers with α and
40 β .

According to statistics, there are a total of 23 GABA(A) receptors with different combinations of subunits. The specific isoform of the receptors are related to the distribution area of neurons in the brain. For example, the GABA (A) receptors containing γ -subunit are mainly located inside the synapses, while the GABA (A) receptors containing
45 δ -subunit are mainly distributed outside the synapses (extrasynaptic).

(Activation of GABA(A) Receptors: Views from Outside the Synaptic Cleft, Neuron, Vol 56, 2007, 763-770); Study (Endogenous neurosteroids regulate GABA(A) receptors through two discrete transmembrane sites: Nature. Vol 444.23 November. 2006.486-489) has showed that endogenous steroids such as tetrahydrodeoxycorticosterone (THDOC) can directly activate the GABA receptor $\alpha_1\beta_2\gamma_2$ in the synapses (hereinafter referred as GABA_{A1}) at high concentration (micro-molar level), while potentiating the GABA receptor $\alpha_1\beta_2\gamma_2$ at lower concentration (submicro-molar level).

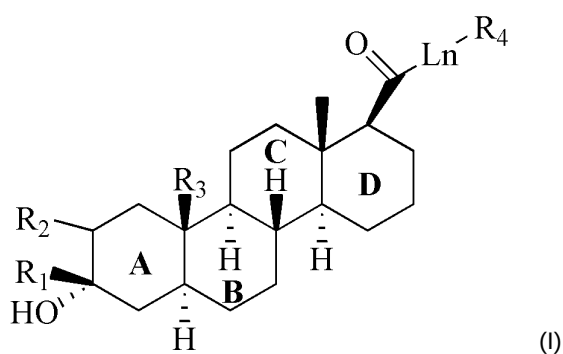


- 10 However, there are no reports about steroid compounds that can directly activate receptor subtypes outside the synapses (such as $\alpha_4\beta_3\delta$, hereinafter referred as GABA_{A4}).

SUMMARY OF THE INVENTION

- One of the purposes of the invention is to provide steroid compounds which can treat neurological diseases more effectively. Compounds of the invention are expected to provide good potency, pharmacokinetic (PK) properties, oral bioavailability, formulatability, stability, safety, clearance and/or metabolism, and reduce side effects, like anesthesia and sedation.

- In view of the above purposes, the invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



- wherein R₁ is hydrogen, substituted or unsubstituted C1-6 alkyl, substituted or unsubstituted C1-6 alkoxy, substituted or unsubstituted C2-6 alkenyl, substituted or unsubstituted C2-6 alkynyl, or substituted or unsubstituted C3-6 carbocyclyl;

R₂ is hydrogen, halogen, substituted or unsubstituted C1-6 alkyl, substituted or unsubstituted C1-6 alkoxy, substituted or unsubstituted C2-6 alkenyl, substituted or unsubstituted C2-6 alkynyl, substituted or unsubstituted C3-6 carbocyclyl;

R₃ is hydrogen, unsubstituted C1-6 alkyl or -CH₂OR^a, wherein R^a is hydrogen, substituted or unsubstituted C1-6 alkyl, substituted or unsubstituted C1-6 alkoxy, substituted or unsubstituted C2-6 alkenyl, substituted or unsubstituted C2-6 alkynyl, substituted or unsubstituted C3-6 carbocyclyl;

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L is -C(Rb)(Rb)-, each Rb is independently hydrogen or C1-C6 alkyl, n is an integer of 0 to 3;

R₄ is halogen or substituted or unsubstituted heteroaryl or heterocyclyl.

- 10 Further preferred compounds, wherein R₁ is hydrogen, substituted or unsubstituted C1-6 alkyl; R₂ is hydrogen, substituted or unsubstituted C1-6 alkyl; R₃ is hydrogen, an unsubstituted C1-6 alkyl; Rb is hydrogen, n is an integer of 1 to 2; R₄ is heteroaryl, optionally substituted with cyano, nitro, hydroxy, halo, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 haloalkyl, -C(O)Rd, -C(O)N(Re)(Rf), -C(O)O(Rd), -N(Re)(Rf), -OC(O)N(Re)(Rf), -OC(O)O(Rd), -OC(O)Rd, -S(O)₀₋₂Rd, -S(O)₀₋₂ORd or -S(O)₀₋₂N(Re)(Rf); each Rd is independently hydrogen or C1-C6 alkyl; each Re and Rf is
- 15 independently hydrogen, C1-C6 alkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl.

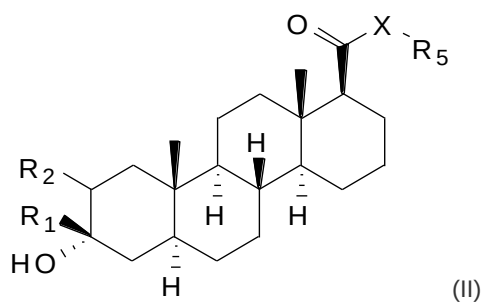
Further preferred compounds, wherein R₁ is hydrogen or methyl; R₂ is hydrogen; R₃ is hydrogen; L is -CH₂-; R₄ is a monocyclic or bicyclic-heteroaryl or a monocyclic or bicyclic-heterocyclyl, each heteroatom is oxygen or nitrogen or sulfur, the bicyclic ring can be spiro or fused.

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Further preferred compounds, R₄ is a five- or six-membered heteroaryl containing 2-4 nitrogen atoms and optionally substituted with cyano, nitro, hydroxy, halo, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 haloalkyl.

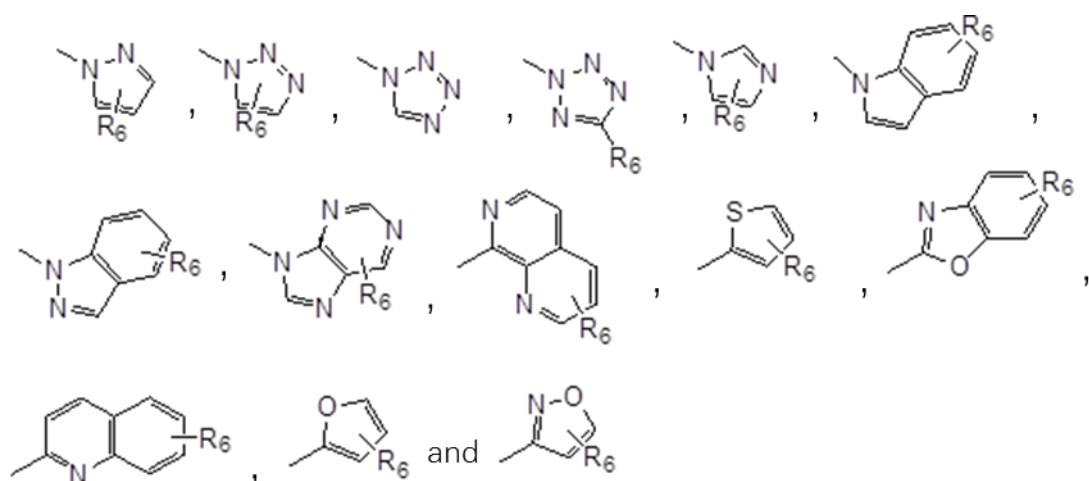
Or as shown in the compound of formula II:

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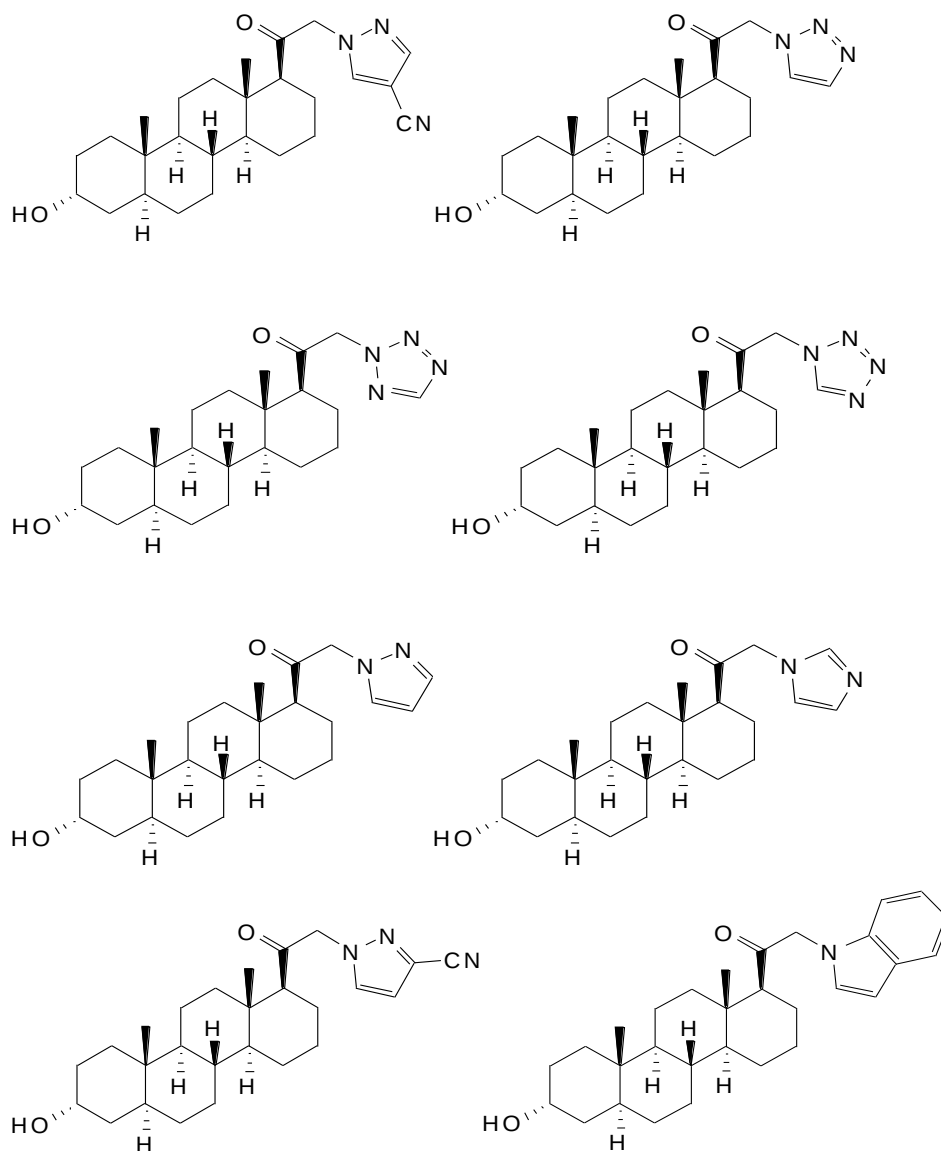
wherein R₁ is hydrogen, or C1-3 alkyl group substituted or unsubstituted with halogen; R₂ is hydrogen, halogen, C1-6 alkyl group substituted or unsubstituted with halogen or C1-6 alkoxy group substituted or unsubstituted with halogen;

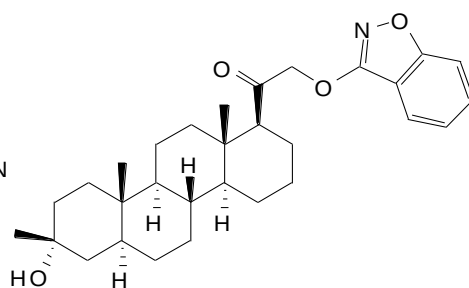
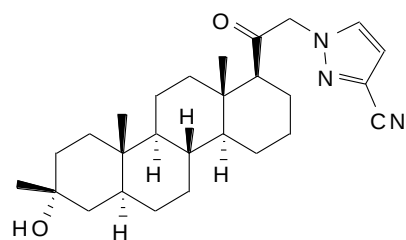
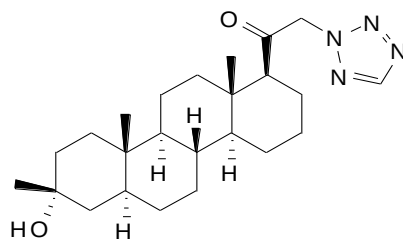
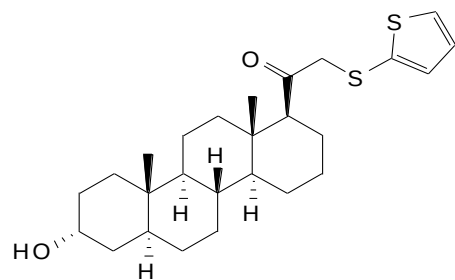
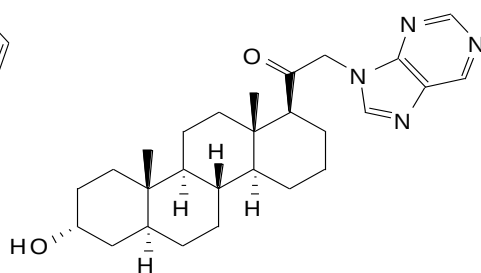
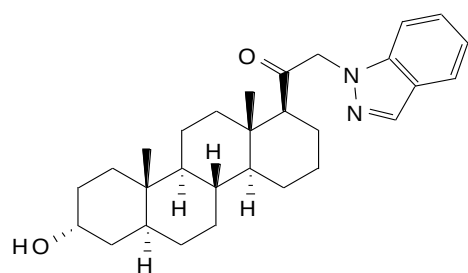
- 30 X is CH₂, N, O or S; R₅ is selected from the following groups:



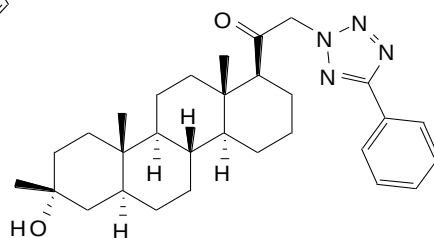
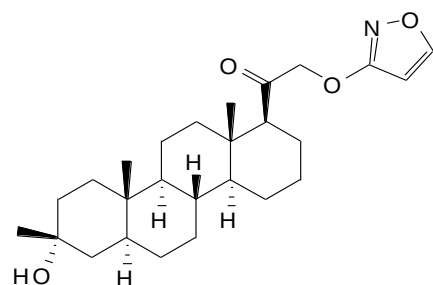
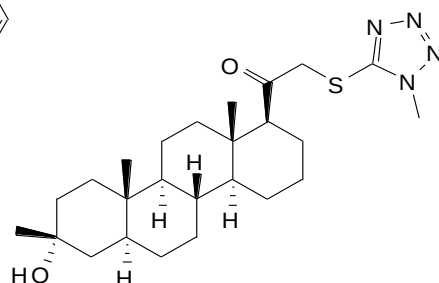
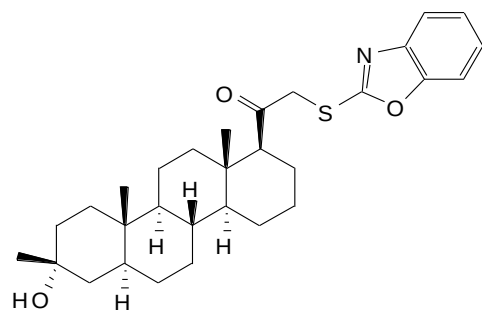
wherein R_6 is H, halogen, CN, CF_3 , NO_2 , C1-6 alkyl substituted or unsubstituted with halogen or C1-6 alkoxy substituted or unsubstituted with halogen.

5 Alternatively, the preferred compound is selected from:

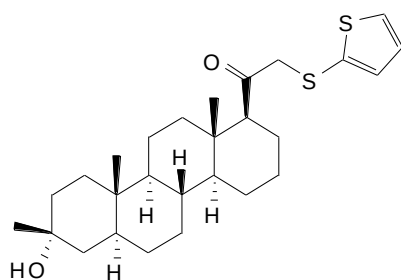




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The present invention also provides pharmaceutical compositions, comprising any of the above compounds or pharmaceutically acceptable salts thereof, or pharmaceutically acceptable excipients.

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The present invention also provides compounds described herein which can act as GABA modulators, such as positively or negatively effecting GABA (A) receptors. As modulators of the excitability of central nervous system (CNS), such compounds are expected to have CNS-activity by their ability to modulate GABA (A) receptors.

- 10 The present invention further provides the application of compounds described herein or the pharmaceutical compositions in preventing or treating neurological diseases; wherein the neurological disease is preferably selected from a sleep disorder, a mood disorder, a schizophrenia spectrum disorder, a spasmodic disorder, a memory and/or cognitive disorder, a movement disorder, a personality disorder, an autism spectrum disorder, pain, traumatic brain injury, a vascular disease, a substance abuse disorder and/or withdrawal syndrome or tinnitus; wherein the mood
- 15 disorder is depression; wherein the depression is destructive mood disorder, severe depressive disorder, persistent depressive disorder, premenstrual dysphoria, substance or drug-induced disorder, disorder due to physical diseases, other specific depressive disorder and nonspecific depressive disorder, preferably mild depression, moderate depression, severe depression or postpartum depression. Preferably severe depression or postpartum depression. And the above compounds or pharmaceutical compositions can be administered orally, subcutaneously, intravenously
- 20 or intramuscularly.

Definition

Chemical Definition

- Compounds described herein may include one or more asymmetric centers, and thus can exist in various isomeric
- 25 forms, e.g., enantiomers and/or diastereomers. For example, the compounds described herein can be in the form of an individual enantiomer, diastereomer or geometric isomer, or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. Isomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred isomers can be prepared by asymmetric syntheses.

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- The following terms are intended to have the following meanings provided therewith, and are useful in understanding the description and the intended scope of the present invention. When describing the present invention, it may include compounds, pharmaceutical compositions containing the compounds, and the compounds and compositions of the test method. For definitions of terms involved in the present invention, reference may be made to the following
- 35 description, any part defined below may be substituted with many substituents, and the corresponding definitions are within their scope listed below, including such substituted parts. Unless otherwise specified, the term "substituted" is as defined below.

"Alkyl" refers to a straight-chain or branched saturated hydrocarbon group having from 1 to 20 carbon atoms ("C1-20

alkyl"). In some embodiments, an alkyl group has 1 to 12 carbon atoms ("C1-12 alkyl"). In some embodiments, an alkyl group has 1 to 10 carbon atoms ("C1-10 alkyl"). In some embodiments, an alkyl group has 1 to 9 carbon atoms ("C1-9 alkyl"). In some embodiments, an alkyl group has 1 to 8 carbon atoms ("C1-8 alkyl"). In some embodiments, an alkyl group has 1 to 7 carbon atoms ("C1-7 alkyl"). In some embodiments, an alkyl group has 1 to 6 carbon atoms ("C1-6 alkyl", also referred to herein as "lower alkyl"). In some embodiments, an alkyl group has 1 to 5 carbon atoms ("C1-5 alkyl"). In some embodiments, an alkyl group has 1 to 4 carbon atoms ("C1-4 alkyl"). In some embodiments, an alkyl group has 1 to 3 carbon atoms ("C1-3 alkyl"). In some embodiments, an alkyl group has 1 to 2 carbon atoms ("C1-2 alkyl"). In some embodiments, an alkyl group has 1 carbon atom ("C1 alkyl"). In some embodiments, an alkyl group has 2 to 6 carbon atoms ("C2-6 alkyl"). Examples of C1-6 alkyl group include methyl (C1), ethyl (C2), n-propyl (C3), isopropyl (C3), n-butyl (C4), tert-butyl (C4), sec-butyl (C4), iso-butyl (C4), n-pentyl (C5), 3-pentanyl (C5), neopentyl (C5), 3-methyl-2-butanyl (C5), tertiary amyl (C5), and n-hexyl (C6). Additional examples of alkyl group include n-heptyl (C7), n-octyl (C8) and the like. Unless otherwise specified, each instance of an alkyl group is independently optionally substituted, i.e., unsubstituted (an "unsubstituted alkyl") or substituted (a "substituted alkyl") with one or more substituents; e.g., for instance from 1 to 5 substituents, 1 to 3 substituents, or 1 substituent. In certain embodiments, the alkyl group is unsubstituted C1-10 alkyl (e.g., —CH₃). In certain embodiments, the alkyl group is substituted C1-10 alkyl.

"Alkenyl" refers to a straight-chain or branched hydrocarbon group having from 2 to 20 carbon atoms, one or more carbon-carbon double bonds (e.g., 1, 2, 3, or 4 carbon-carbon double bonds), and optionally one or more carbon-carbon triple bonds (e.g., 1, 2, 3, or 4 carbon-carbon triple bonds) ("C2-20 alkenyl"). In certain embodiments, alkenyl does not contain any triple bonds. In some embodiments, an alkenyl group has 2 to 10 carbon atoms ("C2-10 alkenyl"). In some embodiments, an alkenyl group has 2 to 9 carbon atoms ("C2-9 alkenyl"). In some embodiments, an alkenyl group has 2 to 8 carbon atoms ("C2-8 alkenyl"). In some embodiments, an alkenyl group has 2 to 7 carbon atoms ("C2-7 alkenyl"). In some embodiments, an alkenyl group has 2 to 6 carbon atoms ("C2-6 alkenyl"). In some embodiments, an alkenyl group has 2 to 5 carbon atoms ("C2-5 alkenyl"). In some embodiments, an alkenyl group has 2 to 4 carbon atoms ("C2-4 alkenyl"). In some embodiments, an alkenyl group has 2 to 3 carbon atoms ("C2-3 alkenyl"). In some embodiments, an alkenyl group has 2 carbon atoms ("C2 alkenyl"). The one or more carbon-carbon double bonds can be internal (such as in 2-butenyl) or terminal (such as in 1-butenyl). Examples of C2-4 alkenyl group include ethenyl (C2), 1-propenyl (C3), 2-propenyl (C3), 1-butenyl (C4), 2-butenyl (C4), butadienyl (C4), and the like. Examples of C2-6 alkenyl group include the aforementioned C2-4 alkenyl groups as well as pentenyl (C5), pentadienyl (C5), hexenyl (C6), and the like. Additional examples of alkenyl include heptenyl (C7), octenyl (C8), octatrienyl (C8), and the like. Unless otherwise specified, each instance of an alkenyl group is independently optionally substituted, i.e., unsubstituted (an "unsubstituted alkenyl") or substituted (a "substituted alkenyl") with one or more substituents e.g., for instance from 1 to 5 substituents, 1 to 3 substituents, or 1 substituent. In certain embodiments, the alkenyl group is unsubstituted C2-10 alkenyl. In certain embodiments, the alkenyl group is substituted C2-10 alkenyl.

"Alkynyl" refers to a straight-chain or branched hydrocarbon group having from 2 to 20 carbon atoms, one or more carbon-carbon triple bonds (e.g., 1, 2, 3, or 4 carbon-carbon triple bonds), and optionally one or more carbon-carbon double bonds (e.g., 1, 2, 3, or 4 carbon-carbon double bonds) ("C2-20 alkynyl"). In certain embodiments, alkynyl does not contain any double bonds. In some embodiments, an alkynyl group has 2 to 10 carbon atoms ("C2-10 alkynyl"). In some embodiments, an alkynyl group has 2 to 9 carbon atoms ("C2-9 alkynyl"). In some embodiments, an alkynyl group has 2 to 8 carbon atoms ("C2-8 alkynyl"). In some embodiments, an alkynyl group has 2 to 7 carbon atoms ("C2-7 alkynyl"). In some embodiments, an alkynyl group has 2 to 6 carbon atoms ("C2-6 alkynyl"). In some embodiments, an alkynyl group has 2 to 5 carbon atoms ("C2-5 alkynyl"). In some embodiments, an alkynyl group

has 2 to 4 carbon atoms ("C2-4 alkynyl"). In some embodiments, an alkynyl group has 2 to 3 carbon atoms ("C2-3 alkynyl"). In some embodiments, an alkynyl group has 2 carbon atoms ("C2 alkynyl"). The one or more carbon-carbon triple bonds can be internal (such as in 2-butyne) or terminal (such as in 1-butyne). Examples of C2-4 alkynyl group include, without limitation, ethynyl (C2), 1-propynyl (C3), 2-propynyl (C3), 1-butyne (C4), 2-butyne (C4), and the like. Examples of C2-6 alkynyl group include the aforementioned C2-4 alkynyl group as well as pentynyl (C5), hexynyl (C6), and the like. Additional examples of alkynyl group include heptynyl (C7), octynyl (C8), and the like. Unless otherwise specified, each instance of an alkynyl group is independently optionally substituted, i.e., unsubstituted (an "unsubstituted alkynyl") or substituted (a "substituted alkynyl") with one or more substituents; e.g., for instance from 1 to 5 substituents, 1 to 3 substituents, or 1 substituent. In certain embodiments, the alkynyl group is unsubstituted C2-10 alkynyl. In certain embodiments, the alkynyl group is substituted C2-10 alkynyl.

