

DESCRIPTION

NOVEL COMPOUND AS KCNQ POTASSIUM CHANNEL AGONIST, PREPARATION METHOD AND USE THEREOF

Technical Field

The present invention belongs to the field of pharmacy. In particular, the present invention relates to a class of novel compounds agonist which can be used as potassium ion channels agonist, the preparation method thereof, and the use of the compounds agonist or the pharmaceutically acceptable salts thereof or the pharmaceutical composition thereof in the preparation of medicaments for treating nervous system diseases, such as epilepsy, convulsion, neuropathic pain, acute ischemic stroke or neurodegenerative diseases.

Background

Epilepsy is a syndrome of neurological function disorder resulted from paroxysmal and abnormal discharging of brain neurons, which has disturbances in motion, sensation, consciousness, mental condition and vegetative nervous system. The incidence rate of epilepsy is approximately 1%, which severely threatening life and health of mankind. The neurological electrophysiological basis for epilepsy is excessive and synchronous discharging of neurons, i.e. epileptiform discharging, which mainly results from excitatory disorder of neurons. The neuronal excitability is determined on the balance of activities of potassium ion channels, sodium ion channels and calcium ion channels. If such balance is disturbed, neuronal excitability disorder and further epilepsy seizures are likely to happen.

Potassium ion channels play an important role in adjustment of neuronal excitation, wherein the ionic mechanism is that in concentration of potassium ions in cells is higher than that out of cells, when the depolarization of membrane potential occurs, potassium ion channels are activated, therefore, potassium ions having positive charges outflow off cells, such that membrane potential becomes negative (polarization or hyperpolarization) and neuronal excitability decreases. Recently, genetic research on epilepsy declares that abnormality of potassium ion channels will directly lead to epilepsy (Wulff, H. Et al. Chem Rev **2008**, *108* (5),

1744-73.), such as benign familial neonatal convulsions (BFNC).

Besides to protein kinase and G-protein coupled receptor, voltage-gated ion channels (VGICs) is the third kind of signal transduction molecules (Harmar, A. J. et al., *Nucleic Acids Res* **2009**, 37 (Database issue), D680-5.). Among voltage-gated ion channels' 78 of family members, more than a half are potassium ion channels, which are divided into 4 types according to their function and structural features: inward rectifier potassium ion channels (K_{ir}); two pore potassium ion channels (K_{2p}); Ca^{2+} -activated potassium ion channels (K_{Ca}) and voltage-gated potassium ion channels (K_V) (Wulff, H. et al., *Nat Rev Medicament Discov* **2009**, 8 (12), 982-1001.).

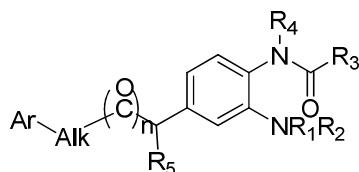
Voltage-gated potassium ion channels (K_V) is an important member of potassium ion channels superfamily, which has 12 members ($K_{V1.X}$ - $K_{V12.X}$). Wherein, KCNQ channels is the 7th member of the voltage-gated potassium ion channels (K_V7), which includes five subtypes, namely, KCNQ1 to KCNQ5. Compared with other potassium ion channels, the activation threshold of KCNQ channels is low, in other words, KCNQ channels can be opened at a voltage lower than action potential threshold (-60mV), KCNQ channels has an slow activation process and will not inactivated on persistent depolarization. Those characters endow KCNQ channels with a basal level in adjustment of cellular excitability. The opening of KCNQ channels may decrease the cellular excitability, while inhibiting them will evoke membrane potential depolarization of neurocytes, such that the excitability is increased and more neural impulses are evoked..

Based on the said advantageous of KCNQ-targeted sites, the agonist thereof is deemed to be an effective medicament for treating epilepsy. By activating potassium ion channels to decrease neuronal excitability, such agonists of potassium ion channels not only can be used in treatment of epilepsy but also can be used in treatment of other diseases induced by exorbitant neuronal excitability, such as convulsion, neuropathic pain, acute ischemic stroke or neurodegenerative diseases.

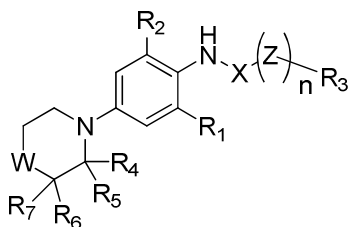
Reported KCNQ agonists are mainly as follows.