"Aryl" refers to a monocyclic or polycyclic (e.g., bicyclic or tricyclic) $4n+2$ aromatic ring system (e.g., having 6, 10, or 14 π electrons shared in a cyclic array) having 6-14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system ("C6-14 aryl"). In some embodiments, an aryl group has a ring of six carbon atoms ("C6 aryl"; e.g., phenyl). In some embodiments, an aryl group has ten ring carbon atoms ("C10 aryl"; e.g., naphthyl such as 1-naphthyl and 2-naphthyl). In some embodiments, an aryl group has fourteen ring carbon atoms ("C14 aryl"; e.g., anthracenyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms continue to designate the number of carbon atoms in the aryl ring system. Typical aryl groups include, but are not limited to, groups derived from aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, coronene, fluoranthene, fluorene, hexacene, hexaphene, hexalene, as-indacene, s-indacene, indane, indene, naphthalene, octacene, octaphene, octalene, ovalene, penta-2,4-diene, pentacene, pentalene, pentaphene, perylene, phenalene, phenanthrene, picene, pleiadene, pyrene, pyranthrene, rubicene, triphenylene, and trinaphthalene. Particularly aryl groups include phenyl, naphthyl, indenyl, and tetrahydronaphthyl. Unless otherwise specified, each instance of an aryl group is independently optionally substituted, i.e., unsubstituted (an "unsubstituted aryl") or substituted (a "substituted aryl") with one or more substituents. In certain embodiments, the aryl group is unsubstituted C6-14 aryl. In certain embodiments, the aryl group is substituted C6-14 aryl.

"Heteroaryl" refers to a radical of a 5-10 membered monocyclic or bicyclic $4n+2$ aromatic ring system (e.g., having 6 or 10 π electrons shared in a cyclic array) having ring carbon atoms and 1-4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen and sulfur ("5-10 membered heteroaryl"). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Heteroaryl bicyclic ring systems can include one or more heteroatoms in one or both rings. "Heteroaryl" includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the point of attachment is on the heteroaryl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heteroaryl ring system. "Heteroaryl" also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designates the number of ring members in the fused (aryl/heteroaryl) ring system. Bicyclic heteroaryl groups wherein one ring does not contain a heteroatom (e.g., indolyl, quinolyl, carbazolyl, and the like) the point of attachment can be on either ring, i.e., either the ring bearing a heteroatom (e.g., 2-indolyl) or the ring that does not contain a heteroatom (e.g., 5-indolyl). In some embodiments, a heteroaryl group is a 5-10 membered aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-10 membered heteroaryl"). In some

embodiments, a heteroaryl group is a 5-8 membered aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-8 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5-6 membered aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-6 membered heteroaryl"). In some embodiments, the 5-6 membered heteroaryl has 1-3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5-6 membered heteroaryl has 1-2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5-6 membered heteroaryl has 1 ring heteroatom selected from nitrogen, oxygen, and sulfur. Unless otherwise specified, each instance of a heteroaryl group is independently optionally substituted, i.e., unsubstituted (an "unsubstituted heteroaryl") or substituted (a "substituted heteroaryl") with one or more substituents. In certain embodiments, the heteroaryl group is unsubstituted 5-14 membered heteroaryl. In certain embodiments, the heteroaryl group is substituted 5-14 membered heteroaryl. Exemplary 5-membered heteroaryl groups containing one heteroatom include, without limitation, pyrrolyl, furanyl and thiophenyl. Exemplary 5-membered heteroaryl groups containing two heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5-membered heteroaryl groups containing three heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5-membered heteroaryl groups containing four heteroatoms include, without limitation, tetrazolyl. Exemplary 6-membered heteroaryl groups containing one heteroatom include, without limitation, pyridinyl. Exemplary 6-membered heteroaryl groups containing two heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6-membered heteroaryl groups containing three or four heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively. Exemplary 7-membered heteroaryl groups containing one heteroatom include, without limitation, azepinyl, oxepinyl, and thiopinyl. Exemplary 5,6-bicyclic heteroaryl groups include, without limitation, indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzthiazolyl, benzisothiazolyl, benzthiadiazolyl, indoliziny, and purinyl. Exemplary 6,6-bicyclic heteroaryl groups include, without limitation, naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl.

"Carbocyclyl" or "carbocyclic" refers to a radical of a non-aromatic cyclic hydrocarbon group having from 3 to 10 ring carbon atoms ("C3-10 carbocyclyl") and zero heteroatoms in the non-aromatic ring system. In some embodiments, a carbocyclyl group has 3 to 8 ring carbon atoms ("C3-8 carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 6 ring carbon atoms ("C3-6 carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 10 ring carbon atoms ("C5-10 carbocyclyl"). Exemplary C3-6 carbocyclyl groups include, but not limited to: cyclopropyl (C3), cyclopropenyl (C3), cyclobutyl (C4), cyclobutenyl (C4), cyclopentyl (C5), cyclopentenyl (C5), cyclohexyl (C6), cyclohexenyl (C6), cyclohexadienyl (C6), and the like. Exemplary C3-8 carbocyclyl groups include, but are not limited to: the above-mentioned C3-6 carbocyclic group, and cycloheptyl (C7), cycloheptenyl (C7), cycloheptadienyl (C7), cycloheptatrienyl (C7), cyclooctyl (C8), cyclooctenyl (C8), bicyclo [2.2.1] heptyl (C7), bicyclo [2.2.2] octyl (C8), and the like. Exemplary C3-10 carbocyclyl groups include, but are not limited to: the above-mentioned C3-8 carbocyclyl group, and cyclononyl (C9), cyclononenyl (C9), cyclodecyl (C10), cyclodecenyl (C10), octahydro-1H-indenyl (C9), decahydronaphthyl (C10), spiro [4.5] decyl (C10), etc. As illustrated by the foregoing examples, in some embodiments, a carbocyclyl group is a monocyclic ring ("monocyclic carbocyclyl") or a carbocyclyl group that comprises a fused ring system, a bridged ring system, or a spiro ring system, such as a bicyclic ring system ("bicyclic carbocyclyl"), and can be saturated or a partially unsaturated carbocyclyl group. "Carbocyclyl" also includes ring systems in which the aforementioned carbocyclic ring is fused to one or more aryl or heteroaryl groups, wherein the point of attachment is on the carbocyclic ring, and in such cases the number of carbons continues to represent the

number of carbons in the carbocyclic ring system. Unless otherwise specified, each of the carbocyclyl groups is independently optionally substituted, i.e., unsubstituted (an "unsubstituted carbocyclyl") or substituted with one or more substituents (a "substituted carbocyclyl"). In some embodiments, carbocyclyl is unsubstituted C3-10 carbocyclyl. In some embodiments, carbocyclyl is substituted C3-10 carbocyclyl. In some embodiments, "carbocyclyl" is a monocyclic, saturated carbocyclyl ("C3-10 cycloalkyl") having from 3 to 10 ring carbon atoms. In some embodiments, a cycloalkyl group has 3 to 8 ring carbon atoms ("C3-8 cycloalkyl"). In some embodiments, a cycloalkyl group has 3 to 6 ring carbon atoms ("C3-6 cycloalkyl"). In some embodiments, a cycloalkyl group has 5 to 6 ring carbon atoms ("C5-6 cycloalkyl"). In some embodiments, cycloalkyl groups have 5 to 10 ring carbon atoms ("C5-10 cycloalkyl"). Examples of C5-6 cycloalkyl include cyclopentyl (C5) and cyclohexyl (C6). Examples of the C3-6 cycloalkyl group include the above-mentioned C5-6 cycloalkyl groups, as well as cyclopropyl (C3) and cyclobutyl (C4). Examples of the C3-8 cycloalkyl group include the above-mentioned C3-6 cycloalkyl group, as well as cycloheptyl (C7) and cyclooctyl (C8). Unless otherwise specified, each instance of a cycloalkyl group is independently unsubstituted (an "unsubstituted cycloalkyl") or substituted (a "substituted cycloalkyl") with one or more substituents. In some embodiments, cycloalkyl is unsubstituted C3-10 cycloalkyl. In some embodiments, cycloalkyl is substituted C3-10 cycloalkyl.

"Heterocyclyl" or "heterocyclic" refers to a radical of a 3 to 10 membered non-aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, sulfur, boron, phosphorus, and silicon ("3-10 membered heterocyclyl"). In heterocyclic groups containing one or more nitrogen atoms, the point of attachment can be carbon or a nitrogen atom, as valency permits. A heterocyclyl group can either be monocyclic ("monocyclic heterocyclyl") or a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl"), and can be saturated or can be partially unsaturated. Heterocyclyl bicyclic ring systems can include one or more heteroatoms in one or both rings. "Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more carbocyclyl groups wherein the point of attachment is either on the carbocyclyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. Unless otherwise specified, each instance of heterocyclyl is independently optionally substituted, i.e., unsubstituted (an "unsubstituted heterocyclyl") or substituted (a "substituted heterocyclyl") with one or more substituents. In certain embodiments, the heterocyclyl group is unsubstituted 3-10 membered heterocyclyl. In certain embodiments, the heterocyclyl group is substituted 3-10 membered heterocyclyl. In some embodiments, a heterocyclyl group is a 5-10 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, sulfur, boron, phosphorus, and silicon ("5-10 membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5-8 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-8 membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5-6 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-6 membered heterocyclyl"). In some embodiments, the 5-6 membered heterocyclyl has 1-3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5-6 membered heterocyclyl has 1-2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5-6 membered heterocyclyl has one ring heteroatom selected from nitrogen, oxygen, and sulfur.

"Hetero" when used to describe a compound or group present on a compound means that one or more carbon atoms in the compound or group have been replaced with a nitrogen, oxygen, or sulfur heteroatom. Hetero can be applied

to any of the hydrocarbonyl described above: for example, alkyl, e.g., heteroalkyl; cycloalkyl groups, e.g., heterocyclyl groups; aryl, e.g., heteroaryl; cycloalkenyl groups, e.g., cycloheteroalkenyl and the like; having from 1 to 5 heteroatoms, in particular 1 to 3 heteroatoms.

- 5 "Alkoxy" refers to the group —OR where R is substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl. Particular alkoxy groups are methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, and 1,2-dimethylbutoxy. Particular alkoxy groups are lower alkoxy, i.e. with between 1 and 6 carbon atoms. Further particular alkoxy groups have between 1 and 4 carbon atoms. Exemplary substituted alkoxy groups include, but are not limited to, -O-(CH₂)_t(C₆-C₁₀ aryl), -O-(CH₂)_t(5-10 membered heteroaryl), -O-(CH₂)_t(C₃-C₁₀ cycloalkyl), and -O-(CH₂)_t(4-10 membered heterocyclyl), wherein t is an integer from 0 to 4, and any substituted groups include aryl, heteroaryl, cycloalkyl or heterocyclyl groups present, can themselves be substituted by unsubstituted a C₁-C₄ alkyl group, halo, a unsubstituted C₁-C₄ alkoxy group, a unsubstituted C₁-C₄ haloalkyl group, a unsubstituted C₁-C₄ hydroxyalkyl group, or a unsubstituted C₁-C₄ haloalkoxy group or a hydroxy group.

"Carboxy" refers to the -C(O)OH.

"Cyano" refers to the -CN.

- 20 "Halo" or "halogen" refers to a fluoro atom (F), a chloro atom (Cl), a bromo atom (Br), and an iodo atom (I). In certain embodiments, the halo group is either a fluoro atom or a chloro atom.

"Hydroxy" refers to the -OH.

"Nitro" refers to the -NO₂.

Other definitions

- 25 The term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al., describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds of the present invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and N+(C₁-4alkyl)₄ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing changes of the control in plasma and brain over time.

Figure 2 is a graph showing changes of KH022 in plasma and brain over time.

5

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

In order to better explain the technical scheme of the invention, the invention provides synthesis or biological examples of a part of compounds, and does not further limit the protection scope of the invention.

10 Materials and methods

The compounds provided herein can be prepared from easily available starting materials using the following general methods and procedures. It is understood that under typical or preferred process conditions (i.e., reaction temperature, time, mole ratio of reactants, solvent, pressures, etc.), other process conditions can be also used unless otherwise specified. Optimum reaction conditions can vary with the particular reactants or solvents used, but such
15 conditions can be determined by those skilled in the art based on routine optimization.

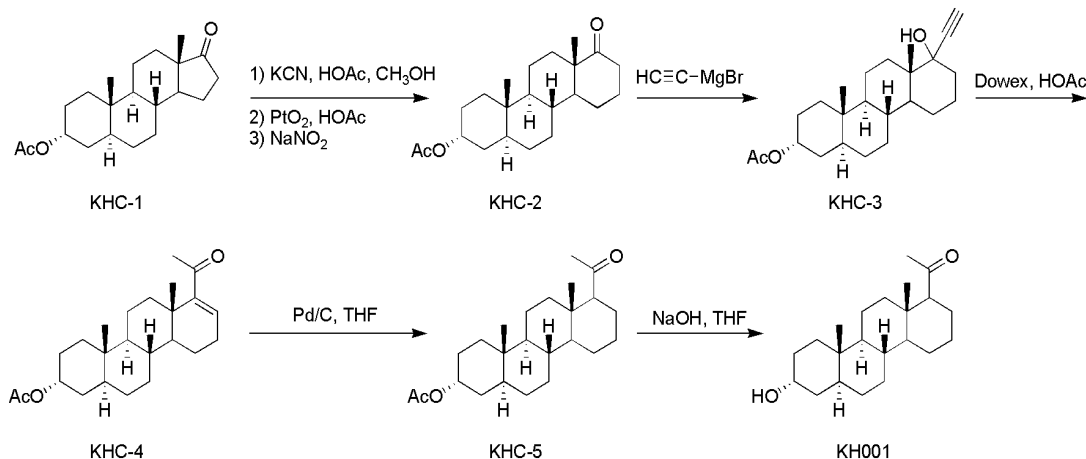
In addition, it is obvious to those skilled in the art that conventional protecting groups can be required to prevent undesirable reactions of certain functional groups. How to select suitable protecting groups for particular functional groups and how to select suitable conditions for protection and de-protection are well known in the art.

20

The compounds provided herein can be isolated and purified by known standard methods, including, but not limited to, recrystallization, column chromatography, HPLC, or Supercritical Fluid Chromatography (SFC). The preparation details of the representative pyrazoles herein are provided in the following reaction schemes.

25 The compounds provided herein can be prepared according to methods described in the art using appropriate reagents, starting materials and purification methods known to those skilled in the art.

Example 1: Synthesis of compound KH001:



30 Compound KHC-2: KCN (600.0g, 9.2mol), acetic acid (640mL) was added to a solution of compound KHC-1 (100.0 g, 0.3mol) in methanol (4L) and the reaction was stirred overnight at room temperature (RT). The reaction solution was poured into water and extracted with ethyl acetate (EA). The organic phase was dried over anhydrous sodium sulfate and concentrated under vacuum to attain the crude product which was dissolved in acetic acid (2.5L). Platinum
35 dioxide (24.0g, 0.1mol) was added to the reaction solution and hydrogenation was carried out with H₂ gas. TLC showed the reaction completion and the reaction solution was filtered, then the filtrate solution was concentrated to

about 200mL, diluted with ice water (2.4L). NaNO_2 (46.0g, 0.7mol) aqueous solution was slowly added dropwise to the mixture, then the mixture was, stirred for 15 hours at RT and extracted with EA. The combined organic layers were washed with brine, dried, concentrated, and purified by silica gel column flash chromatography to give KHC-2(57.3g, 55% yield).

5

Compound KHC-3: Ethynylmagnesium bromide (395M L, 0.5M) was added dropwise to a solution of compound KHC-2 (57.3g,164.5mmol) in THF (1L) under N_2 atmosphere at -78°C, then gradually warmed to RT, stirred overnight at RT. Saturated NH_4Cl aqueous solution was slowly added to the above mixture, followed by extraction with EA, washing of the organic phase with brine, drying over anhydrous Na_2SO_4 , concentration, and purification of the resulting crude product by silica gel column flash chromatography to give compound KHC-3(33.7g, 55% yield).

10

Compound KHC-4: Compound KHC-3 (33.7g,90.5mmol) was dissolved in a mixed solvent of acetic acid (200mL) and water (20mL), followed with heating reflux by addition of Dowex-50 (10.0g). After the reaction completion, cooling to RT, filtration of the resin, washing with EA, neutralization of the filtrate with 40% NaOH aqueous solution, washing of the organic layer with saturated brine, drying over anhydrous Na_2SO_4 , concentration, and purification of the resulting crude product by silica gel column flash chromatography to give Compound KHC-4 (23.6g, 70% yield).

15

Compound KHC-5: Compound KHC-4 (23.6g, 63.3mmol) and palladium black (1.2g) in THF (200mL) were hydrogenated with H_2 gas, after stirring overnight at RT, the mixture was filtered through a pad of celite, washed with EA, and the filtrate was concentrated in vacuo to provide the crude compound which was recrystallized from acetone to give compound KHC-5(19.0g, 80% yield).

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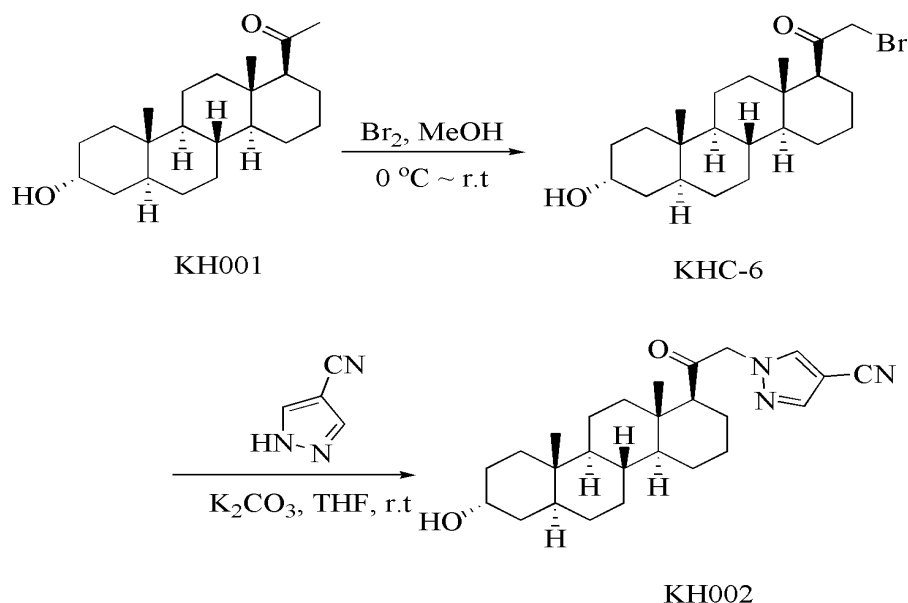
Compound KH001: NaOH aqueous solution (50ML, 1M) was added slowly to a solution of compound KHC-5 (19.0g, 50.8mmol) in THF (200ml) and stirring overnight at RT, the pH of the reaction solution was adjusted with dilute HCl and then the solution was extracted with EA. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 , and concentrated to give the crude product which was purified by silica gel column flash chromatography to give Compound KH001 (13.5g, 80% yield).

25

^1H NMR(400MHz, CDCl_3), δ 4.06(s,1H), 2.32-2.28(dd,1H), 2.15(s,3H),1.84-1.77(m,2H), 1.74-1.61(m,5H), 1.61-1.54(m,3H), 1.48-1.46(m,1H), 1.33-1.14(m,10H), 1.02-0.95(m,1H), 0.93(s,3H), 0.85-0.79(m,3H), 0.75(s,3H).

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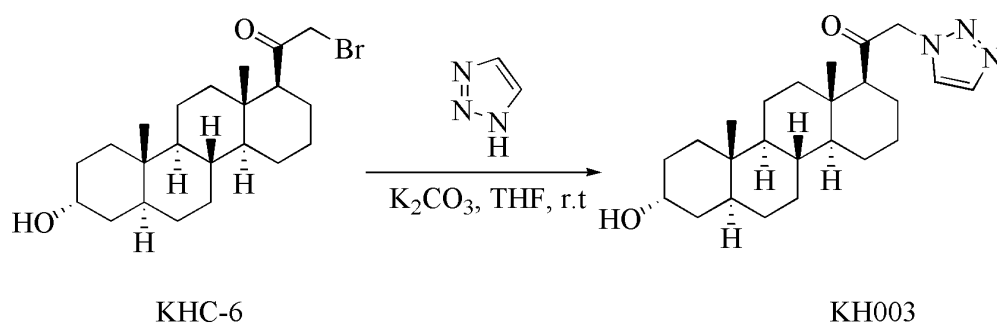
Example 2: Synthesis of compound KH002:



Compound KHC-6: Compound KH001 (1.5g, 4.5mmol) synthesized in example 1 was dissolved in CH₃OH (40mL) until the solution was clear, then the solution was cooled to 5°C in the wet ice. Subsequently, bromine (1.5g, 9.2mmol) was added dropwise to the cooled solution. The ice-bath was removed and the reaction was carried out at RT for 4 hours, the reaction solution became pale yellow from the orange color. TLC monitoring of the reaction showed that the reaction was complete, water (30mL) was added to the reaction solution, extraction was carried out with EA (2x35mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by silica gel column flash chromatography (eluent: CH₂Cl₂) to give a white solid (1.0g, 56.7% yield).

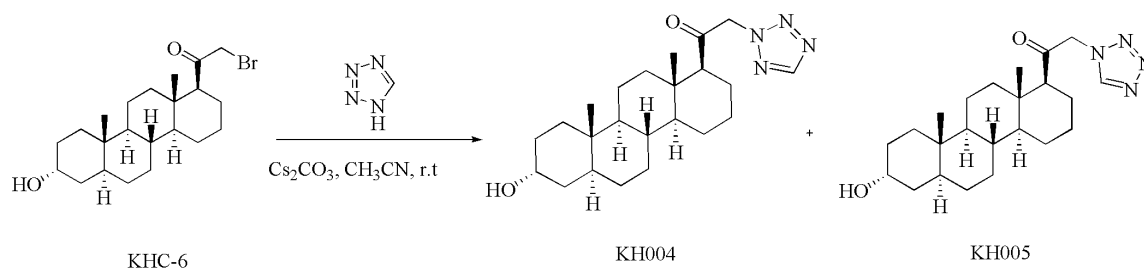
- Compound KH002: Compound KHC-6 (80mg, 0.2mmol) was dissolved in THF (5mL) until the solution was clear, then K₂CO₃ (83mg, 0.6mmol) and 4-cyanopyrazole (56mg, 0.6mmol) was added. Reaction was performed at room temperature. overnight. TLC monitoring of the reaction showed that the reaction was complete, then the reaction was washed with 10mL of water and extracted with EA (2x20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄ and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by silica gel column flash chromatography (eluent: CH₂Cl₂/ CH₃OH=100:1) and then recrystallized EA/PE=1:10 to attain white solid (36mg, 42.5% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.82 (s, 1H), 7.80 (s, 1H), 5.06-4.93 (m, 2H), 4.05-4.04 (m, 1H), 2.32 (dd, 1H), 1.88-1.83 (m, 2H), 1.79-1.66 (m, 4H), 1.64-1.58 (m, 2H), 1.54-1.44 (m, 4H), 1.39-1.13 (m, 9H), 1.04-0.97 (m, 1H), 0.94 (s, 3H), 0.85-0.75 (m, 3H), 0.74 (s, 3H).

Example 3: Synthesis of compound KH003:



- Compound KH003: Compound KHC-6 (50mg, 0.1mmol) was dissolved in THF (5mL) until the solution was clear, then K₂CO₃(50mg, 0.3mmol) and 1H-triazole (25mg, 0.3mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete, then the reaction was washed with 10ml water, extracted with EA (2x20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄ and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by silica gel column flash chromatography (eluent: CH₂Cl₂/ CH₃OH=150:1) and then recrystallized (EA/PE=1:10) to attain white solid (17mg, 35.5% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.76 (s, 1H), 7.60 (s, 1H), 5.31-5.17 (m, 2H), 4.06-4.05 (m, 1H), 2.37 (d, 1H), 1.87-1.61 (m, 8H), 1.52-1.10 (m, 13H), 1.04-0.98 (m, 1H), 0.95 (s, 3H), 0.92-0.79 (m, 3H), 0.74 (s, 3H).

Example 4: Synthesis of compound KH004 and KH005:

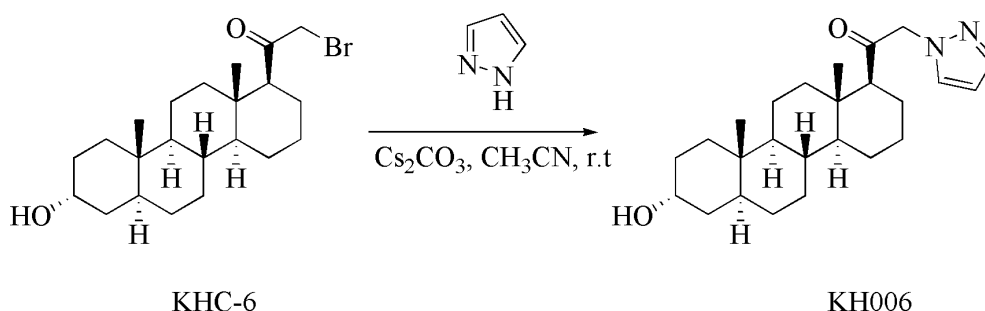


Compound KH004 and KH005: Compound KHC-6 (103 mg, 0.2 mmol) was dissolved in CH₃CN (15mL) until the solution was clear, then Cs₂CO₃ (244mg, 0.7mmol) and 1H-tetrazolium (53mg, 0.7mmol) was added. Reaction was performed at room temperature. TLC monitoring of the reaction showed that the reaction was complete, the reaction was washed with 10mL water and extracted with EA (2×25mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄ and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by silica gel column flash chromatography (eluent: CH₂Cl₂/ CH₃OH=150:1 to 90:1) to give two compounds which were respectively recrystallized (EA/PE=1:10) to attain white solid one (24mg, 24.0%) and white solid two (36mg, 36.0% yield).

White solid 1 (KH004) ¹H NMR (400 MHz, CDCl₃) δ 8.57 (s, 1H), 5.50 (s, 2H), 4.06-4.05 (m, 1H), 2.35 (dd, 1H), 1.92-1.81 (m, 3H), 1.74-1.68 (m, 3H), 1.64-1.57 (m, 4H), 1.46-1.11 (m, 10H), 1.05-0.98 (m, 2H), 0.96 (s, 3H), 0.88-0.78 (m, 3H), 0.75 (s, 3H).

White solid 2 (KH005) ¹H NMR (400 MHz, CDCl₃) δ 8.71 (s, 1H), 5.38 -5.23 (m, 2H), 4.06-4.05 (m, 1H), 2.41 (dd, 1H), 2.28 (dd, 1H), 1.91-1.72 (m, 4H), 1.68-1.51 (m, 7H), 1.47-1.11 (m, 10H), 1.06-0.99 (m, 1H), 0.95 (s, 3H), 0.91-0.78 (m, 3H), 0.75 (s, 3H).

Example 5: Synthesis of compound KH006:

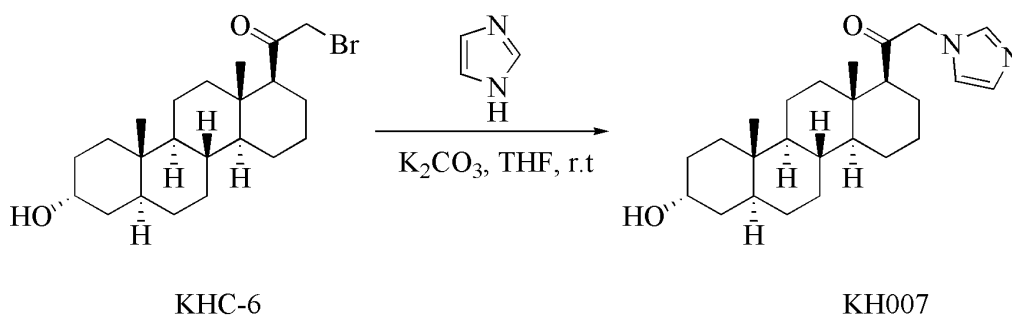


Compound KH006: Compound KHC-6 (50mg, 0.1mmol) was dissolved in CH₃CN (8mL), then Cs₂CO₃ (117mg, 0.3mmol) and 1H-pyrazole (24mg, 0.3mmol) was added, Reaction was performed at room temperature overnight.

TLC monitoring of the reaction showed that the reaction was complete. The reaction was washed with water 10mL and extracted with EA (2×20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give a crude pale yellow solid which was purified by flash chromatography on silica gel column (eluent: CH₂Cl₂/CH₃OH =100:1) and recrystallized (EA/PE=1:10) to attain a white solid (24mg, 50.2% yield).

¹H NMR (400 MHz, CDCl₃) δ 7.56 (d, 1H), 7.39 (d, 1H), 6.34 (s, 1H), 5.03-4.92 (m, 2H), 4.05-4.04 (m, 1H), 2.28 (dd, 1H), 1.86-1.73 (m, 4H), 1.71-1.61 (m, 3H), 1.60-1.49 (m, 4H), 1.48-1.09 (m, 10H), 1.03-0.97 (m, 1H), 0.95 (s, 3H), 0.81-0.76 (m, 3H), 0.74 (s, 3H).

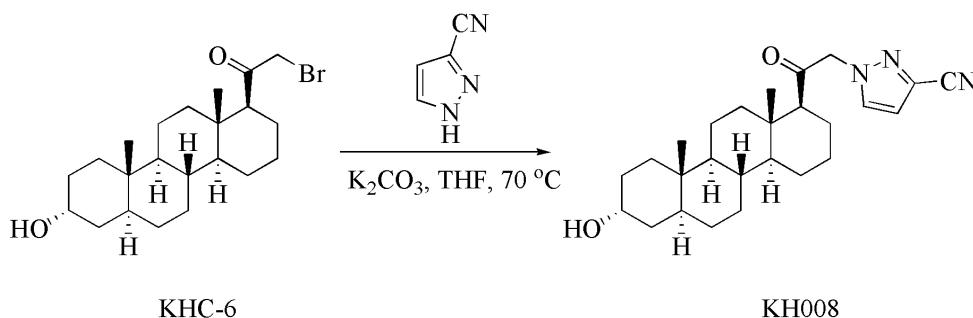
Example 6: Synthesis of compound KH007:



Compound KH007: Compound KHC-6 (150mg, 0.3mmol) was dissolved in THF (10mL), then K₂CO₃ (152mg, 1.1mmol) and 1H-imidazole (75mg, 1.1mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. Water (10mL) was added to the reaction solution, extraction was carried out with EA (2x20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by silica gel column flash chromatography (eluent: CH₂Cl₂/CH₃OH=30:1), followed by recrystallization (EA) to give white solid (35mg, 24.1% yield).

¹H NMR (400 MHz, CDCl₃) δ 7.47 (s, 1H), 7.11 (s, 1H), 6.85 (s, 1H), 4.74(d, 2H), 4.06-4.05 (m, 1H), 2.30 (dd, 1H), 1.88-1.68 (m, 4H), 1.64-1.56 (m, 3H), 1.52-1.45 (m, 4H), 1.40-1.11 (m, 10H), 1.04-0.97 (m, 1H), 0.95 (s, 3H), 0.83-0.77 (m, 3H), 0.75 (s, 3H).

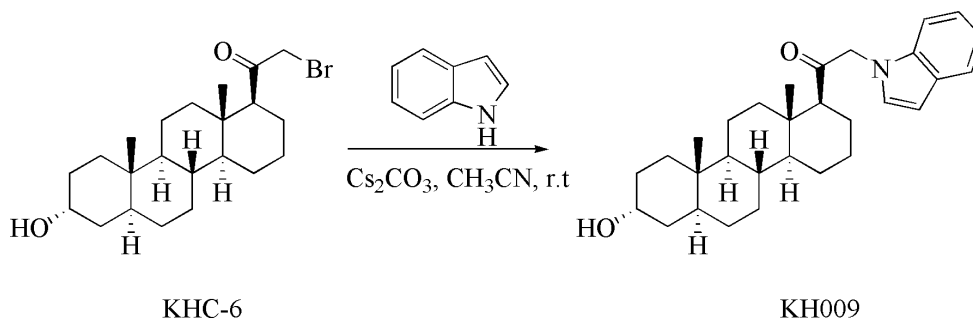
Example 7: Synthesis of compound KH008:



Compound KH008: Compound KHC-6 (100mg, 0.2mmol) was dissolved in THF (10mL), then K₂CO₃ (97mg, 0.7mmol) and 3-cyanopyrazole (68mg, 0.7mmol) was added. Reaction was performed at 70°C over 2 hours. TLC monitoring of the reaction showed that the reaction was complete, water (10mL) was added to the reaction solution, extraction was carried out with EA (2x20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by TLC (eluent: PE/ ACETONE=3:1), followed by recrystallization (CH₃CN) to give white solid (20mg, 19.4% yield).

¹H NMR (400 MHz, CDCl₃) δ 7.44 (d, 1H), 6.72 (d, 1H), 6.34 (s, 1H), 5.00 (q, 2H), 4.06-4.05 (m, 1H), 2.32 (dd, 1H), 1.88-1.68 (m, 4H), 1.63-1.60 (m, 3H), 1.50-1.45 (m, 4H), 1.41-1.11 (m, 10H), 1.04-0.97 (m, 1H), 0.95 (s, 3H), 0.87-0.79 (m, 3H), 0.75 (s, 3H).

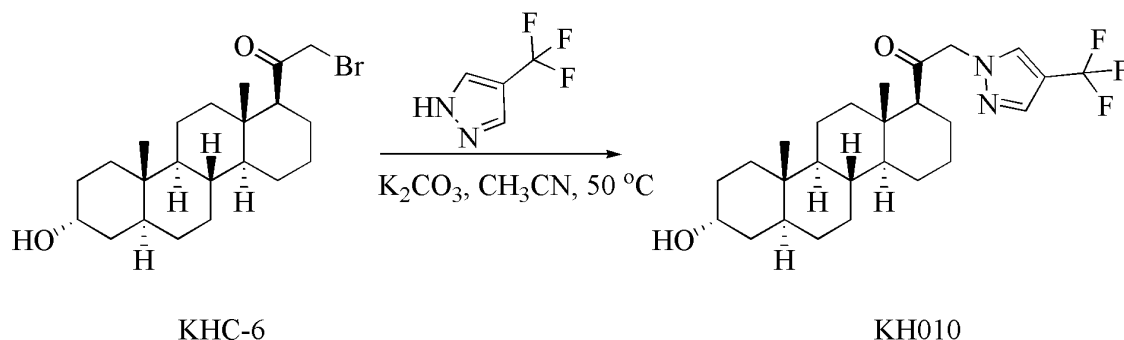
Example 8: Synthesis of compound KH009:



Compound KH009: Compound KHC-6 (50mg, 0.1mmol) was clearly dissolved in CH₃CN (5mL), then Cs₂CO₃ (120mg, 0.3mmol) and 1H-indole (68mg, 0.7mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. Water (10mL) was added to the reaction solution, extraction was carried out with EA (2x20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by TLC (eluent: CH₂Cl₂/EA=10:1) to give pale yellow solid (34 mg, 62.4% yield).

^1H NMR (400 MHz, CDCl_3) δ 7.65-7.63 (m, 1H), 7.21-7.18 (m, 1H), 7.13-7.10 (m, 2H), 7.01-7.00 (m, 1H), 6.57 (d, 1H), 4.83 (d, 2H), 4.06-4.05 (m, 1H), 2.30 (dd, 1H), 1.82-1.72 (m, 4H), 1.64-1.61 (m, 3H), 1.51-1.45 (m, 4H), 1.41-1.11 (m, 10H), 1.01-0.96 (m, 1H), 0.96 (s, 3H), 0.92-0.77 (m, 3H), 0.74 (s, 3H).

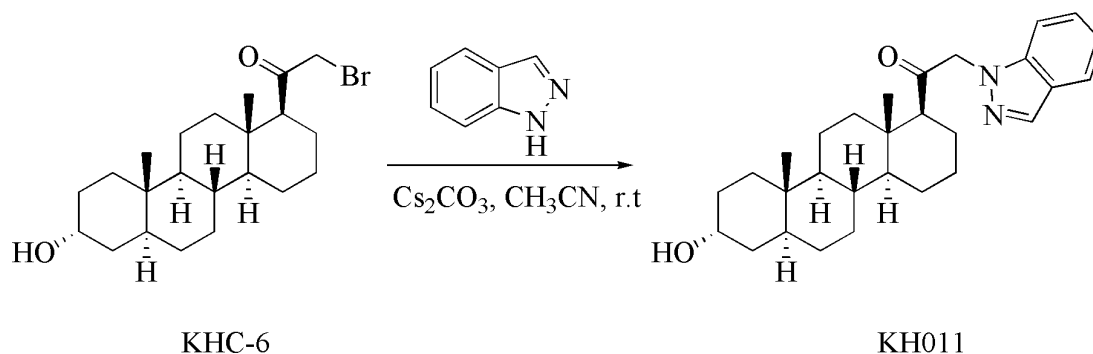
5 Example 9: Synthesis of compound KH010:



Compound KH010: Compound KHC-6 (100mg, 0.2mmol) was dissolved in CH_3CN (5mL), then K_2CO_3 (101mg, 0.7mmol) and 4- (trifluoromethyl) -1H-pyrazole (35mg, 0.2mmol) was added, Reaction was performed at 50°C over 2 hours. TLC monitoring of the reaction showed that the reaction was complete. Water (10mL) was added to the reaction solution, extraction was carried out with EA (2x20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na_2SO_4 , and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by silica gel column flash chromatography (eluent: PE/ ACETONE= 7:1), followed by recrystallization (CH_3CN) to give white solid (61mg, 53.1% yield).

^1H NMR (400 MHz, CDCl_3) δ 7.71 (d, 2H), 4.98 (q, 2H), 4.06-4.05 (m, 1H), 2.32 (dd, 1H), 1.87-1.68 (m, 4H), 1.64-1.57 (m, 3H), 1.52-1.45 (m, 4H), 1.41-1.11 (m, 10H), 1.04-0.97 (m, 1H), 0.95 (s, 3H), 0.87-0.82 (m, 3H), 0.74 (s, 3H).

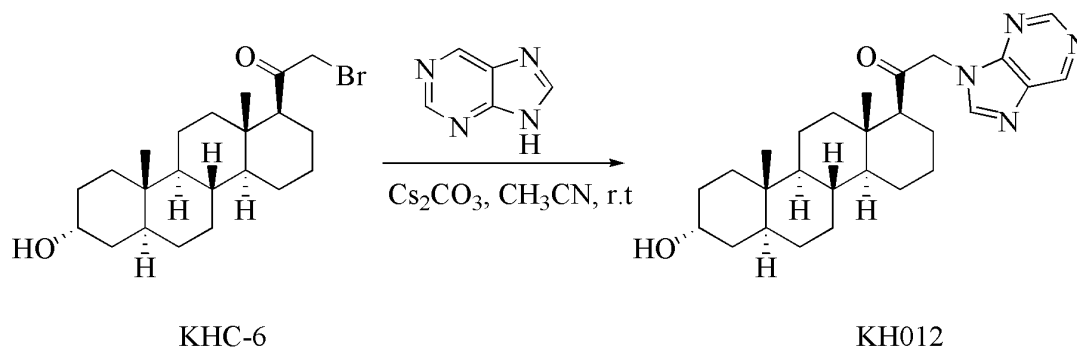
Example 10: Synthesis of compound KH011:



Compound KH011: Compound KHC-6 (50mg, 0.1mmol) was dissolved in CH_3CN (5mL), then Cs_2CO_3 (120mg, 0.3mmol) and 1H-indazole (43mg, 0.3mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. Water (10mL) was added to the reaction solution, extraction was carried out with EA (2x20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na_2SO_4 , and the solvent was distilled under reduced pressure to give crude pale yellow solid which was purified by TLC (eluent: PE/ ACETONE=3:1), followed by recrystallization (CH_3CN) to give white solid (20mg, 36.6%).

^1H NMR (400 MHz, CDCl_3) δ 8.11 (s, 1H), 7.78 (d, 1H), 7.45-7.41 (m, 1H), 7.23-7.19 (m, 2H), 5.31-5.20 (m, 2H), 4.06-4.05 (m, 1H), 2.35 (dd, 1H), 1.87-1.72 (m, 4H), 1.67-1.63 (m, 3H), 1.54-1.45 (m, 4H), 1.42-1.14 (m, 10H), 1.01-0.98 (m, 1H), 0.96 (s, 3H), 0.93-0.78 (m, 3H), 0.75 (s, 3H).

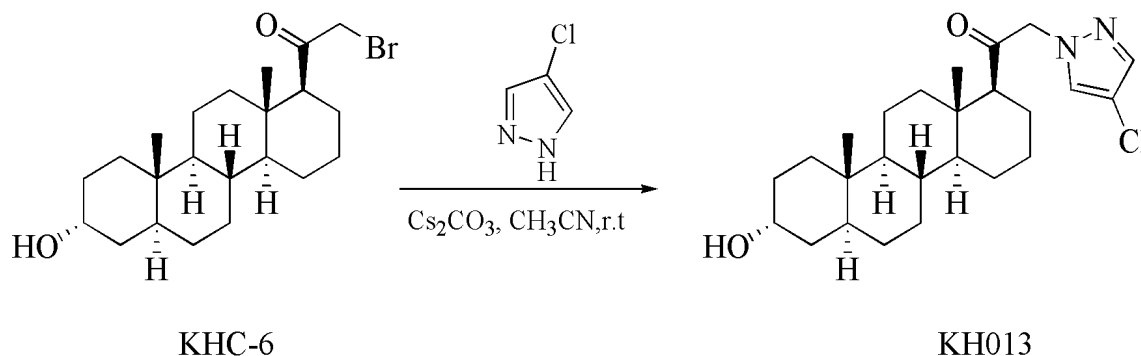
Example 11: Synthesis of compound KH012:



Compound KH012: Compound KHC-6(50mg, 0.1mmol) was dissolved in CH₃CN (5mL), then Cs₂CO₃ (120mg, 0.3mmol) and 1H-purine (44mg, 0.3mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. Water (10mL) was added to the reaction solution, the mixture was extracted with EA(2x20mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄ and the solvent was distilled under reduced pressure to give crude product was obtained as a pale yellow solid which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=20:1) followed by recrystallisation (CH₃CN) to give a white solid (5mg, 9.1% yield).

- ¹H NMR (400 MHz, CDCl₃) δ 9.22 (s, 1H), 9.06(s, 1H), 8.25(s, 1H), 5.18 (q, 2H), 4.07-4.06 (m, 1H), 2.49 (dd, 1H), 1.93-1.72 (m, 4H), 1.68-1.65 (m, 3H), 1.58-1.45 (m,4H), 1.42-1.13 (m, 10H), 1.06-1.00 (m, 1H), 0.96 (s, 3H), 0.90-0.80 (m, 3H), 0.74 (s, 3H).

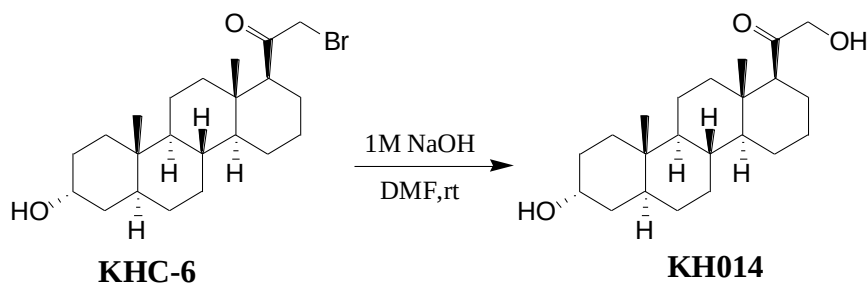
Example 12: Synthesis of compound KH013:



Compound KH013: Compound KHC-6 (80mg, 0.2mmol) was dissolved in CH₃CN (8mL), then Cs₂CO₃ (196mg, 0.6mmol) and 4-chloropyrazole (62mg, 0.6mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete, 15mL water was added to the reaction mixture, the mixture was extracted with EA (2x30mL), the combined organic layers were washed with saturated brine, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude pale yellow solid, which was purified by silica gel column flash chromatography (eluent: CH₂Cl₂/CH₃OH=100:1) and then recrystallized (EA/PE=1:10) to give a white solid (28mg, 32.3% yield).

- ¹H NMR (400MHz, CDCl₃) δ 7.46 (s, 1H), 7.38 (s, 1H), 4.96-4.83 (m, 2H), 4.06-4.05 (m, 1H), 2.19 (dd, 1H), 1.86-1.71 (m, 4H), 1.64-1.55 (m, 3H), 1.51-1.45 (m,4H), 1.45-1.10 (m, 10H), 1.03-0.96 (m, 1H), 0.95 (s, 3H), 0.86-0.77 (m, 3H), 0.74 (s, 3H).

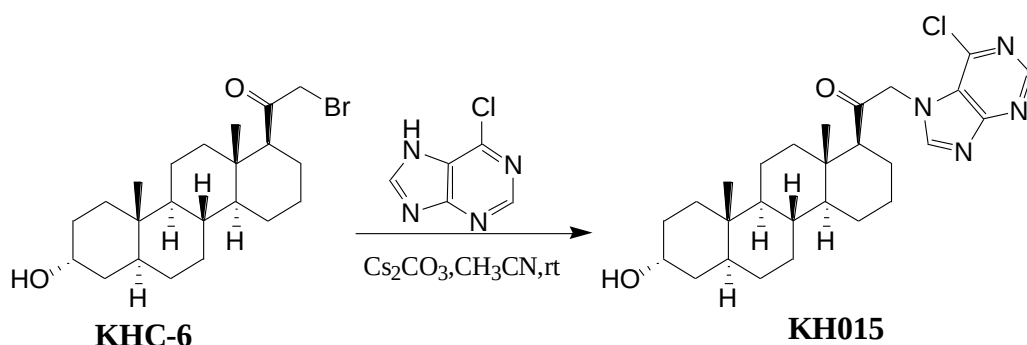
Example 13: Synthesis of compound KH014:



Compound KH014: compound KHC-6 (60mg, 0.15mmol) was dissolved in DMF (5mL) then NaOH (1mL, 1.0mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 × 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=50:1) to give a white solid (12mg, 22.92% yield).

¹H NMR (400 MHz, DMSO-*d*₆) δ 4.94 - 4.89 (m, 1H), 4.18 (t, 1H), 4.06 - 4.02 (m, 2H), 3.80 (t, 1H), 2.27 (dd, 1H), 1.77 - 0.65 (m, 27H), 0.68 (s, 3H).