1) A compound of following structure is disclosed in Patent No. US5384330, which

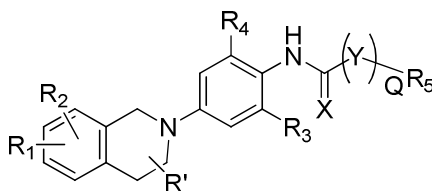
characterized in that it has a benzene ring substituted by two amino groups at positions next to each other.



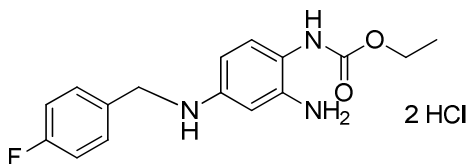
2) Agonists of KCNQ channels having following structure are described in Patent No. WO2005/087754 A1, which characterized in that it has a benzene ring substituted by two amino groups at positions opposite to each other, wherein, the nitrogen atom in one of the amino group is in a saturated ring (or a heterocycle when W is O), while the two hydrogen atoms at the positions next to another nitrogen atom on the benzene ring are substituted by R₁ or R₂.



3) Patent No. WO2008024398-B1 records compound of following structure, which has a similar structure to that of compounds in Patent No. WO2005/087754, except for an additional ring fused to the saturated ring.



At present, the most representative KCNQ agonist is commercially available retigabine having following structure, which is an anti-epilepsy medicament developed by GSK (GlaxoSmithKline):



Retigabine is the first KCNQ agonist, which has been investigated in a systematic procedure. By activating KCNQ2-5, retigabine is mainly used for treatment of adults suffered from partial seizures of epilepsy. Retigabine can be absorbed in short time, its maximum concentration in plasma will be reached 1.5h to 2h after administration of single dosage.

Retigabine has an electron-rich benzene ring substituted by three nitrogen atoms, wherein two nitrogen atoms are at positions next to each other, and one nitrogen atom is present in the form of amino. Such structural feature causes retigabine to be oxidized and deteriorated in synthesis and storage. In addition, the inventors of the present invention have found that the concentration of retigabine in mice's brain tissue is not high in research on tissue distribution of the medicament, which may influence the maximum effectiveness thereof. Thereby, it is necessary to develop an agonist of potassium ion channels, which has higher activity and more stable property, especially contributes to increase the concentration distribution in brain tissue, so as to be used in developing of medicaments for treating neuropathic diseases, such as epilepsy, convulsion, neuropathic pain, acute ischemic stroke or neurodegenerative diseases.

The inventors of the present application also find that when the nitrogen atom in -NH-(secondary amine) of retigabine is further substituted, the resulting compound not only retains or increase the retigabine's activity of opening potassium ion channels, but also has a higher distribution concentration in brain tissue than that of retigabine. Consequently, the compound of the present invention exhibits a better treatment effect.

Summary of the invention

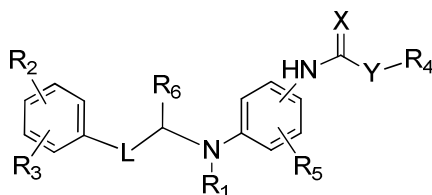
One object of the present invention is to provide a class of novel compounds or pharmaceutically acceptable salts thereof which can be used as agonists of KCNQ potassium channels.

Another object of the present invention is to provide a method for preparing the compounds said above.

Yet another object of the present invention is to provide a pharmaceutical composition comprising the compounds or pharmaceutically acceptable salt thereof as an active ingredient and pharmaceutically acceptable auxiliary substances.

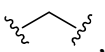
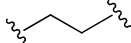
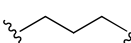
Still another object of the present invention is to provide a use of the compounds, pharmaceutically acceptable salt thereof or pharmaceutical composition thereof in preparing medicaments for treating nervous system diseases etc.

The compounds according to the present invention have the structure of following general formula I:



I

wherein,

L is not present, or L is a linking radical selected from the group consisting of , , and , optionally substituted by C₁-C₄ alkyl;

R₁ is a radical selected from the group consisting of H, C₁-C₆ alkyl, C₃-C₆ cycloalkyl, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, C₁-C₆ alkylamino carbonyl, C₂-C₈ alkenyl, C₅-C₇ cycloalkenyl, C₂-C₈ alkynyl and C₆-C₁₀ aryl; wherein, the C₁-C₆ alkyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkoxy, C₁-C₄ alkylcarbonyl, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl; the C₂-C₈ alkenyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, C₁-C₄ alkoxy, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl; and the C₂-C₈ alkynyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, C₁-C₄ alkoxy, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl;