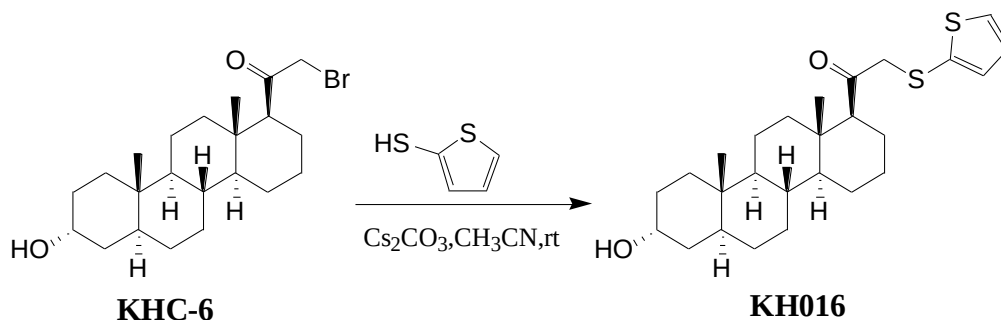
Example 14: Synthesis of compound KH015:



Compound KH015: Compound KHC-6 (60mg, 0.15mmol) was dissolved in CH₃CN (5mL), then Cs₂CO₃ (98mg, 0.30mmol) and 6-chloropurine (46mg, 0.30mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 × 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH= 40:1) to give a white solid (39mg, 53.61% yield).

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.60 (s, 1H), 8.37 (s, 1H), 5.41 (q, 2H), 4.20 (d, 1H), 3.79 (t, 1H), 2.56 (dd, 1H), 1.80 - 0.75 (m, 27H), 0.69 (s, 3H).

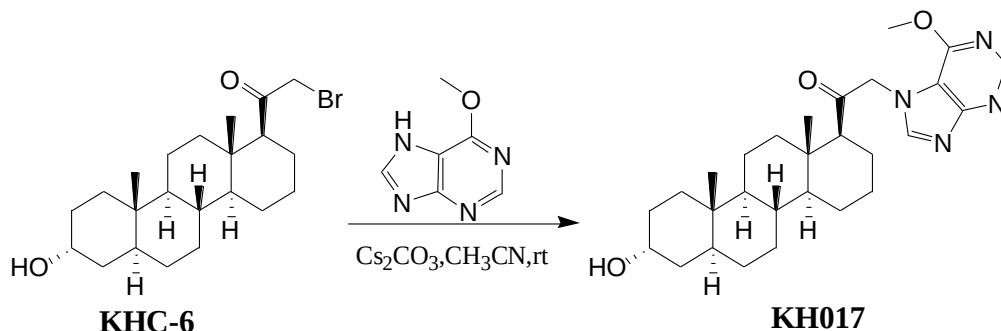
Example 15: Synthesis of compound KH016:



Compound KH016: KHC-6 (60mg, 0.15mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (98mg, 0.30mmol) and 2-mercaptothiophene (17mg, 0.30mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH= 40:1) to give a white solid (19mg, 28.34% yield).

¹H NMR (400 MHz, CDCl₃) δ 7.35 (d, 1H), 7.14 (d, 1H), 6.95 (dd, 1H), 4.04 (t, 1H), 3.62 (q, 2H), 2.45 (dd, 1H), 1.65 – 0.81 (m, 27H), 0.73 (s, 3H).

10 Example 16: Synthesis of compound KH017:

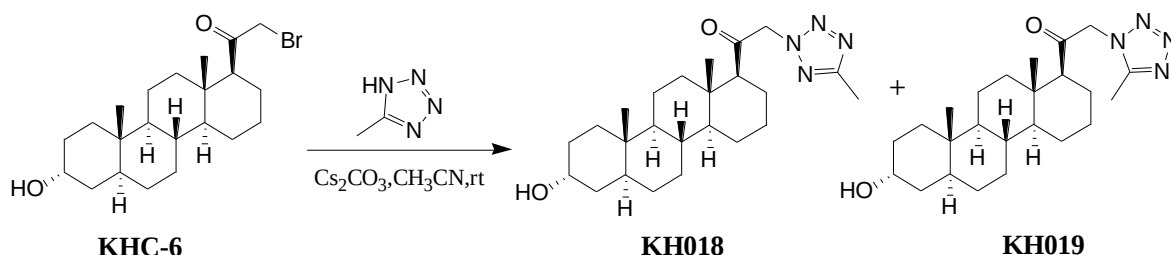


Compound KH017: KHC-6 (60mg, 0.15mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (98mg, 0.30mmol) and 6-methoxypurine (45mg, 0.30mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL water 10mL was added to the reaction mixture, the mixture extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH= 40:1) to give a white solid (46mg, 63.76% yield).

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.57 (s, 1H), 8.32 (s, 1H), 5.37 (q, 2H), 4.20 (d, 1H), 3.91 (s, 3H), 3.79 (t, 1H), 2.53 (dd, 1H), 1.79 – 0.75 (m, 27H), 0.69 (s, 3H).

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Example 17: Synthesis of compound KH018 and KH019:

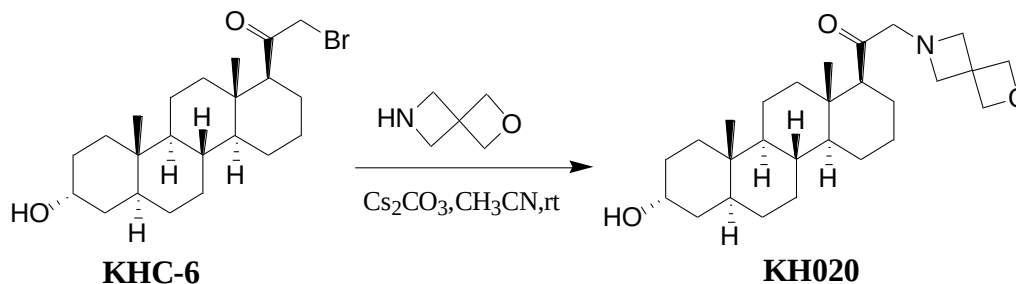


Compound KH018 and KH019: KHC-6 (60mg, 0.15mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (98mg, 0.30mmol) and 5-methyl-1H-tetrazole (25mg, 0.30mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH= 40:1) to give white solid KH6070053-1(13mg, 20.88%) and KH6070053-2(27mg, 43.37%) as white solids, respectively.

30 ¹H NMR (400 MHz, CDCl₃) δ 4.39 (s, 2H), 4.05 (t, 1H), 2.56 (s, 3H), 2.34 (dd, 1H), 1.92 – 0.76 (m, 27H), 0.74 (s, 3H).

¹H NMR (400 MHz, CDCl₃) δ 5.15 (dd, 2H), 4.06 (t, 1H), 2.43 (s, 3H), 2.39 (dd, 1H), 1.95 – 0.78 (m, 27H), 0.75 (s, 3H).

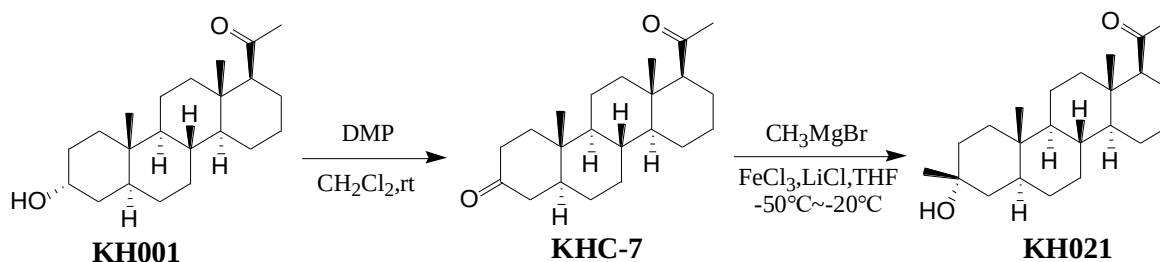
Example 18: Synthesis of compound KH020:



Compound KH020: KHC-6 (60mg, 0.15mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (98mg, 0.30mmol) and 2-oxa-6-aza-spiro [3,3]Heptane (30mg, 0.30mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH= 40:1) to give a white solid (21mg, 32.56% yield).

¹H NMR (400 MHz, CDCl₃) δ 4.75 (s, 4H), 4.04 (t, 1H), 3.57 (dd, 4H), 3.40 (dd, 2H), 2.13 (dd, 1H), 1.90 – 0.75 (m, 27H), 0.73 (s, 3H).

Example 19: Synthesis of compound KH021:

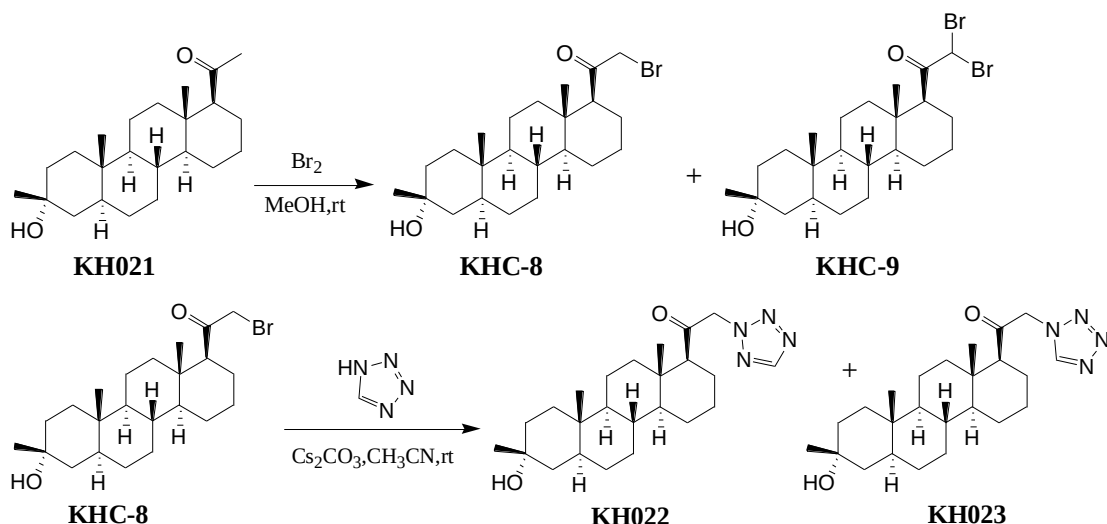


Compound KHC-7: KH001 (0.70g, 2.11mmol) was dissolved in 100mL CH₂Cl₂ and DMP (1.78g, 4.20mmol) was added in batches. Reaction was performed at room temperature overnight under N₂ atmosphere. TLC monitoring of the reaction showed that the reaction was complete. 40mL of water was added to the reaction mixture, the mixture was extracted with EA (2x80mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by flash chromatography on silica gel (eluent: PE/Acetone = 40:1 → 30:1) to give KHC-7 as a white solid (0.62g, 89.47% yield).

Compound KH021: FeCl₃ (0.61g, 3.75mmol) and LiCl (0.34g, 8.07mmol) were mixed and added into a 100mL of three-necked flask under N₂ atmosphere, then anhydrous THF (40mL) was added and the mixture was stirred at RT for 5 minutes and cooled to -45°C. Then CH₃MgBr (15.20mL, 15.20mmol) was added dropwise and the mixture was stirred at -40°C for 15 minutes, then compound KHC-7 (0.62g, 1.88mmol) dissolved in anhydrous THF was added dropwise. The reaction was heated to about -20°C and stirred for 2 hours. TLC monitoring of the reaction showed that the reaction was complete. The reaction solution was quenched with 40mL saturated NH₄Cl aqueous solution and the mixture was extracted with EA (2x80mL). The combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by flash chromatography on silica gel (eluent: PE/acetone=40:1 → 35:1) to give a white solid KH021 (0.52g, 78.50% yield).

¹H NMR (400 MHz, CDCl₃) 2.29 (dd, 1H), 2.13 (s, 3H), 1.88 – 1.25 (m, 21H), 1.20 (s, 3H), 0.95 (s, 3H), 0.88 – 0.78 (m, 3H), 0.70 (s, 3H).

Example 20: Synthesis of compound KH022 and KH023:



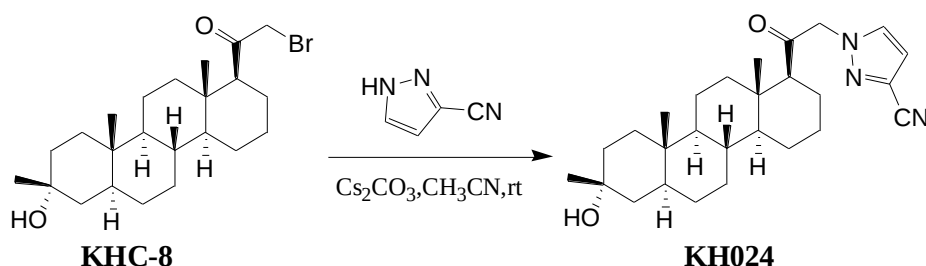
Compounds KHC-8 and KHC-9: Compound KH021 (1.25 g, 3.60 mmol) was dissolved in 40 mL of methanol until clear, then bromine (0.86 g, 5.40 mmol) was added dropwise. After 4 hours of reaction at room temperature, the reaction solution turned from orange to light yellow, and TLC monitoring showed that the reaction was complete. The reaction mixture was added with 30 ml of water, extracted with EA (2 x 65 mL), the combined organic layers were washed with saturated sodium chloride, dried over anhydrous sodium sulfate, and the solvent was distilled under reduced pressure to obtain a light yellow crude solid, which was purified by flash chromatography on silica gel (elute with dichloromethane) to obtain white solid KHC-8 (0.93 g, 60.78%) and white solid KHC-9 (0.36 g, 19.89%).

Compounds KH022 and KH023: Compound KHC-8 (100 mg, 0.24 mmol) was dissolved in 5 mL of acetonitrile until clear, Cs_2CO_3 (234 mg, 0.72 mmol) and 1H-tetrazole (50 mg, 0.72 mmol) were added. Reaction was performed at room temperature overnight, and TLC monitoring showed that the reaction was complete. The reaction mixture was added with 10 ml of water, extracted with ethyl acetate (2 x 20mL), the combined organic layers were washed with saturated sodium chloride, dried over anhydrous sodium sulfate, and the solvent was distilled under reduced pressure to obtain crude product. Thin layer chromatography with thick silica gel preparation plate was used for purification (elute with dichloromethane/methanol = 30:1) to obtain a white solid KH022 (20 mg, 20.08%) and a white solid KH023 (47 mg, 47.19%), respectively.

^1H NMR (400 MHz, CDCl_3) δ 8.56 (s, 1H), 5.54 – 5.45 (m, 2H), 2.35 (dd, 1H), 1.89 – 1.25 (m, 21H), 1.20 (s, 3H), 0.95 (s, 3H), 0.88 – 0.80 (m, 3H), 0.72 (s, 3H).

^1H NMR (400 MHz, CDCl_3) δ 8.71 (s, 1H), 5.30 (dd, 2H), 2.35 (dd, 1H), 1.83 – 1.23 (m, 21H), 1.20 (s, 3H), 0.95 (s, 3H), 0.87 – 0.78 (m, 3H), 0.72 (s, 3H).

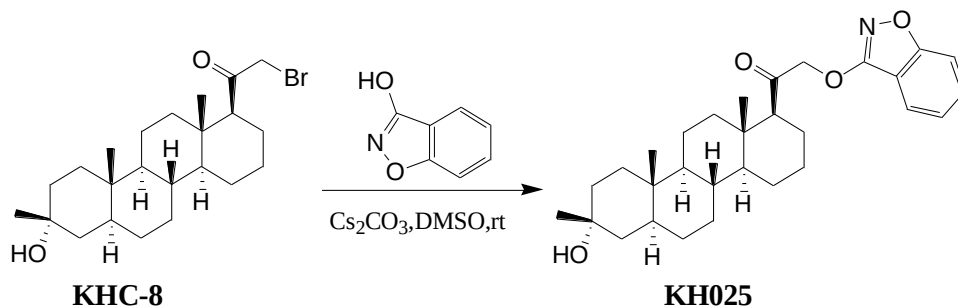
Example 21: Synthesis of compound KH024:



Compounds KH024: KHC-8 (60mg, 0.14mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (91mg, 0.28mmol) and 3-cyanopyrazole (26mg, 0.28mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH= 40:1) to give a white solid KH024 (16mg, 26.09%).

¹H NMR (400 MHz, CDCl₃) δ 7.45 (d, 1H), 6.73 (d, 1H), 5.06 (q, 2H), 2.30 (dd, 1H), 1.86 – 1.23 (m, 21H), 1.20 (s, 3H), 0.95 (s, 3H), 0.87 – 0.77 (m, 3H), 0.72 (s, 3H).

10 Example 22: Synthesis of compound KH025:

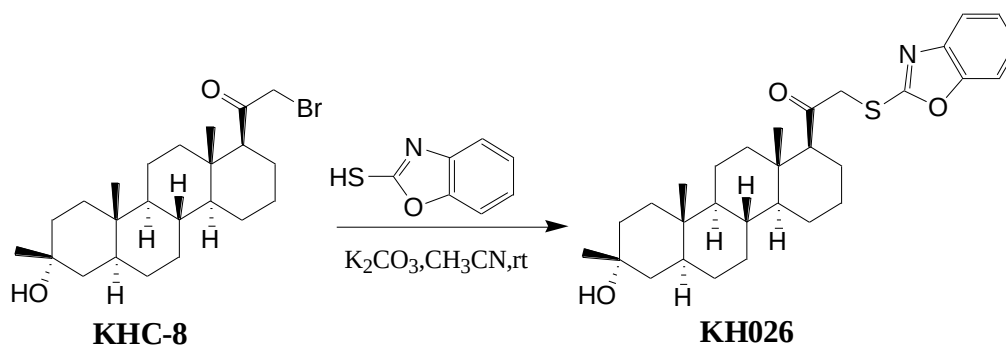


Compounds KH025: KHC-8 (100mg, 0.24mmol) was dissolved in DMSO 5mL, then Cs₂CO₃ (235mg, 0.72mmol) and 3-hydroxybenzooxazole (97mg, 0.72mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone= 5:1) to give a white solid KH025 (70mg, 60.76%).

¹H NMR (400 MHz, CDCl₃) δ 7.72 (d, 1H), 7.58 – 7.49 (m, 1H), 7.42 (d, 1H), 7.29 (d, 1H), 5.11 – 4.95 (m, 2H), 2.35 (dd, 1H), 1.87 – 1.22 (m, 21H), 1.20 (s, 3H), 0.99 (s, 3H), 0.87 – 0.80 (m, 3H), 0.72 (s, 3H).

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Example 23: Synthesis of compound KH026:

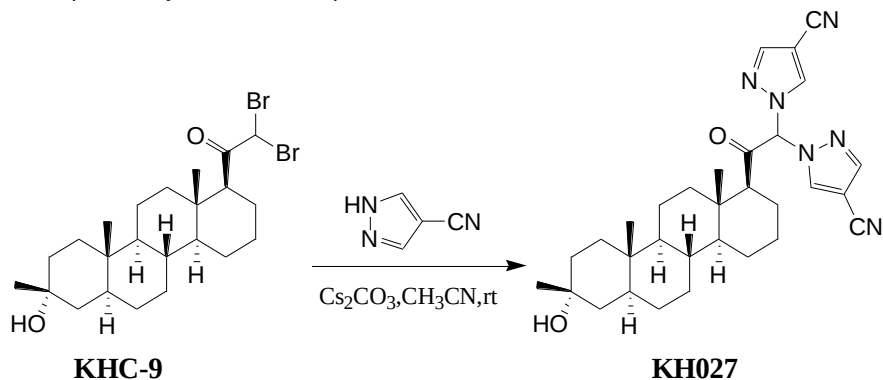


Compounds KH026: KHC-8 (53mg, 0.12mmol) was dissolved in CH₃CN 5mL, then K₂CO₃ (100mg, 0.72mmol) and 2-mercaptobenzoxazole (24mg, 0.16mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone= 6:1) to give a white solid KH026 (30mg, 50.40%).

¹H NMR (400 MHz, CDCl₃) δ 7.55 (d, 1H), 7.42 (d, 1H), 7.34 – 7.24 (m, 2H), 4.33 (m, 2H), 2.54 (dd, 1H), 1.83 – 1.23 (m, 21H), 1.18 (s, 3H), 0.94 (s, 3H), 0.85 – 0.78 (m, 3H), 0.70 (s, 3H).

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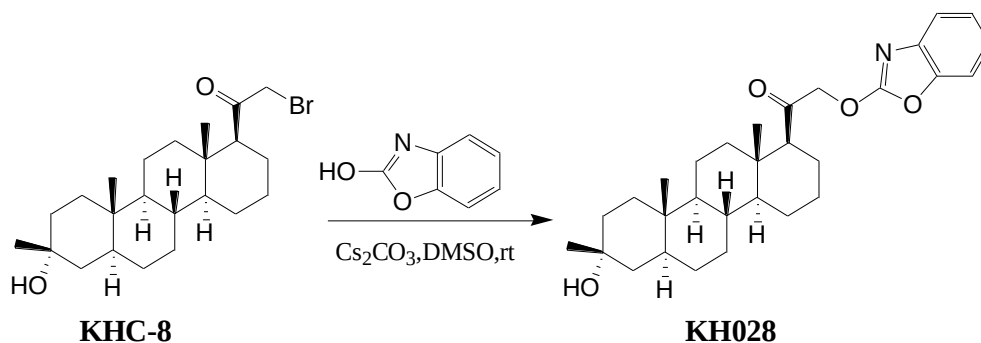
Example 24: Synthesis of compound KH027:



Compounds KH027: KHC-9 (110mg, 0.22mmol) was dissolved in 10mL CH₃CN, then Cs₂CO₃ (215mg, 0.66mmol) and 4-cyanopyrazole (61mg, 0.66 mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring showed that the reaction was complete, 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH= 6:1) to give a white solid KH027 (76mg, 65.30%).

- ¹H NMR (400 MHz, CDCl₃) δ 8.23 (s, 1H), 8.13 (s, 1H), 7.92 (s, 1H), 7.83 (s, 1H), 7.03 (s, 1H), 2.11 (dd, 1H), 1.83 – 1.23 (m, 21H), 1.20 (s, 3H), 0.91 (s, 3H), 0.82 – 0.75 (m, 3H), 0.70 (s, 3H).

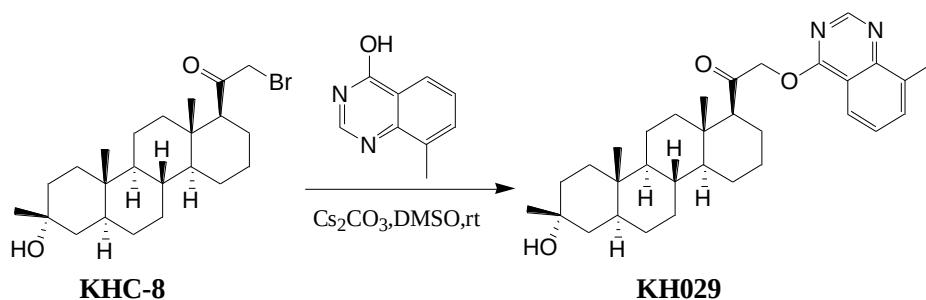
Example 25: Synthesis of compound KH028:



- Compounds KH028: KHC-8 (70mg, 0.16mmol) was dissolved in 8mL DMSO, then Cs₂CO₃ (160mg, 0.49mmol) and 2-hydroxybenzoxazole (66mg, 0.49mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH= 60:1) to give a white solid KH028 (55mg, 71.61%).

¹H NMR (400 MHz, CDCl₃) δ 7.24 – 7.18 (m, 1H), 7.17 – 7.06 (m, 2H), 6.75 – 6.64 (m, 1H), 4.58 (dd, 2H), 2.38 (dd, 1H), 1.92 – 1.22 (m, 21H), 1.20 (s, 3H), 0.97 (s, 3H), 0.89 – 0.78 (m, 3H), 0.72 (s, 3H).

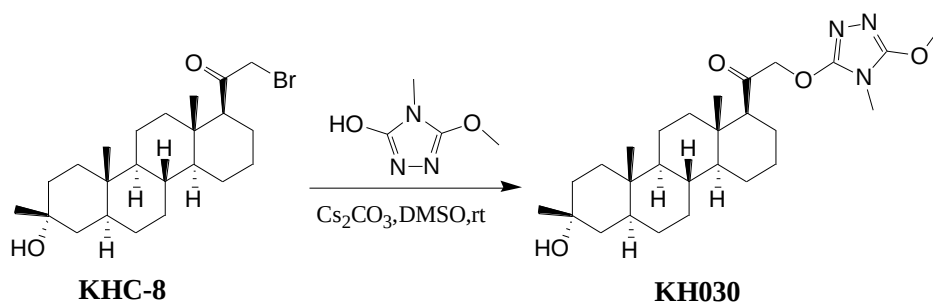
Example 26: Synthesis of compound KH029:



Compounds KH029: KHC-8 (60 mg, 0.14 mmol) was dissolved in 5 mL DMSO, then Cs_2CO_3 (138 mg, 0.42 mmol) and 8-methyl-4-hydroxyquinazoline (68 mg, 0.42 mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring showed that the reaction was complete. 10 mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na_2SO_4 , and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}=60:1$) to give a white solid KH029 (50mg, 70.72%).

^1H NMR (400 MHz, CDCl_3) δ 8.13 (d, 1H), 7.90 (s, 1H), 7.61 (d, 1H), 7.39 (t, 1H), 4.81 (s, 2H), 2.63 (s, 3H), 2.43 (dd, 1H), 1.88 – 1.22 (m, 21H), 1.20 (s, 3H), 0.97 (s, 3H), 0.90 – 0.81 (m, 3H), 0.72 (s, 3H).

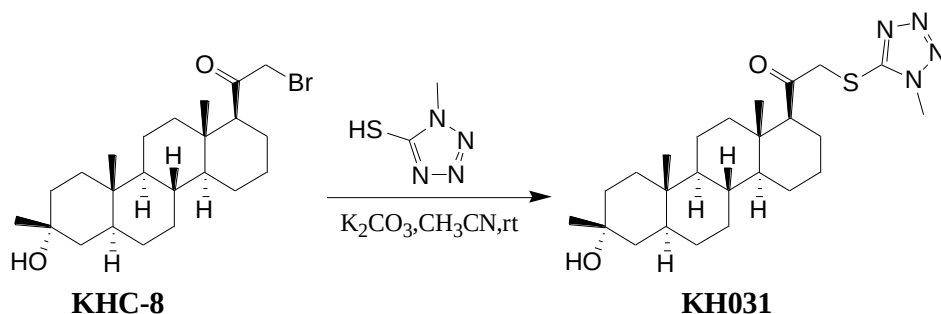
Example 27: Synthesis of compound KH030:



Compounds KH030: KHC-8 (50mg, 0.12mmol) was dissolved in 5mL DMSO, then Cs_2CO_3 (78mg, 0.24mmol) and 3-hydroxy-4-methyl-5-methoxy-4H-1,2,4-triazole (31mg, 0.24 mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na_2SO_4 , and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=4:1) to give a white solid KH030 (20mg, 35.16%).

^1H NMR (400 MHz, CDCl_3) δ 4.47 (q, 2H), 3.93 (s, 3H), 3.12 (s, 3H), 2.28 (dd, 1H), 1.86 – 1.22 (m, 21H), 1.19 (s, 3H), 0.94 (s, 3H), 0.85 – 0.78 (m, 3H), 0.70 (s, 3H).

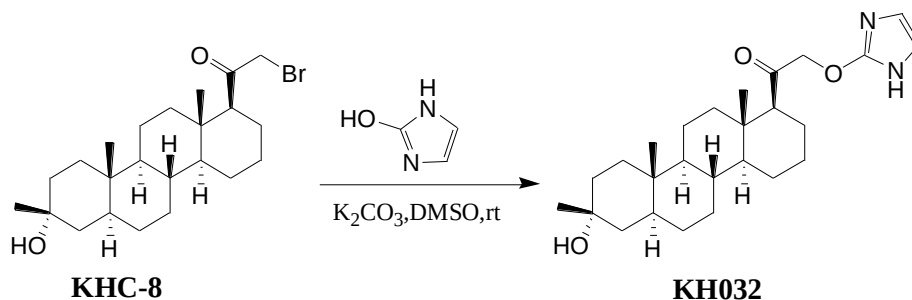
Example 28: Synthesis of compound KH031:



Compounds KH031: KHC-8 (60mg, 0.14mmol) was dissolved in 5mL CH₃CN, then K₂CO₃ (58mg, 0.42mmol) and 1-methyl-5-mercapto-1H-tetrazole (68mg, 0.42 mmol) was added. Reaction was performed at room temperature. TLC monitoring showed that the reaction was complete. 10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=4:1) to give a white solid KH031(50mg, 77.47%).

¹H NMR (400 MHz, CDCl₃) 4.40 (dd, 2H), 3.97 (s, 3H), 2.47 (dd, 1H), 1.88 – 1.22 (m, 21H), 1.19 (s, 3H), 0.93 (s, 3H), 0.87 – 0.79 (m, 3H), 0.71 (s, 3H).

10 Example 29: Synthesis of compound KH032:

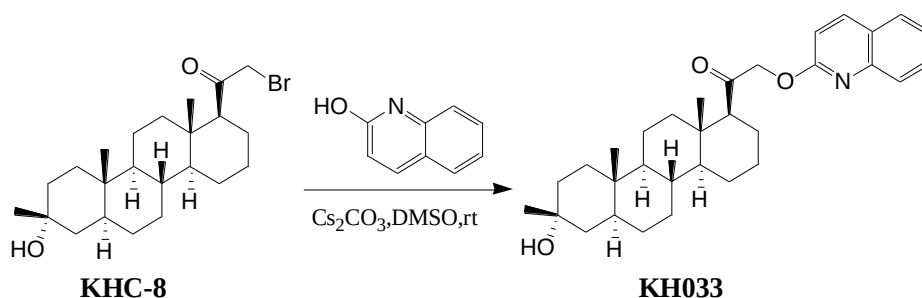


Compounds KH032: KHC-8 (50mg, 0.12mmol) was dissolved in 5mL DMSO, then K₂CO₃ (50mg, 0.36mmol) and 1-hydroxy-1H-imidazole (30mg, 0.36mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=2:1) to give a white solid KH032(11mg, 21.37%).

¹H NMR (400 MHz, CDCl₃) δ 9.26 (s, 1H), 6.32 (s, 1H), 6.17 (s, 1H), 4.53 – 4.32 (m, 2H), 2.31 (dd, 1H), 1.86 – 1.25 (m, 21H), 1.21 (s, 3H), 0.95 (s, 3H), 0.88 – 0.78 (m, 3H), 0.71 (s, 3H).

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Example 30: Synthesis of compound KH033:

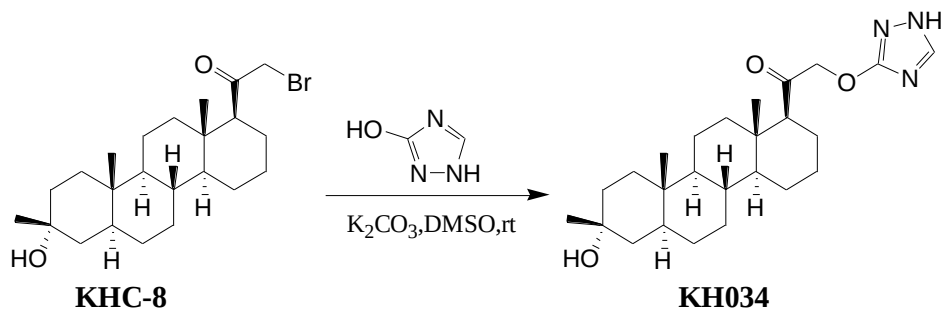


Compounds KH033: KHC-8 (60mg, 0.14mmol) was dissolved in 5mL DMSO, then Cs₂CO₃ (138mg, 0.42mmol) and 2-hydroxyquinoline (61mg, 0.42mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=4:1) to give a white solid KH033(15mg, 21.87%).

¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, 1H), 7.75 (dd, 2H), 7.61 (t, 1H), 7.39 (t, 1H), 7.05 (d, 1H), 5.15 (s, 2H), 2.43 (dd, 1H), 1.98 – 1.23 (m, 21H), 1.21 (s, 3H), 0.97 (s, 3H), 0.91 – 0.78 (m, 3H), 0.74 (s, 3H).

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Example 31: Synthesis of compound KH034:



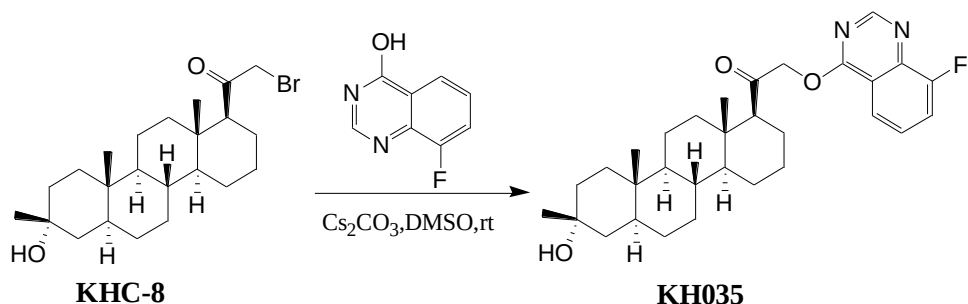
Compounds KH034: KHC-8 (60mg, 0.14mmol) was dissolved in DMSO 5mL, then K_2CO_3 (58mg, 0.42mmol) and 3-hydroxy-1H-1,2,4-triazole (36mg, 0.42mmol) was added. Reaction was performed at room temperature overnight.

- 5 TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na_2SO_4 , and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: $CH_2Cl_2/CH_3OH=40:1$) to give a white solid KH034(40mg, 66.45%).

1H NMR (400 MHz, $CDCl_3$) δ 9.30 (s, 1H), 7.42 (s, 1H), 4.47 (q, 2H), 2.33 (dd, 1H), 1.87 – 1.25 (m, 21H), 1.20 (s, 3H),

- 10 0.95 (s, 3H), 0.87 – 0.79 (m, 3H), 0.71 (s, 3H).

Example 32: Synthesis of compound KH035:

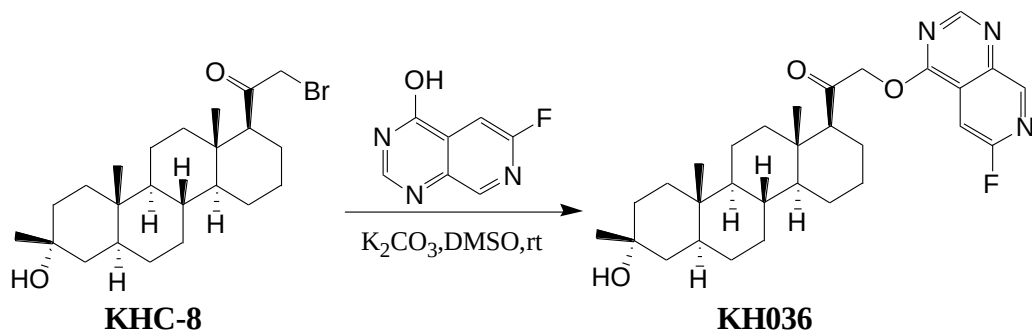


Compounds KH035: KHC-8 (80mg, 0.19mmol) was dissolved in DMSO 6mL, then Cs_2CO_3 (184mg, 0.56mmol) and 8-fluoro-4-hydroxyquinazoline (92mg, 0.56 mmol) was added. Reaction was performed at room temperature overnight.

- 15 TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na_2SO_4 , and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=2:1) to give a white solid KH035(65mg, 67.21%).

- 20 1H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, 1H), 7.88 (s, 1H), 7.55 – 7.40 (m, 2H), 4.79 (m, 2H), 2.43 (dd, 1H), 1.88 – 1.22 (m, 21H), 1.21 (s, 3H), 0.96 (s, 3H), 0.90 – 0.81 (m, 3H), 0.72 (s, 3H).

Example 33: Synthesis of compound KH036:

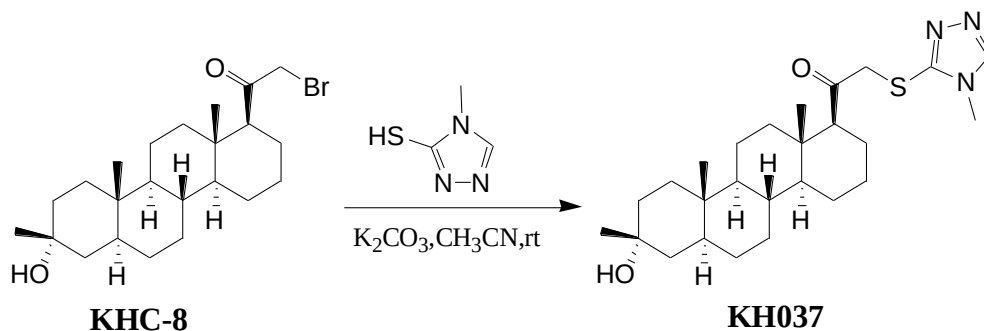


Compounds KH036: KHC-8 (60mg, 0.14mmol) was dissolved in DMSO 5mL, then K₂CO₃ (58mg, 0.42mmol) and 6-fluoropyridine [3,4-d] Pyrimidin-4-one (69mg, 0.42mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with

5 saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=40:1) to give a white solid KH036(40mg, 56.02%).

¹H NMR (400 MHz, CDCl₃) δ 8.82 (s, 1H), 7.80 (s, 1H), 7.66 (d, 1H), 4.80 (q, 2H), 2.43 (dd, 1H), 1.89 – 1.23 (m, 21H), 1.20 (s, 3H), 0.95 (s, 3H), 0.90 – 0.80 (m, 3H), 0.71 (s, 3H).

10 Example 34: Synthesis of compound KH037:



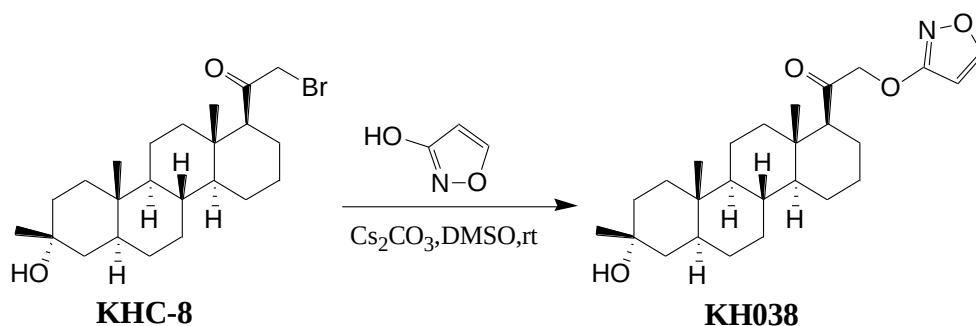
Compounds KH037: KHC-8(60mg, 0.14mmol) was dissolved in CH₃CN 5mL, then K₂CO₃ (58mg, 0.42mmol) and 4-methyl-4H-3-mercapto-1, 2, 4-triazole (49mg, 0.42mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was

15 added to the reaction mixture, the mixture was extracted with EA (2x20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=3:2) to give a white solid KH037 (53mg, 82.30%).

¹H NMR (400 MHz, CDCl₃) δ 8.16 (s, 1H), 4.34 (dd, 2H), 3.65 (s, 3H), 2.45 (dd, 1H), 1.88 – 1.22 (m, 21H), 1.19 (s, 3H), 0.91 (s, 3H), 0.87 – 0.79 (m, 3H), 0.70 (s, 3H).

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Example 35: Synthesis of compound KH038:



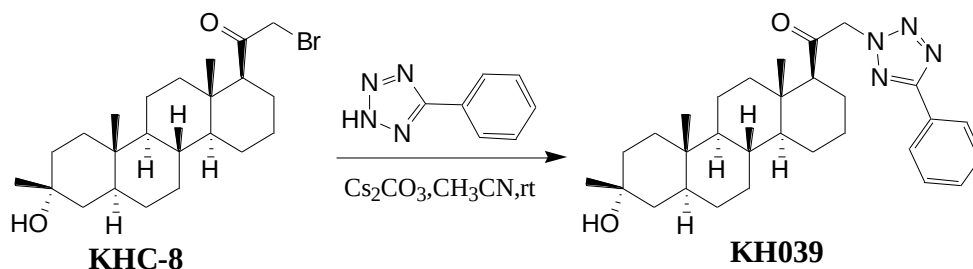
Compounds KH038: KHC-8(60mg, 0.14mmol) was dissolved in DMSO 5mL, then Cs₂CO₃ (138mg, 0.42mmol) and 3-hydroxyisoxazole (36mg, 0.42mmol) was added. Reaction was performed at room temperature overnight. TLC

25 monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=15:1) to give a white solid KH038(39mg, 65.00%).

¹H NMR (300 MHz, CDCl₃) δ 8.12 (d, 1H), 6.06 (d, 1H), 4.87 (s, 2H), 2.29 (dd, 1H), 1.90 – 1.22 (m, 21H), 1.20 (s, 3H),

30 0.97 (s, 3H), 0.90 – 0.74 (m, 3H), 0.71 (s, 3H).

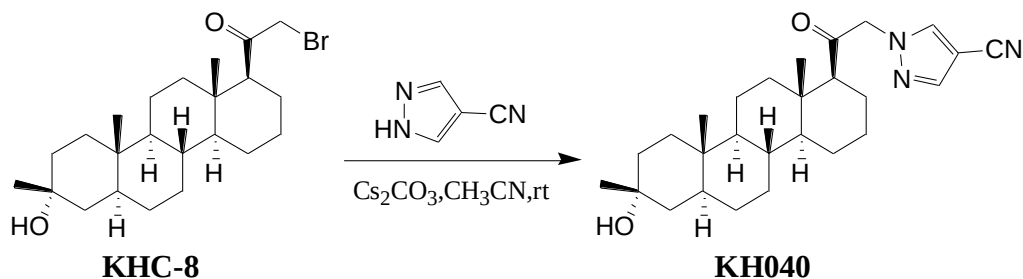
Example 36: Synthesis of compound KH039:



Compounds KH039: KHC-8(75mg, 0.18mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (171mg, 0.53mmol) and 5-phenyltetrazole (77mg, 0.53mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=3:1) to give a white solid KH039(79mg, 89.39%).

¹H NMR (400 MHz, CDCl₃) δ 8.19 – 8.07 (m, 2H), 7.55 – 7.42 (m, 3H), 5.54 – 5.40 (m, 2H), 2.37 (dd, 1H), 1.92 – 1.23 (m, 21H), 1.21 (s, 3H), 0.97 (s, 3H), 0.90 – 0.78 (m, 3H), 0.73 (s, 3H).

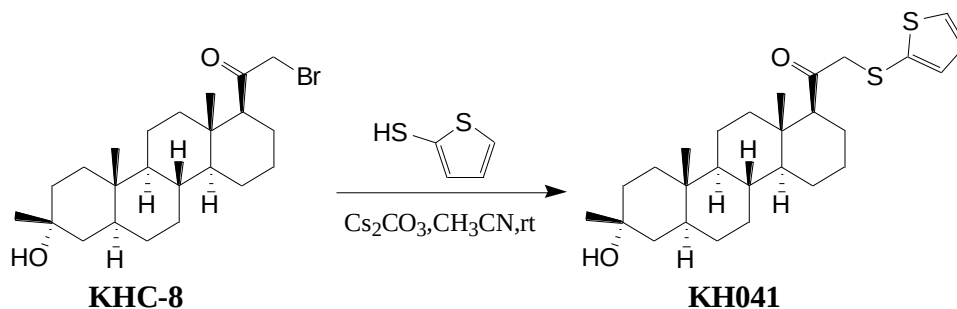
Example 37: Synthesis of compound KH040:



Compounds KH040: KHC-8(60mg, 0.14mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (91mg, 0.28mmol) and 4-cyanopyrazole (26mg, 0.28mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=40:1) to give a white solid KH040(16mg, 26.09%).

¹H NMR (400 MHz, CDCl₃) δ 7.82 (s, 1H), 7.81 (s, 1H), 5.00 (q, 2H), 2.32 (dd, 1H), 1.88 – 1.22 (m, 21H), 1.20 (s, 3H), 0.94 (s, 3H), 0.87 – 0.79 (m, 3H), 0.71 (s, 3H).

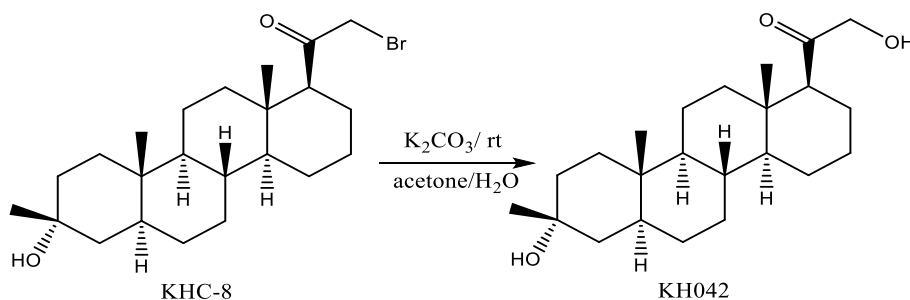
Example 38: Synthesis of compound KH041:



Compounds KH041: KHC-8(70mg, 0.16mmol) was dissolved in 5mL CH₃CN, then Cs₂CO₃ (160mg, 0.49mmol) and 2-mercaptothiophene (57mg, 0.49mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/PE=1:1) to give a grey solid KH041(27mg, 35.53%).

¹H NMR (400 MHz, CDCl₃) δ 7.35 (dd, 1H), 7.14 (dd, 1H), 6.95 (dd, 1H), 3.62 (dd, 2H), 1.90 – 1.21 (m, 21H), 1.20 (s, 3H), 0.90 (s, 3H), 0.84 - 0.74 (m, 3H), 0.70 (s, 3H).

10 Example 39: Synthesis of compound KH042:

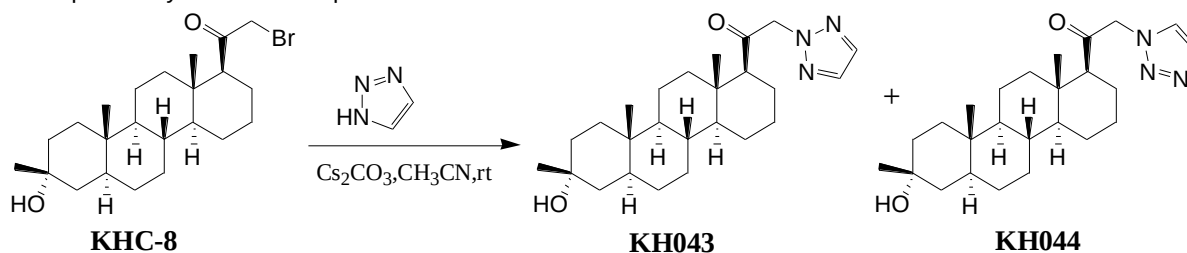


Compounds KH042: KHC-8 (150mg, 0.35mmol) was dissolved in 15mL of water and 30mL of acetone, then K₂CO₃ (49mg, 0.35mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 30mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=50:1) to give a white solid KH042(21mg, 16.54%).

¹H NMR (400 MHz, DMSO-*d*₆) δ 4.92 (t, 1H), 4.06 - 4.04 (m, 2H), 3.85 (t, 1H), 2.27 (dd, 1H), 1.82 – 1.10 (m, 21H), 1.05 (s, 3H), 0.87 (s, 3H), 0.85 - 0.70 (m, 3H), 0.65 (s, 3H).

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Example 39: Synthesis of compound KH043 and KH044:



Compounds KH043 and KH044: KHC-8 (60mg, 0.14mmol) was dissolved in 5ml CH₃CN, then Cs₂CO₃ (91mg, 0.28mmol) and 1H-triazole (15mg, 0.21mmol) was added. Reaction was performed at room temperature overnight.

TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=40:1) to give a white solid KH6070100-1(12mg, 20.70%) and a white solid KH6070100-2(27mg, 46.58%) respectively.

¹H NMR (400 MHz, CDCl₃) δ 7.68 (s, 2H), 5.34 – 5.20 (m, 2H), 2.25 (dd, 1H), 1.88 – 1.23 (m, 21H), 1.20 (s, 3H), 0.95 (s, 3H), 0.89 – 0.79 (m, 3H), 0.71 (s, 3H).

¹H NMR (400 MHz, CDCl₃) δ 7.76 (s, 1H), 7.61 (s, 1H), 5.25 (dd, 2H), 2.37 (dd, 1H), 1.86 – 1.22 (m, 21H), 1.20 (s, 3H), 0.94 (s, 3H), 0.86 – 0.76 (m, 3H), 0.71 (s, 3H).

Example 40: Synthesis of compound KH045:

Compounds KHC-11: Compound KHC-10 (150.0g, 183.8mmol) was dissolved in THF 500mL and 10% Pd/C (6.0g) was added to the solution. Then the solution was hydrogenated under H₂ atmosphere and kept stirring at RT for 24 hours, subsequently filtered. The filtrate was concentrated under vacuum, to obtain crude product which was subsequently stirred with solvent (eluent: PE:acetone=1:1) to obtain compound KHC-11 (150.0g, 99.26% yield).

Compound KHC-12: KHC-11(150.0g, 547.4mmol) was dissolved in 1000 mL CH₃OH, then I₂ (14.0g, 54.8mmol) was added to the solution, the solution was heated to 60 °C and kept stirring for 12 hours. Then it was concentrated under vacuum and the residue was purified by silica gel column flash chromatography (eluent: PE: EA=10:1) to obtain a white solid KHC-12(130.0g, 74.20%).

Compound KHC-13: KHC-12 (130.0g, 406.2mmol) was dissolved in 1000 mL DMF, and t-BuOK (91.0g, 812.4mmol) trimethyl iodonium sulfide (165.8g, 812.7mmol) was added to the solution, the solution kept stirring at RT for 12 hours under N₂ Atmosphere. Then brine was added to the reaction mixture, the mixture was extracted with EA (3 x 1L), the organic layers were combined, and concentrated under vacuum to obtain a white solid KHC-13(120.0g, 88.50%).

Compound KHC-14: KHC-13 (120.0g, 359.4mmol) was dissolved in 900mL ethanol and 160 ml of water NaN₃ (70.2g, 1077.9mmol) and NH₄Cl (67.2g, 1257.9mmol) was added to the solution, the solution kept stirring at 90°C overnight. Brine was added to the reaction mixture, the mixture was extracted with EA (3x1L), the organic layers were combined and concentrated under vacuum. The residue was purified by silica gel column flash chromatography (eluent: PE: EA=10:1) to obtain a white solid KHC-14 (130.0g, 95.59%).

Compound KHC-15: KHC-14 (125.6g, 332.4mmol) was dissolved in 1000mL CH₃CN, NaI (249.3g, 1662.0mmol) was added to the solution, the solution kept stirring at RT for half an hour, then TMSCl (144.9, 1329.6mmol) was added dropwise to the reaction solution and the reaction kept stirring at RT for 4 hours. Brine was added to the reaction mixture, the mixture was extracted with EA (3 x 1L), the organic layers were combined and concentrated under vacuum. The residue was purified by silica gel column flash chromatography (eluent: PE: EA=10:1) to obtain a white solid KHC-15 (72.0g, 75.20%).

Compound KHC-16: KHC-15 (72.0g, 250.0mmol) was dissolved in 1000 mL CH₃OH, I₂ (19.0g, 75.0mmol) was added to the solution, the reaction solution was heated to 60 °C and kept stirring for 12 hours, then the reaction mixture was concentrated under vacuum. The residue was purified by silica gel column flash chromatography (eluent: PE: EA=10:1) to obtain a white solid KHC-16(53.0g, 63.50%).

Compound KHC-17: Taking trimethylsilyl acetylene (77.8g, 793.0mmol) into 1000mL THF, n-butyl lithium solution (1.6M, 329.0mmol, 205.6ml) was added at -78°C and the reaction solution kept stirring for 2 hours. Then compound KHC-16 (53.0g, 158.6mmol) in 400mL THF was added into the reaction solution. The mixture was stirred at -78°C for 2 hours and was gradually heated to 0°C, then it was poured into saturated NH₄Cl solution, and the mixture was extracted with EA (800mL x 3). The organic layers were combined and was concentrated under vacuum, the residue was purified by chromatography column (eluent: PE:EA = 10:1) to obtain a white solid KHC-17 (56.0g, 81.55%).

Compound KHC-18: KHC-17 (56.0g, 129.3mmol) in 800mL THF, were mixed with 1.0M TBAF (129.3mmol, 129.3ml) at 0°C and the mixture kept stirring for 1 hours. Brine was added to the reaction mixture, the mixture was extracted with EA (3 x 1L). The organic layers were combined and was concentrated under vacuum, the residue was purified by silica gel column flash chromatography (eluent: PE: EA=5:1) to obtain a white solid KHC-18 (43.0g, 92.30%).

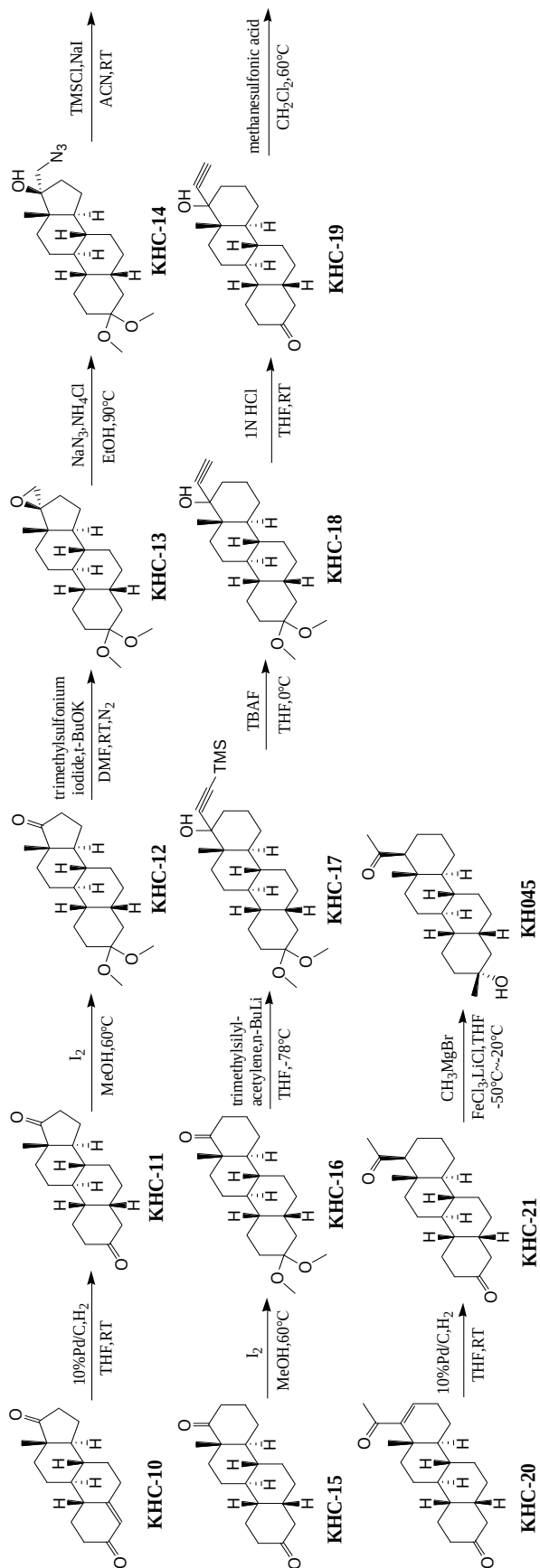
Compound KHC-19: KHC-18 (38.0g, 105.6mmol) in 800mL THF, were mixed with 1.0M HCl to PH=3 and the mixture kept stirring at RT for 12 hours. Brine (1.0L) was added to the reaction mixture, the mixture was extracted with EA (3 x 1L). The organic layers were combined and was concentrated under vacuum. The residue was subsequently
5 purified by silica gel column flash chromatography (eluent: PE: EA=5:1) to obtain a white solid KHC-19 (32.0g, 96.50%).

Compound KHC-20: and methanesulfonic acid (148.0g, 1541.6mmol) were mixed with 1000mL CH₂Cl₂, KHC-19 (32.0g, 101.9mmol) in CH₂Cl₂ 1000ml was added dropwise to the solution, the reaction solution kept stirring at 60°C
10 for 2 hours and was cooled to RT. Brine (1.0L) was added to the reaction mixture, the mixture was extracted with CH₂Cl₂ (3 x 1.0L). The organic layers were combined and was concentrated under vacuum. The residue was purified by silica gel column flash chromatography (eluent: PE: EA=10:1) to obtain a white solid KHC-20 (20.0g, 62.50%).

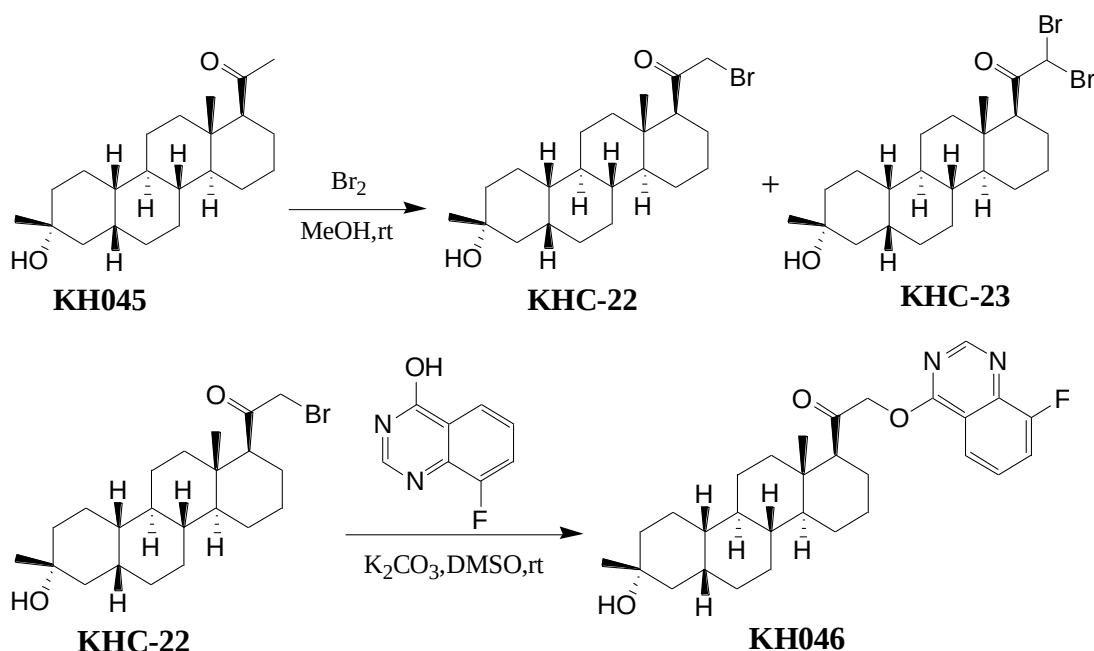
Compound KHC-21: Compound KHC-20 (20.0g, 63.7mmol) was dissolved in 500mL THF and 10% Pd/C (3.0g) was
15 added to the solution. Then the solution was hydrogenated under H₂ atmosphere. The reaction solution kept stirring at RT for 2 days, then filtered. The filtrate was concentrated under vacuum, to obtain crude product which was subsequently purified by silica gel column flash chromatography (eluent: PE: EA=15:1) to obtain a white solid KHC-21 (17.0g, 84.40%).

Compound KH045: To a mixture of FeCl₃ (23.0g, 142.0mmol) and LiCl (13.5g, 321.4mmol) in a 2000mL three-necked flask under N₂, 800mL anhydrous THF was added, the mixture kept stirring at RT for 20 minutes, then cooled to -40°C, CH₃MgBr (3M, 190ML, 570.0mmol) was dropwise added to the solution, then the solution kept stirring at -40°C for 30min. KHC-21(17.0g, 53.8mmol) in anhydrous THF (200mL) was added dropwise to the above reaction solution, then the solution was heated to -20°C and kept stirring for 4 hours. TLC monitoring of the reaction showed that the
25 reaction was complete, the reaction solution was quenched with saturated NH₄Cl (40mL) and the mixture was extracted with EA (3 X 1.0L). The combined organic layers were washed with saturated brine I, dried over anhydrous Na₂SO₄, and concentrated under vacuum to obtain crude product which was subsequently purified by flash chromatography on silica gel (eluent: PE/EA=15:1) to give a white solid KH045 (10.2g, 57.10% yield).

¹H NMR (400 MHz, CDCl₃) δ 2.34 – 2.25 (m, 1H), 2.14 (s, 3H), 2.00 (t, J = 11.4 Hz, 1H), 1.87 – 1.78 (m, 1H), 1.75 –
30 1.54 (m, 9H), 1.51 – 1.43 (m, 2H), 1.39 – 1.30 (m, 4H), 1.27 – 1.13 (m, 7H), 1.09 – 0.84 (m, 8H).



Example 41: Synthesis of compound KH046:



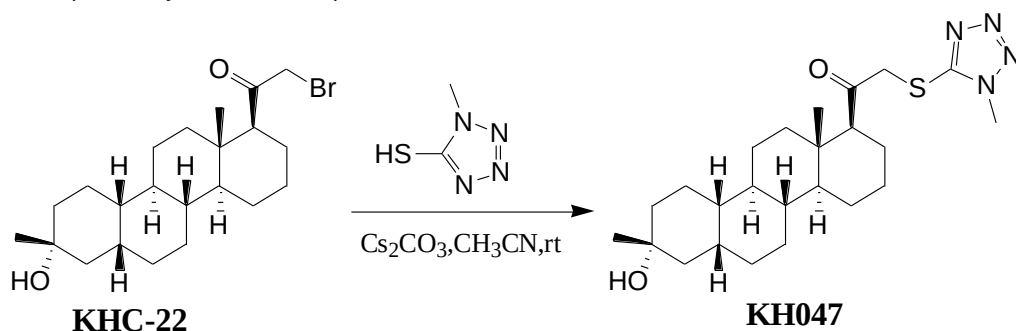
- 5 Compound KHC-22 and Compound KHC-23: Compound KH045 (1.50g, 4.50mmol) was dissolved in 40mL CH_3OH , then Br_2 (1.08g, 6.75mmol) was added dropwise to the solution. The reaction solution turned from orange to light yellow at RT for 4 hours, and TLC monitoring of the reaction showed that the reaction was complete. 30mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 65mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na_2SO_4 , and the solvent was distilled under reduced pressure to give crude product which was purified by flash chromatography on silica gel (eluent: CH_2Cl_2) to give white solid KHC-22 (1.19g, 64.34%) and white solid KHC-23 (0.33g, 17.84%).

Compound KH046: KHC-22 (50mg, 0.12mmol) was dissolved in 5mL DMSO, then K_2CO_3 (21mg, 0.15mmol) and 8-fluoro-4-hydroxyquinazoline (25mg, 0.15 mmol) was added. Reaction was performed at room temperature overnight.

- 15 TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na_2SO_4 , and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=4:1) to give a white solid KH046 (33mg, 55.56%).

- ^1H NMR (400 MHz, CDCl_3) δ 8.06 (d, 1H), 7.88 (s, 1H), 7.55 – 7.40 (m, 2H), 4.80 (q, 2H), 2.44 (dd, 1H), 2.04 - 2.01 (m, 1H), 1.91 - 1.88 (m, 1H), 1.84 – 1.16 (m, 21H), 1.15 – 0.80 (m, 8H).

Example 42: Synthesis of compound KH047

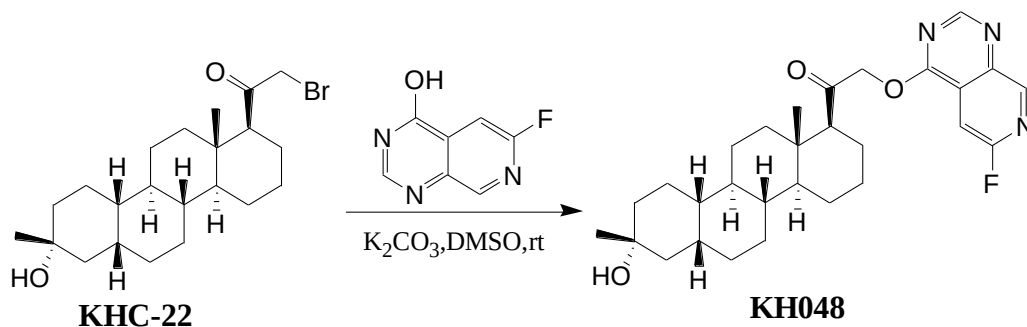


Compound KH047: KHC-22 (50mg, 0.12mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (49mg, 0.15mmol) and 5-mercapto-1-methyl-1H-tetrazolium (17mg, 0.15mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete, 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with

- 5 saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=4:1) to give a white solid KH047 (51mg, 50.12%).

¹H NMR (400 MHz, CDCl₃) 4.40 (dd, 2H), 3.97 (s, 3H), 2.48 (dd, 1H), 2.05 - 1.99 (m, 1H), 1.93 - 1.82 (m, 1H), 1.83 - 1.14 (m, 21H), 1.13 - 0.70 (m, 8H).

10 Example 43: Synthesis of compound KH048



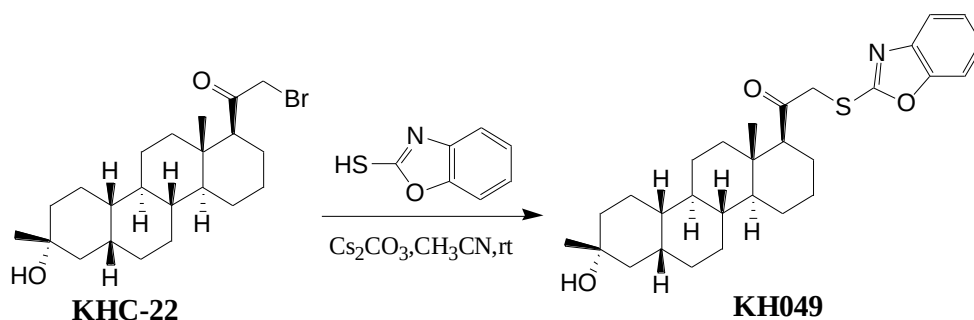
Compound KH048: KHC-22 (60mg, 0.14mmol) was dissolved in DMSO 5mL, then K₂CO₃ (58mg, 0.42mmol) and 6-fluoropyridine [3,4-d] Pyrimidin-4-one (69mg, 0.42mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete, 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with

- 15 saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/CH₃OH=40:1) to give a white solid KH048 (37mg, 50.12%).

¹H NMR (400 MHz, CDCl₃) δ 8.82 (s, 1H), 7.79 (s, 1H), 7.66 (d, 1H), 4.80 (dd, 2H), 2.44 (dd, 1H), 2.05 - 1.99 (m, 1H), 1.93 - 1.82 (m, 1H), 1.83 - 1.14 (m, 21H), 1.13 - 0.70 (m, 8H).

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Example 44: Synthesis of compound KH049

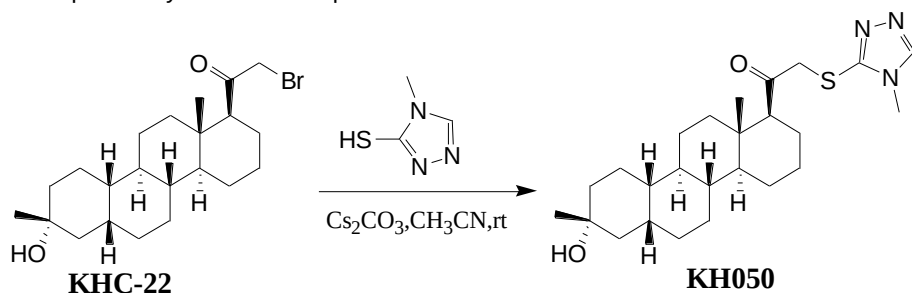


Compound KH049: KHC-22 (60mg, 0.15mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (62mg, 0.19mmol) and 2-mercaptobenzoxazole (29mg, 0.19 mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete, 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/Acetone=4:1) to give a white solid KH049 (42mg, 42.92%).

- 25
- ¹H NMR (400 MHz, CDCl₃) δ 7.55 (d, 1H), 7.42 (d, 1H), 7.34 - 7.24 (m, 2H), 4.33 (dd, 2H), 2.57 (dd, 1H), 2.05 - 1.99 (m, 1H), 1.93 - 1.82 (m, 1H), 1.83 - 1.14 (m, 21H), 1.13 - 0.70 (m, 8H).

30

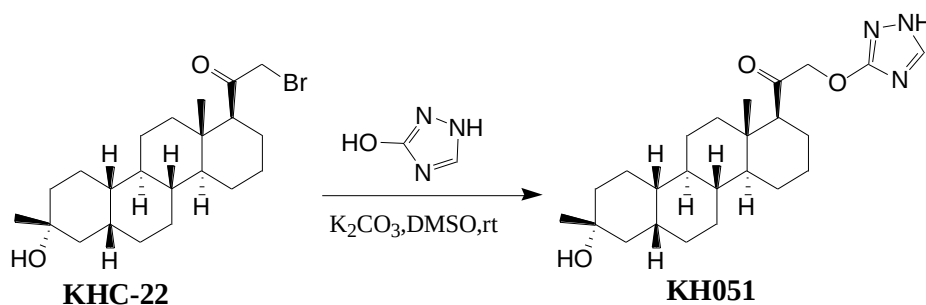
Example 45: Synthesis of compound KH050



Compound KH050: KHC-22 (60mg, 0.15mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (62mg, 0.19mmol) and 3-mercapto-4-methyl-4H-1, 2, 4-triazole (22mg, 0.19 mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=70:1) to give a white solid KH050 (17mg, 25.41%).

¹H NMR (400 MHz, CDCl₃) δ 8.22 (s, 1H), 4.37 (dd, 2H), 3.68 (s, 3H), 2.50 (dd, 1H), 2.10 – 1.97 (m, 1H), 2.06 - 2.02 (m, 1H), 1.79 – 1.14 (m, 21H), 1.12 – 0.79 (m, 8H).

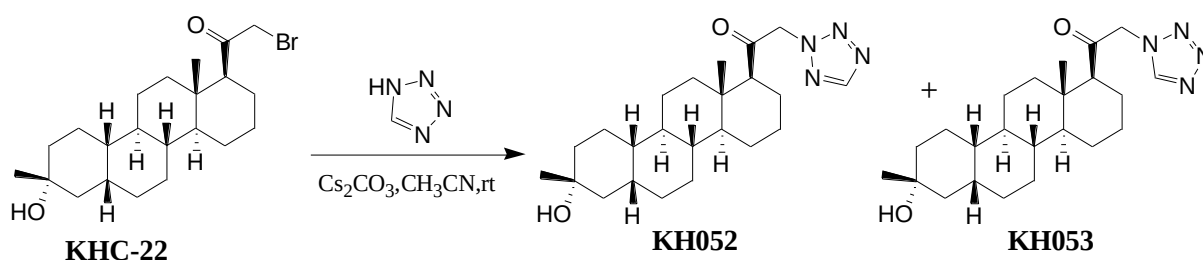
Example 46: Synthesis of compound KH051



Compound KH051: KHC-22 (60mg, 0.15mmol) was dissolved in DMSO 5mL, then K₂CO₃ (58mg, 0.42mmol) and 3-hydroxy-1H-1, 2, 4-triazole (36mg, 0.42mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=40:1) to give a white solid KH051 (27mg, 46.36%).

¹H NMR (400 MHz, CDCl₃) δ 9.57 (s, 1H), 7.42 (s, 1H), 4.46 (q, 2H), 2.34 (dd, 1H), 2.07 – 1.96 (m, 1H), 1.88 - 1.82 (m, 1H), 1.79 (s, 21H), 1.11 – 0.81 (m, 8H).

Example 47: Synthesis of compound KH052 and compound KH053



Compound KH052 and compound KH053: KHC-22 (100mg, 0.24mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (234mg, 0.72mmol) and 1H-tetrazole (50mg, 0.72mmol) was added. Reaction was performed at room temperature

overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=30:1) to give a white solid KH6070069-1 (19mg, 19.74%)

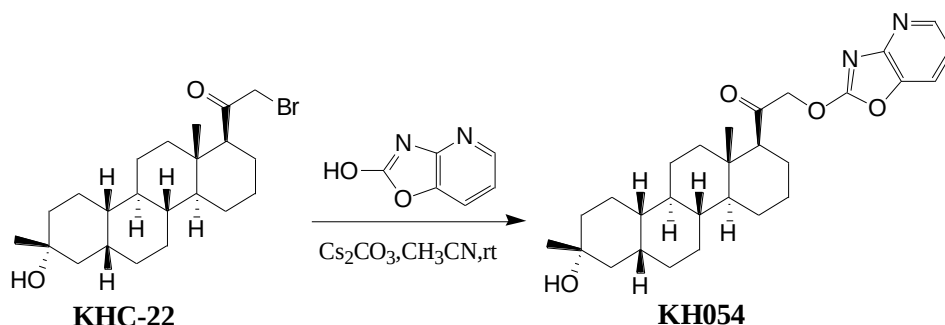
5 and a white solid KH6070069-2(44mg,45.72%), respectively.

¹H NMR (400 MHz, CDCl₃) δ 8.57 (s, 1H), 5.49 (s, 2H), 2.33 (dd, 1H), 2.05 - 2.01 (m, 1H), 1.94 - 1.84 (m, 1H), 1.83 - 1.15 (m, 21H), 1.14 - 0.82 (m, 8H).

¹H NMR (400 MHz, CDCl₃) δ 8.72 (s, 1H), 5.29 (dd, 2H), 2.41 (dd, 1H), 2.06 - 2.01 (m, 1H), 1.95 - 1.85 (m, 1H), 1.85 - 1.15 (m, 21H), 1.14 - 0.82 (m, 8H).

10

Example 48: Synthesis of compound KH054

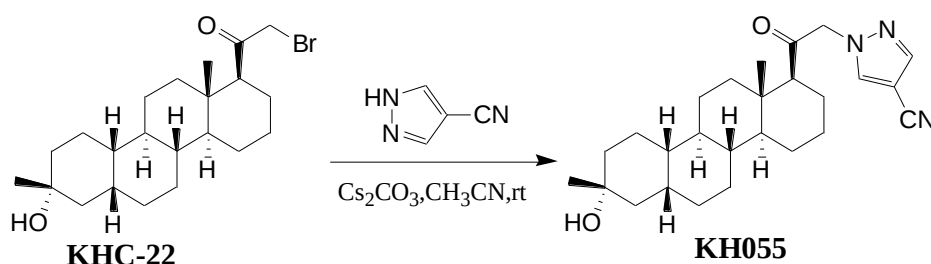


Compound KH054: KHC-22 (60mg, 0.15mmol) was dissolved in 5mL CH₃CN, then Cs₂CO₃ (62mg, 0.19mmol) and 2-hydroxyoxazole [4,5-b] Pyridine (26mg, 0.19mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=70:1) to give a white solid KH054 (17mg, 42.92%).

¹H NMR (400 MHz, CDCl₃) δ 7.25 (d, 1H), 7.01 (d, 1H), 6.69 (t, 1H), 5.11 (q, 2H), 2.40 (dd, 1H), 2.08 - 1.98 (m, 1H), 1.93 - 1.83 (m, 1H), 1.82 - 1.14 (m, 21H), 1.15 - 0.81 (m, 8H).

20

Example 49: Synthesis of compound KH055

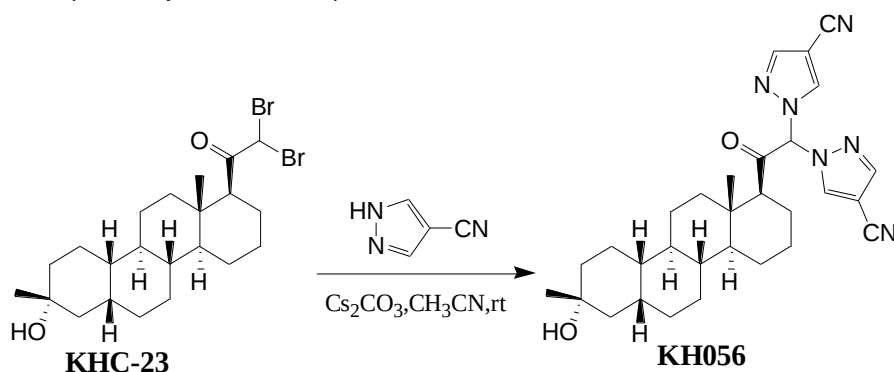


Compound KH055: KHC-22 (60mg, 0.15mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (156mg, 0.48mmol) and 4-cyanopyrazole (45mg, 0.48mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water10mL water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=30:1) to give a white solid KH055 (51mg, 50.12%).

¹H NMR (400 MHz, CDCl₃) δ 7.83 (s, 1H), 7.81 (s, 1H), 4.99 (q, 2H), 2.32 (dd, 1H), 2.05 - 2.01 (m, 1H), 1.92 - 1.83 (m, 1H), 1.82 - 1.15 (m, 21H), 1.14 - 0.81 (m, 8H).

30

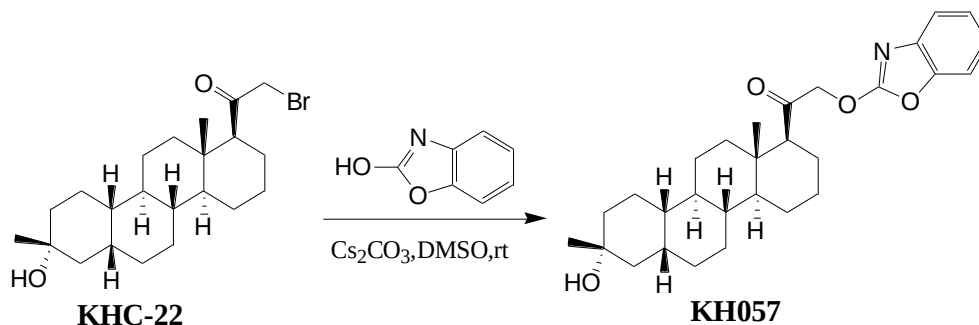
Example 50: Synthesis of compound KH056



Compound KH056: KHC-23 (40mg, 0.10mmol) was dissolved in CH₃CN 5mL, then Cs₂CO₃ (198mg, 0.3mmol) and 4-cyanopyrazole (28mg, 0.30mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: CH₂Cl₂/CH₃OH=30:1) to give a white solid KH056 (12mg, 23.30%).

¹H NMR (400 MHz, CDCl₃) δ 8.22 (s, 1H), 8.14 (s, 1H), 7.92 (s, 1H), 7.84 (s, 1H), 7.02 (s, 1H), 2.10 (dd, 1H), 2.05 - 2.00 (m, 1H), 1.87 – 1.78 (m, 1H), 1.78 – 1.14 (m, 21H), 1.12 – 0.79 (m, 8H).

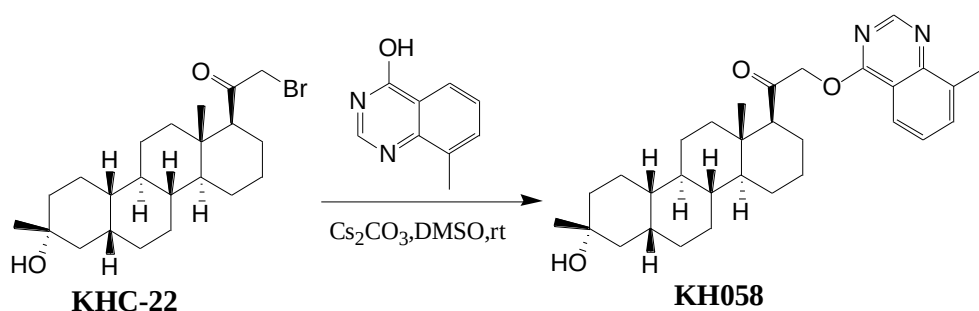
Example 51: Synthesis of compound KH057



Compound KH057: KHC-22 (60mg, 0.15mmol) was dissolved in DMSO 5mL, then Cs₂CO₃ (143mg, 0.44mmol) and 2-hydroxybenzoxazole (59mg, 0.44mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=3:1) to give a white solid KH057 (30mg, 42.92%).

¹H NMR (400 MHz, CDCl₃) δ 7.24 – 7.19 (m, 1H), 7.17 – 7.07 (m, 2H), 6.75 – 6.65 (m, 1H), 4.58 (s, 2H), 2.40 (dd, 1H), 2.08 – 1.98 (m, 1H), 1.93 – 1.83 (m, 1H), 1.82 – 1.14 (m, 21H), 1.15 – 0.81 (m, 8H).

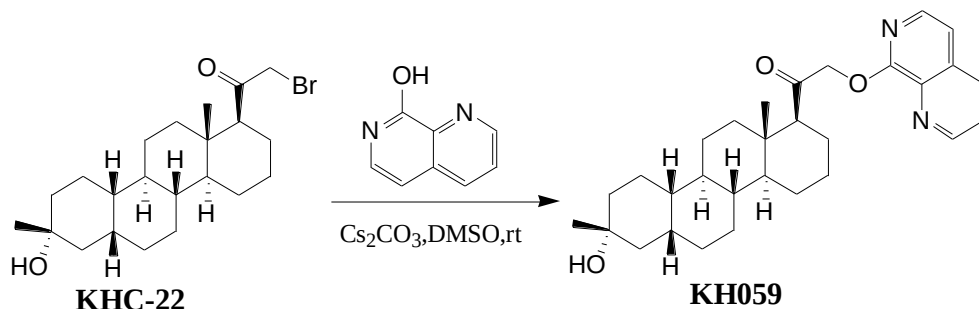
Example 52: Synthesis of compound KH058



Compound KH058: KHC-22 (80mg, 0.19mmol) was dissolved in DMSO 5mL, then Cs₂CO₃ (190mg, 0.58mmol) and 8-methyl-4-quinazolinone (93mg, 0.58 mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=3:1) to give a white solid KH058(28mg, 30.01%).

¹H NMR (400 MHz, CDCl₃) δ 8.13 (d, 1H), 7.88 (s, 1H), 7.61 (d, 1H), 7.39 (t, 1H), 4.80 (dd, 2H), 3.49 (s, 3H), 2.45 (dd, 1H), 2.03 - 2.01 (m, 1H), 1.92 - 1.84 (m, 1H), 1.83 - 1.16 (m, 21H), 1.14 - 0.80 (m, 8H).

10 Example 53: Synthesis of compound KH059

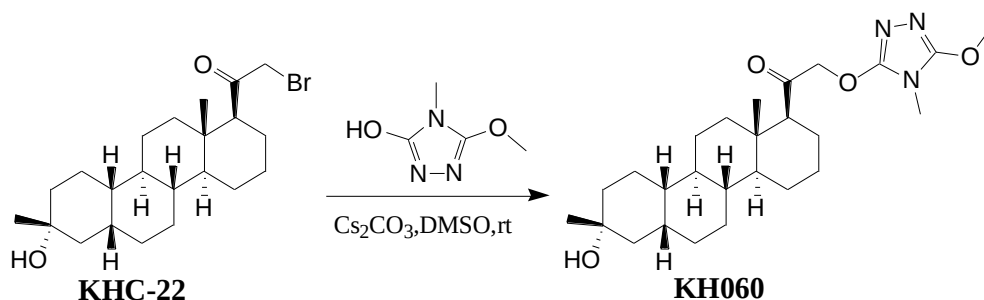


Compound KH059: KHC-22 (80mg, 0.19mmol) was dissolved in DMSO 5mL, then Cs₂CO₃ (190mg, 0.58mmol) and 8-hydroxy-1, 7-naphthyridine (85mg, 0.58 mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=3:1) to give a white solid KH058(28mg, 30.89%).

¹H NMR (400 MHz, CDCl₃) δ 8.86 (d, 1H), 7.88 (d, 1H), 7.54 (dd, 1H), 6.96 (d, 1H), 6.45 (d, 1H), 4.86 (s, 2H), 2.45 (dd, 1H), 2.09 - 1.95 (m, 1H), 1.87 - 1.83 (m, 1H), 1.80 - 1.15 (m, 21H), 1.12 - 0.76 (m, 8H).

20

Example 54: Synthesis of compound KH060

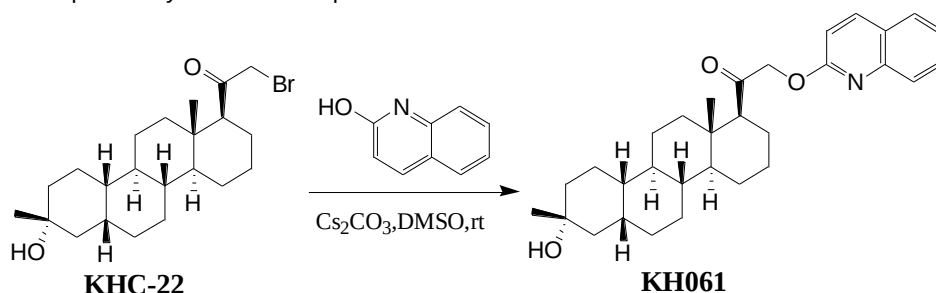


Compound KH060: KHC-22 (100mg, 0.24mmol) was dissolved in 5mL DMSO, then Cs₂CO₃ (156mg, 0.48mmol) and 3-hydroxy-4-methyl-5-methoxy-4H-1, 2, 4-triazole (62mg, 0.48mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=4:1) to give a white solid KH058(56mg, 50.72%).

¹H NMR (400 MHz, CDCl₃) δ 4.54 - 4.40 (m, 2H), 3.93 (s, 3H), 3.12 (s, 3H), 2.29 (dd, 1H), 2.03 - 1.99 (m, 1H), 1.85 - 1.82 (m, 1H), 1.77 - 1.13 (m, 21H), 1.10 - 0.81 (m, 8H).

30

Example 55: Synthesis of compound KH061

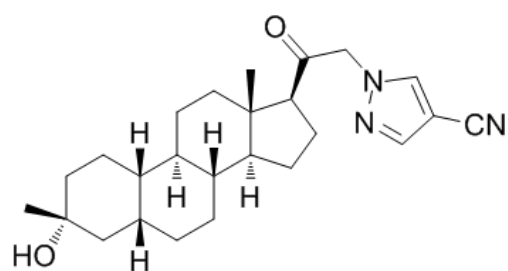


Compound KH061: KHC-22 (80mg, 0.19mmol) was dissolved in 5mL DMSO, then Cs₂CO₃ (156mg, 0.48mmol) and 2-hydroxyquinoline (84mg, 0.58mmol) was added. Reaction was performed at room temperature overnight. TLC monitoring of the reaction showed that the reaction was complete. 10mL of water was added to the reaction mixture, the mixture was extracted with EA (2 x 20mL), the combined organic layers were washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was distilled under reduced pressure to give crude product which was purified by TLC (eluent: PE/acetone=4:1) to give a white solid KH058(45mg, 49.76%).

¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, 1H), 7.75 (dd, 2H), 7.61 (t, 1H), 7.39 (t, 1H), 7.05 (d, 1H), 5.15 (s, 2H), 2.43 (dd, 1H), 2.04 - 2.00 (m, 1H), 1.86 - 1.83 (m, 1H), 1.78 - 1.12 (m, 21H), 1.10 - 0.80 (m, 8H).

Example 56: In vitro cell viability assay

The control compound used in the present invention has the chemical structure described below:



The specific synthesis of the control compound is prepared with reference to CN105339381B.

The present invention applies the two subunits of the recombinant GABA_A receptor: α₁β₂γ₂ and α₄β₃δ to evaluate the potency of the exemplary compounds in vitro by patch-clamp electrophysiology.

56.1 Electrophysiological testing solution

Extracellular and intracellular fluid compositions

Extracellular fluid: 140 mM NaCl, 5 mM CsCl, 2 mM CaCl₂ • 2H₂O, 1 mM MgCl₂ • 6H₂O, 5 mM HEPES, 10mM D-Glucose, pH=7.4 (NaOH).

Intracellular fluid: 130mM CsCl, 0.1mM CaCl₂ • H₂O, 2mM MgCl₂ • 6H₂O, 1.1mM EGTA, 5mM Na₂-ATP, 10 mM HEPES, pH=7.2 (CsOH).

The prepared intracellular fluid was divided into 1 mL per tube and froze and stored at -20°C refrigerator, freshly melted intracellular fluid was used in the experiment every day. All the intracellular fluid was used up within three months.

56.2 Cell Lines with stable expression of ion channels

Ion Channel	Expressed Gene	Gene Info	Cell Line
GABA_{A1} ($\alpha 1\beta 2\gamma 2$)	GABRA1	NM_000806	HEK293
	GABRB2	NM_021911	
	GABRG2	NM_198904	
GABA_{A4} ($\alpha 4\beta 3\delta$)	GABRA4	NM_000809	HEK293
	GABRB3	NM_000814	
	GABRD	NM_000815	

56.3 Cell culture

56.3.1 GABA_A($\alpha 1\beta 2\gamma 2$) cell culture

- 5 The HEK293 cells line stably expressing GABA_A ($\alpha 1\beta 2\gamma 2$) receptors was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 800 $\mu\text{g/mL}$ G418, 200 $\mu\text{g/mL}$ Hygromycin B and 100 $\mu\text{g/mL}$ Zeocin. Cells were grown in a humidified incubator at 37°C with 5% carbon dioxide.

- Cell passage: The old medium was removed, and the cells were washed once with PBS. Then 1 mL 0.25%-Trypsin-EDTA solution was added and incubated at 37 °C for 0.5 min. When the cells are detached from the bottom of the dish, 5 ml of 37 °C pre-warmed complete medium was added. Cell aggregates were dissociated by gentle pipetting. The cell suspension was transferred into a sterile centrifuge tube and then the cell suspension was spun at 1000 rpm for 5 min to collect the cells. The cells were amplified or maintained by seeding 2.5×10^5 cells in a 6 cm cell culture dish (final medium volume: 5 mL).

To maintain electrophysiological performances, cell density must not exceed 80%.

- 15 Before manual patch clamp test, the cells were detached using 0.25%-Trypsin-EDTA solution. Then 8×10^3 cells were plated onto coverslips, and cultured in a 24-well plate (final medium volume: 500 μL) and tested after 18 hours by patch clamp.

56.3.2 GABA_A($\alpha 4\beta 3\delta$) cell culture

HEK293 cell line was cultured in DMEM containing 10% fetal bovine serum and 5% carbon dioxide at 37 °C.

- 20 Cell passage: The old medium was removed, and the cells were washed once with PBS. Then 1 mL 0.25%-Trypsin-EDTA solution was added to digest cells. The culture dish was incubated at 37°C in pre-warmed incubator for 0.5 minute. As soon as cells were detached, about 5 mL of 37°C pre-warmed complete medium was added. Cell aggregates were dissociated by gentle pipetting. The cell suspension was transferred into a sterile centrifuge tube and then the cell suspension was spun at approximately 1000 rpm for 5 minutes. The cells were amplified or maintained by seeding 2.5×10^5 cells in a 6 cm dish (final medium volume: 5 mL). For transfection, 8×10^3 cells were plated onto coverslips, and cultured in a 24-well plate (final medium volume: 5 mL).

- Transfection: Second day, the transfection of GABA_{A4} ($\alpha 4$, $\beta 3$, δ plasmid ratio 1: 1: 1) was performed. The X-tremeGENE HP DAN Transfection Reagents kit were used. The ratio of the plasmid and transfection reagent was 1 μg : 2 μL . The amount of plasmid in the 24-well plate was 0.5 μg per well, and the transfection reagent was 1 μL . The following specific steps were taken for preparing the 12 wells: The 600 μL Opti-MEM was added to a sterile centrifuge tube along with 6 μg of plasmid and was allowed to mix well. Then the 12 μL of transfection reagent was added, mixed well following 15 min incubation at room temperature. The transfection complex was then inoculated into the cell, 50 μL per well, and the transfection complex was lightly stirred and mixed.
- 35 This mixture was changed on the third day, and the patch-clamp was performed on the fourth day.

56.4 Patch-clamp testing

Glass pipette (BF 150-86-10, Sutter Instruments) was pulled using a micropipette puller (P97, Sutter Instruments). The glass pipette was manipulated using a micro-manipulator (MP285, Sutter Instruments) under the microscope (IX71, Olympus). After touching the cell, a slight suction was applied to achieve high seal resistance ($G\Omega$). Fast capacitance (in pF) compensation was made after achieving high seal, and the membrane was broken. Cell capacitance (in pF) compensation was made from whole-cell capacitance compensation after the whole cell mode was achieved. No leak subtraction was made.

The test and control solutions flowed into a recording chamber mounted on the stage of an inverted microscope via a gravity-fed solution delivery system. During the experiment, solutions were withdrawn from the recording chamber by vacuum aspiration. Each concentration was tested at multiple times. All tests were performed at room temperature.

Whole cell model recorded $GABA_{A1}$ receptors current experimental protocol was indicated as follows: When sealing, cell was at a holding potential of -70 mV, with gap-free mode to record the peak current. The cell would be applied with 3 μM GABA to active current. After the test articles application for 30 s, each concentration of test articles was applied with 3 μM GABA followed by 2 minutes washout using extracellular solution. Finally, 100 μM GABA was used as a positive control. The data was collected by EPC-10 amplifier and stored in PatchMaster (HEKA) software.

Whole cell model recorded $GABA_{A4}$ receptors current experimental protocol was indicated as follows: When sealing, cell was at a holding potential of -70 mV, with gap-free mode to record the peak current. The cell would be applied with 10 nM GABA to active current. After the test articles application for 30 s, each concentration of test articles was applied with 10 nM GABA followed by 2 minutes washout using extracellular solution. Finally, 10 μM GABA was used as a positive control. The data was collected by EPC-10 amplifier and stored in PatchMaster (HEKA) software.

56.5 Data quality standard

The following criteria are used to determine whether the data is acceptable:

- (1) Electrode resistance $< 5 M\Omega$
- (2) Seal resistance $> 1 G\Omega$
- (3) The start of access resistance $< 15 M\Omega$
- (4) The end of access resistance $< 15 M\Omega$
- (5) No obvious spontaneous current decay.
- (6) No obvious current leakage when the membrane potential is -70 mv.

56.6 Data analysis

In the detection of $GABA_{A1}$, the currents of 3 μM GABA and different concentrations of test compounds were

respectively normalized to the current of 100 μM GABA $\frac{\text{Peak current compound}}{\text{Peak current vehicle}}$, and then the activation rate corresponding to each concentration was calculated. In the detection of $GABA_{A4}$, the currents of 10 nM GABA and different concentrations of test compounds were respectively normalized to the current of 10 μM

$\frac{\text{Peak current compound}}{\text{Peak current vehicle}}$, and then the activation rate corresponding to each concentration was calculated. Means, Standard Deviation and Standard Error were calculated for each test group. EC50 value was calculated using nonlinear regression:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC50} - X) \times \text{HillSlope}})$$

To fit data to the concentration-response, EC50 was the concentration giving a half-maximum effect and Hill slope was the Hill coefficient. Curve-fitting and EC50 calculations were performed using Graphpad5.0 software.

56.7 Results of experiments in vitro

Table 1: Electrophysiological evaluation of the agonistic effect of the exemplary compounds on the GABA_{A1} receptor

compounds	0.1µM	1µM
KH001	D	B
KH002	E	B
KH003	D	B
KH004	D	A
KH005	D	B
KH006	D	C
KH007	E	C
KH008	D	A
KH009	D	A
KH010	E	D
KH011	D	B
KH012	E	B
KH013	E	E
KH014	E	C
KH015	E	E
KH016	E	C
KH017	E	E
KH018	E	D
KH019	E	E
KH020	E	E
KH021	E	E
KH022	E	C
KH023	E	E
KH024	E	C
KH025	E	B
KH026	E	C
KH027	E	E
KH028	E	E
KH029	E	E
KH030	E	E
KH031	E	C
KH032	E	D
KH033	E	D
KH034	E	E
KH035	E	D
KH036	E	E

KH037	E	E
KH038	E	C
KH039	E	B
KH040	E	D
KH041	E	B
KH042	E	C
KH043	E	D
KH045	E	D
KH047	E	E
KH048	E	E
KH051	E	E
KH052	E	E
The control	E	D

A: >80%, B: 60% -80% (including 60%), C: 40% -60% (including 40%), D: 20%-40% (including 20%), E: 0-20%.

Table 1 showed the potency of the exemplary compounds at concentrations of 0.1 μ M and 1 μ M. The potency was based on the ratio of the peak current generated on the GABA_{A1} receptor to the peak current generated only in 100 μ mol of GABA and the ratio was multiplied by 100%. The peak current generated on the GABA_{A1} receptor was that the exemplary compounds act on the GABA_{A1} receptor together with 3 μ mol GABA at these two concentrations respectively.

10 Table 2: Electrophysiological evaluation of the exemplary compounds at the GABA_{A1} receptor

The exemplary compounds	EC ₅₀ (nmol)	E _{max}
KH002	463.9	74.6%
KH003	478.1	72.8%
KH004	403.7	106.1%
KH022	821.1	93.1%
KH025	619.9	93.7%
The control	1261	84.2%

E_{max} is defined as the ratio of the peak current generated on the GABA_{A1} receptor to the peak current generated only in 100 μ mol of GABA and the ratio is multiplied by 100%. The peak current generated on the GABA_{A1} receptor is that the exemplary compounds act on the GABA_{A1} receptor together with 3 μ mol GABA.

Table 3: Electrophysiological evaluation of the exemplary compounds on the GABA_{A1} receptor

The exemplary compounds	EC ₅₀ (nmol) (without GABA)	E _{max} (without GABA)	EC ₅₀ (nmol) (with 10 nM GABA)	E _{max} (with 10 nM GABA)
KH001	172.8	103.2%	132.2	91.7%
KH004	171.6	158.4%	134.6	171.6%
KH022	N/A	45.8%	385.5	88.6%
KH025	947.1	84.6%	571.9	92.5%
KH039	>4000	120.8%	>4000	124.6%
The control	N/A	113.9%	218.7	148.7%

N/A indicates that the current does not change with concentration.

- 5 E_{max} is defined as the ratio of the peak current generated on the GABA_{A1} receptor to the peak current generated only in GABA and the ratio is multiplied by 100%. The peak current generated on the GABA_{A1} receptor is that the exemplary compounds act alone on the GABA_{A1} receptor or together with 10nmol GABA respectively.

Example 57: pharmacokinetics in brain and plasma after dosing

10 1. Protocol

1.1 tested Drugs

KH022 in the present invention and the control described in example 56

1.2 Animals

The experimental animals are CD-1 mice.

15 1.3 Administration

- Preparation of test compounds with 30% SBECD in water for intragastric administration. On the first day of the experiment, the animals in the first group are intraperitoneally injected with the solution of KH022 or the control with the dosage of 5mL/kg; before the administration, the animals were weighed, and the administration volume was calculated according to the body weight. Whole blood samples (about 0.03 ml per group) were collected at a
- 20 specified time by saphenous vein puncture (or other suitable collection locations). Blood samples were immediately processed for plasma by centrifugation at approximately 4°C, 3,200 g for 10 min, quickly placed in dry ice, and kept below -20°C until LC/MS/MS analysis. At each time point, animals were euthanized using CO₂ and then brain tissue was collected, rinsed and dried with physiological saline, and homogenized with 4 times the brain weight (g) volume of 15mM PBS(pH7.4):MeOH=2:1. After homogenization, samples were transferred to labeled centrifuge tubes and
- 25 quickly placed in dry ice, stored at or below -20°C for LC-MS/MS analysis.

2. Data analysis

- The concentrations of the test compound in dose formulation samples was determined by the LC/UV, and the calibration curve contains at least 6 calibration standards, R² ≥0.999. The concentrations of the test compound in
- 30 biological matrix was determined by the LC/MS/MS method. The retention time of test compounds or internal standards, chromatogram acquisition and chromatogram integration were processed by software Analyst (Applied Biosystems), and data statistics were processed by software Watson LIMS (Thermo Fisher Scientific) or Analyst (Applied Biosystems). The unit of the analyte concentration in the sample is ng/mL, with 3 significant digits reserved,

and all values expressed in percentages (such as % deviation and % coefficient of variation, etc.) were kept to one decimal place. Non-compartmental models of WinNonlin™ Version 6.3 (Pharsight, Mountain View, CA) pharmacokinetic software were used to process plasma concentrations, and linear log trapezoidal method was used to calculate pharmacokinetic parameters.

5

Experimental results

As shown in Figure 1 and Figure 2, KH022 is cleared more slowly in the mouse brain than the control (there is no detection of the control in the brain at 8h) at the same dosage, and the content of KH022 in the mouse brain is 2.5 times of the control at 2h.

10

57.3 Anti-anxiety test in mice

57.3.1 Light-dark shuttle box: The light-dark shuttle box is a standard and well characterized assay for anxiolytic effects of the exemplary compounds. Mice naturally avoid the light of the box because light makes mice evasive and stressful, but the exploration habit of mice also prompts mice to try to explore a bright box. Increased time on the light of the box by the exemplary compounds reflects an anxiolytic effect on mice. The experimental mice were randomly divided into 5 groups based on weight, including a negative control group, two different dosage groups (1 mg/kg and 3 mg/kg) of the tested compounds (KH004 or KH022), and every group had 10 male mice. Mice were administered with corresponding dosage for tested intervention for 5 consecutive days in advance, and conducting anxiolytic experiments after 30min of the last administration. The retention time in the light box was selected as the main index for evaluating the anti-anxiety effect of KH004 and KH022.

20

Experimental results

Table 4: Effect of KH004 and KH022 on the retention time in the light box

Groups	dosage (mg/kg)	Number of mice	The retention time (%)
the negative control group	0	10	26.69±8.38
KH022	1.0	10	50.00±4.59****
	3.0	10	56.66±7.96****
KH004	1.0	10	50.30±6.89****
	3.0	10	53.91±9.37****

25 Note: data in the table are all expressed as mean ± standard deviation (Mean ± SD). Statistical analysis is performed on 10 animals in each experimental group and *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 represents different experimental groups and the negative control group animals.

57.3.2 Elevated Plus Maze (EPM) testing: EPM for exploratory behavior was used to assess general motor and anxiety-like behavior that was consisting of two open arms and two closed arms extending from a common central platform, thus forming anxiety-like symptoms. The anxiolytic compound would relieve the anxiety conflict status of animals in the test system. The experimental mice were randomly divided into 4 groups based on weight, including a negative control group, two different dosage groups (1 mg/kg and 3 mg/kg) of test compounds (KH004 or KH022), and every group had 10 male mice. Mice were administered with corresponding dosage for test intervention for 5

30

consecutive days in advance, and conducting anxiolytic experiments after 30min of the last administration. The percentage of the retention time (latency) of mice open arms were used as the main index to evaluate the anxiolytic effect of KH004 and KH022, the results are shown in Table 5.

5 Table 5: Effect of different dosage of KH004 and KH022 on the retention time of mice open arms

Groups	dosage (mg/kg)	Number of mice	The retention time (%)
the negative control group	0	10	10.79±6.24
KH022	1	10	31.40±16.41**
	3	10	39.46±11.82****
KH004	1	10	27.21±11.15***
	3	10	36.44±13.28****

Note: data in the table are all expressed as mean ± standard deviation (Mean±SD). Statistical analysis is performed on 10 animals in each experimental group and *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 represents different experimental groups and negative control group animals. Conclusion: KH004 and KH022 significantly increase the

10 percent open arm retention time of the animals, showing anxiolytic effect.

57.3.3 Experiments of side effects during administration

Since the drugs which are acting on GABA (A) receptor can produce behavioral anesthesia (side effects), side effects of mice during administration were observed during the course of the elevated plus maze experiment. Each group of 15 10 mice was observed for three behavioral manifestations: gait, mobility and prone behavior. "+" indicates severity, three "+" are the most severe, with time to resume normal action >60 minutes. The positive drug is the control compound as described in example 56 and the results are shown in Table 6.

KH004 and KH022 have less side effects compared to the positive drug in Table 6.

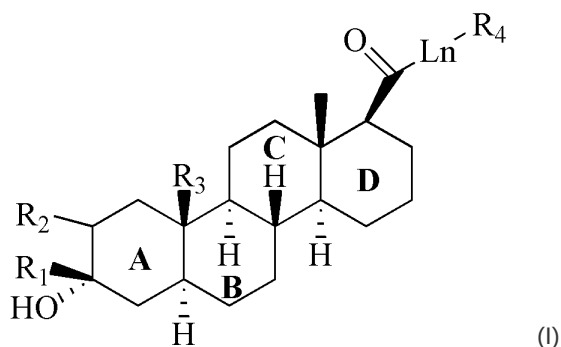
20

Table 6. summary of observation of side effects in animals during administration

The dosage of 3mg/kg	The first day						The second day						The third day						The fourth day					
	Gait		mobility		prone behavior		Gait		mobility		prone behavior		Gait		mobility		prone behavior		Gait		mobility		prone behavior	
	Number	Level	Number	Level	Number	Level	Number	Level	Number	Level	Number	Level	Number	Level	Number	Level	Number	Level	Number	Level	Number	Level	Number	Level
The positive drug	7	++	3	+++	2	+	6	++	0		0		8	+	4	++	0		9	+	5	+	0	
KH004	0		0		0		0		0		0		1	+	0		0		0		0		0	
KH022	3	+	1	++	0		2	+	0		0		1	+	0		0		3	+	1	+	0	

CLAIMS

1. A compound of Formula (I):



5 or a pharmaceutically acceptable salt thereof;
wherein:

R₁ is hydrogen, substituted or unsubstituted C1-6 alkyl, substituted or unsubstituted C1-6 alkoxy, substituted or unsubstituted C2-6 alkenyl, substituted or unsubstituted C2-6 alkynyl, or substituted or unsubstituted C3-6 carbocyclyl;

10 R₂ is hydrogen, halogen, substituted or unsubstituted C1-6 alkyl, substituted or unsubstituted C1-6 alkoxy, substituted or unsubstituted C2-6 alkenyl, substituted or unsubstituted C2-6 alkynyl, substituted or unsubstituted C3-6 carbocyclyl;

R₃ is hydrogen, unsubstituted C1-6 alkyl or -CH₂OR^a, wherein R^a is hydrogen, substituted or unsubstituted C1-6 alkyl, substituted or unsubstituted C1-6 alkoxy, substituted or unsubstituted C2-6 alkenyl, substituted or unsubstituted C2-6 alkynyl, substituted or unsubstituted C3-6 carbocyclyl;

L is -C(Rb)(Rb)-, each Rb is independently hydrogen or C1-C6 alkyl, n is an integer of 0 to 3;

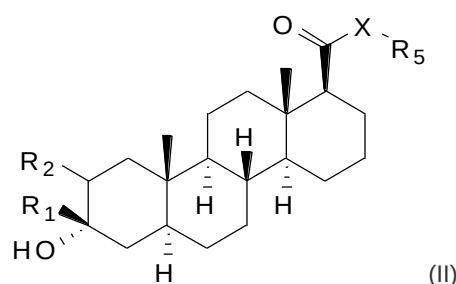
R₄ is halogen or substituted or unsubstituted heteroaryl or heterocyclyl.

2. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein R₁ is hydrogen, substituted or unsubstituted C1-6 alkyl; R₂ is hydrogen, substituted or unsubstituted C1-6 alkyl; R₃ is hydrogen, an unsubstituted C1-6 alkyl; Rb is hydrogen, n is an integer of 1 to 2; R₄ is heteroaryl optionally substituted with cyano, nitro, hydroxy, halo, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 haloalkyl, -C(O)Rd, -C(O)N(Re)(Rf), -C(O)O(Rd), -N(Re)(Rf), -OC(O)N(Re)(Rf), -OC(O)O(Rd), -OC(O)Rd, -S(O)₀₋₂Rd, -S(O)₀₋₂ORd or -S(O)₀₋₂N(Re)(Rf); each Rd is independently hydrogen or C1-C6 alkyl; each Re and Rf is independently hydrogen, C1-C6 alkyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl.

3. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein R₁ is hydrogen or methyl; R₂ is hydrogen; R₃ is hydrogen; L is -CH₂-; R₄ is a monocyclic or bicyclic-heteroaryl or a monocyclic or bicyclic-heterocyclyl, each heteroatom is oxygen or nitrogen or sulfur, the bicyclic ring is spiro or fused; the heteroaryl or heterocyclyl is optionally substituted with cyano, nitro, hydroxy, halo, C1-C6 alkoxy, C1-C6 haloalkyl, -S- or -O-.

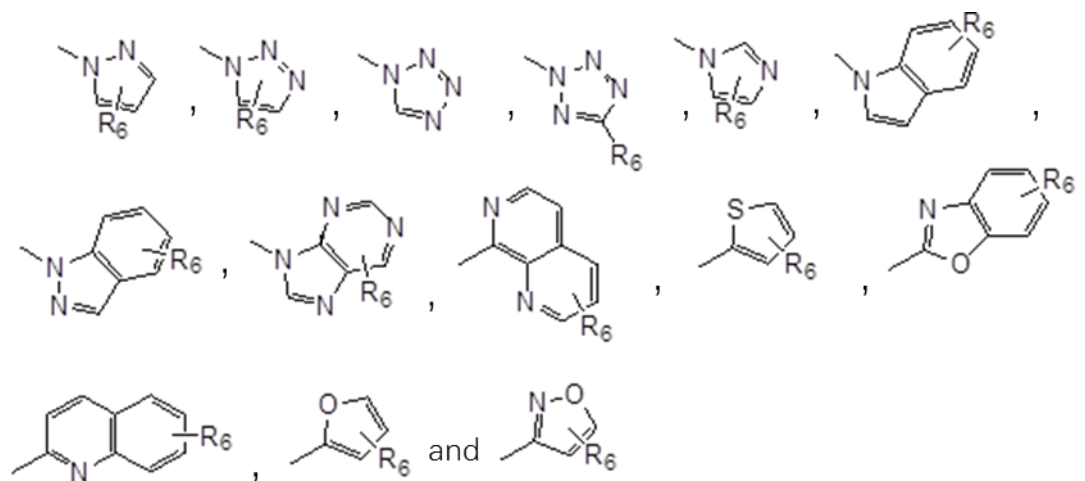
4. The compound of claim 3 or a pharmaceutically acceptable salt thereof, wherein R₄ is a five- or six-membered heteroaryl containing 2-4 nitrogen atoms and is optionally substituted with cyano, nitro, hydroxyl, halo, C1-C6 alkyl, C1-C6 alkoxy, or C1-C6 haloalkyl.

5. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein the compound is a compound as shown in formula II:



5 wherein:

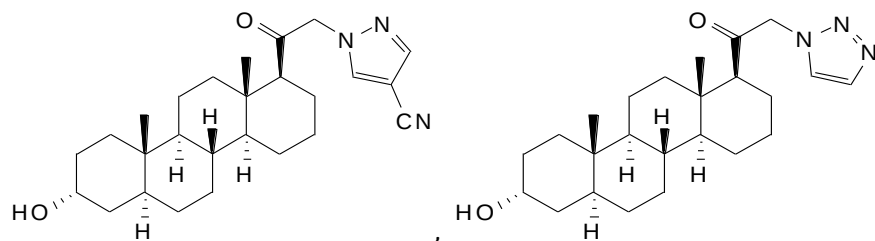
R₁ is hydrogen or C1-3 alkyl substituted or unsubstituted with halogen; R₂ is hydrogen, halogen, C1-6 alkyl unsubstituted or substituted with halogen or C1-6 alkoxy unsubstituted or substituted with halogen; X is CH₂, N, O or S; R₅ is selected from the group consisting of:

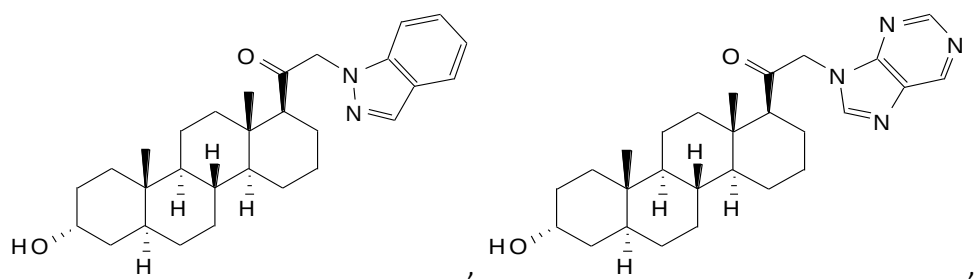
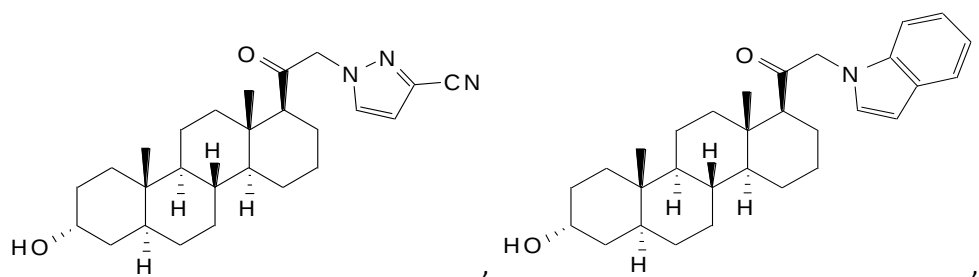
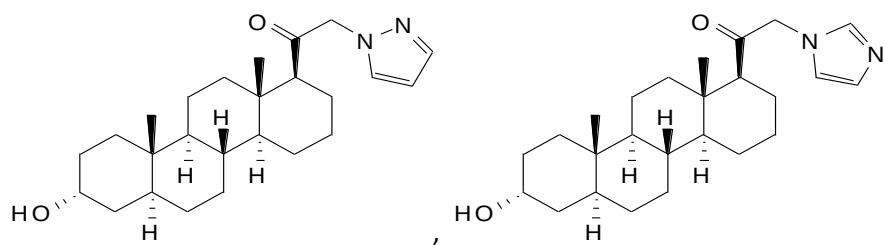
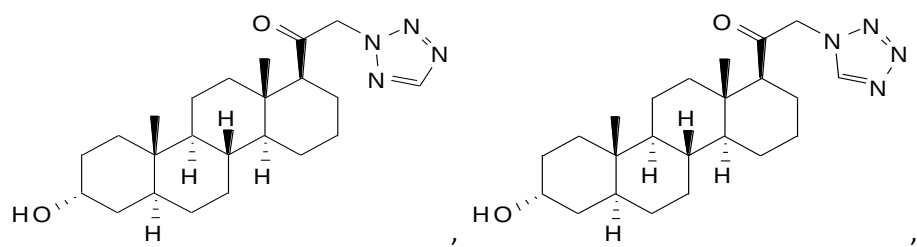


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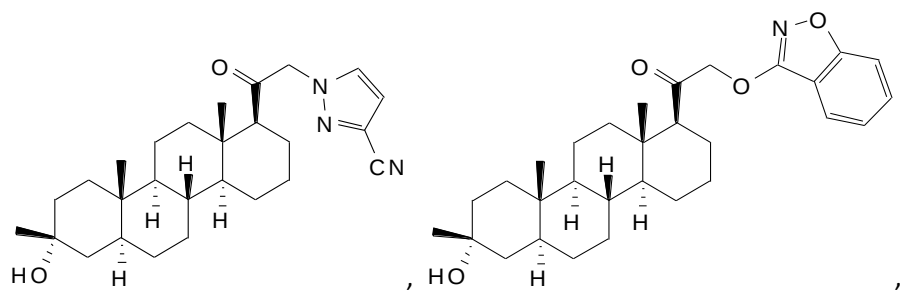
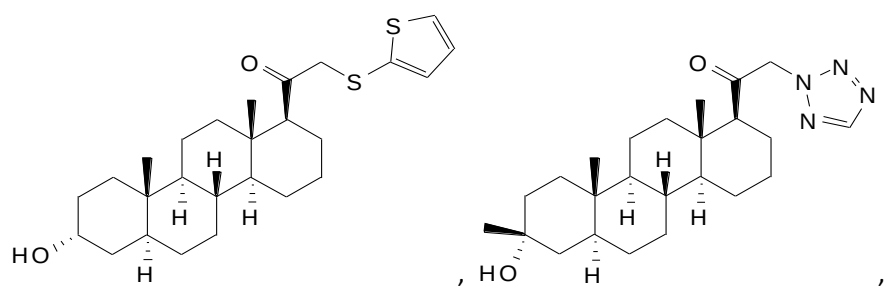
wherein R₆ is H, halogen, CN, CF₃, NO₂, C1-6 alkyl unsubstituted or substituted with halogen or C1-6 alkoxy unsubstituted or substituted with halogen.

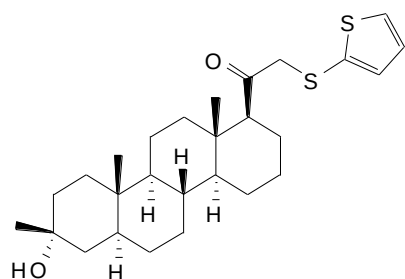
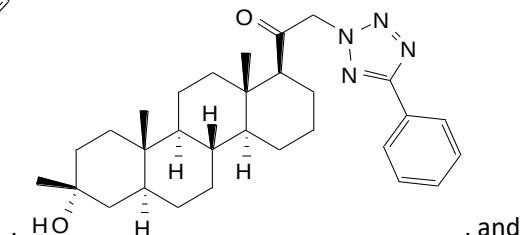
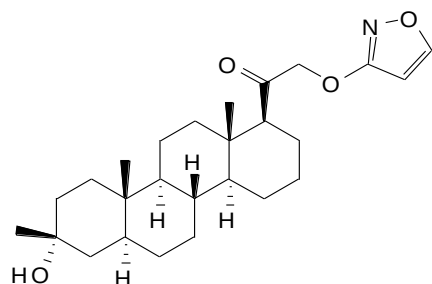
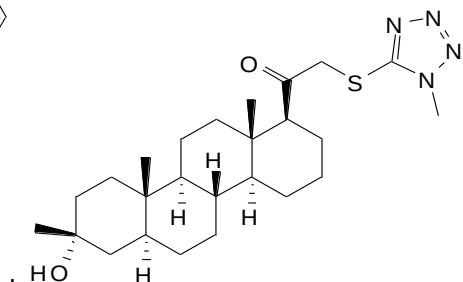
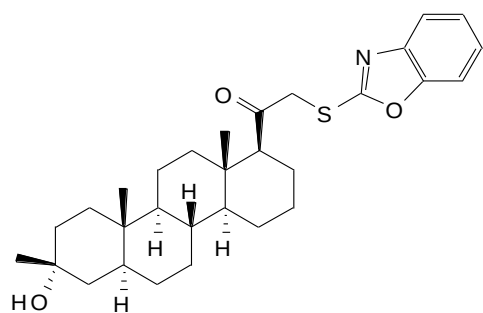
6. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein the compound is selected from the group consisting of:





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7. A pharmaceutical composition comprising a compound of any one of claims 1 to 6, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

10 8. Use of the compound of any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof and the pharmaceutical composition of claim 7 in the preparation of a medicament for preventing or treating a neurological disease.

9. The use of claim 8, wherein the neurological disease is selected from the group consisting of sleep disorders, mood disorders, schizophrenia spectrum disorders, spastic disorders, memory disorders and/or cognitive disorders, movement disorders, personality disorders, autism spectrum disorders, pain, traumatic brain injury, vascular diseases, substance abuse disorders and truncation syndromes or tinnitus.

10. The use of claim 9, wherein the mood disorder is depression, wherein the depression is destructive mood disorder, severe depressive disorder, persistent depressive disorder, premenstrual dysphoric disorder, substance or drug induced disorder, disorder due to other body diseases, or other specific depressive disorder and nonspecific depressive disorder.

11. The use of claim 9, wherein the neurological disease is mild depression, moderate depression, severe depression, or postpartum depression.

12. The pharmaceutical composition of claim 7, wherein the pharmaceutical composition is administered orally, subcutaneously, intravenously, or intramuscularly.