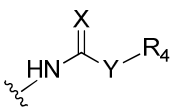
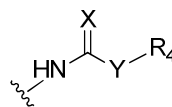
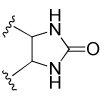
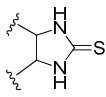
R₂ is a radical selected from the group consisting of halogen atom and C₁-C₄ alkoxy;

R₃ is a radical selected from the group consisting of H, halogen atom, trifluoromethyl, cyano, nitro, C₁-C₄ alkylcarbonyl and C₁-C₄ alkoxy carbonyl;

X is O, S or NH;

Y is not present, or Y is O or NR₇, wherein R₇ is a radical selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl and C₃-C₈ cycloalkyl;

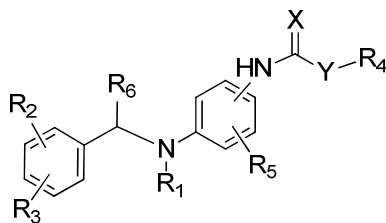
R₄ is a radical selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl and C₆-C₁₀ aryl;

R₅ is a radical selected from the group consisting of H, halogen atom, amino, C₁-C₆ alkylamino and , or R₅, together with adjacent , forms a fused-ring structure of  or  ;

R₆ is H, C₁-C₆ alkyl or C₃-C₆ cycloalkyl;

in the above said definitions of substituents, the halogen atom or the halogen atom in halogenated group is F, Cl or Br, and the halogenated radical is one substituted by F, Cl or Br.

More preferably, the compounds according to the present invention have a structure of general formula II:



II

wherein,

R₁ is a radical selected from the group consisting of H, C₁-C₆ alkyl, C₃-C₆ cycloalkyl, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, C₁-C₆ alkylaminocarbonyl, C₂-C₈ alkenyl, C₅-C₇ cycloalkenyl, C₂-C₈ alkynyl and C₆-C₁₀ aryl; wherein, the C₁-C₆ alkyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl; the C₂-C₈ alkenyl is unsubstituted or optionally substituted by

hydroxyl, amino, C₁-C₄ alkylcarbonyl, C₁-C₄ alkoxy, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl; the C₂-C₈ alkynyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, C₁-C₄ alkoxy, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl;

R₂ is a radical selected from the group consisting of halogen atom and C₁-C₄ alkoxy;

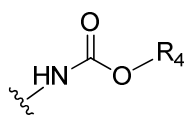
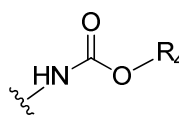
R₃ is a radical selected from the group consisting of H, halogen atom, trifluoromethyl and nitro;

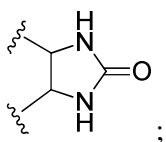
X is O;

Y is not present or Y is O;

R₄ is a radical selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl and C₆-C₁₀ aryl;

R₅ is a radical selected from the group consisting of H, halogen atom, amino and

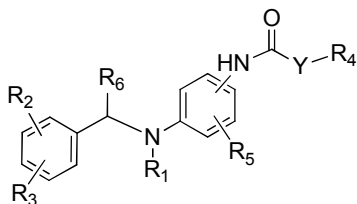
, or R₅, together with adjacent , forms a fused-ring structure of



R₆ is H, C₁-C₆ alkyl or C₃-C₆ cycloalkyl;

in the above said definitions of substituents, the halogen atom or the halogen atom in halogenated group is F, Cl or Br.

Most preferably, the compounds according to the present invention have a structure of general formula III:



III

wherein,

R₁ is a radical selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₈ alkenyl, C₅-C₇

cycloalkenyl and C₂-C₈ alkynyl; wherein, the C₁-C₆ alkyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, halogen atom, phenyl or halogenated phenyl; the C₂-C₈ alkenyl is unsubstituted or optionally substituted by hydroxyl, amino, halogen atom, phenyl or halogenated phenyl; the C₂-C₈ alkynyl is unsubstituted or optionally substituted by hydroxyl, amino, halogen atom, phenyl or halogenated phenyl;

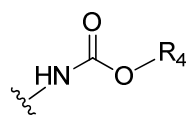
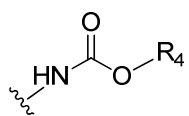
R₂ is a radical selected from the group consisting of F, Cl and methoxyl;

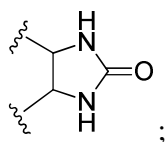
R₃ is a radical selected from the group consisting of H, halogen atom and trifluoromethyl;

Y is not present or Y is O;

R₄ is a radical selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl and C₆-C₁₀ aryl;

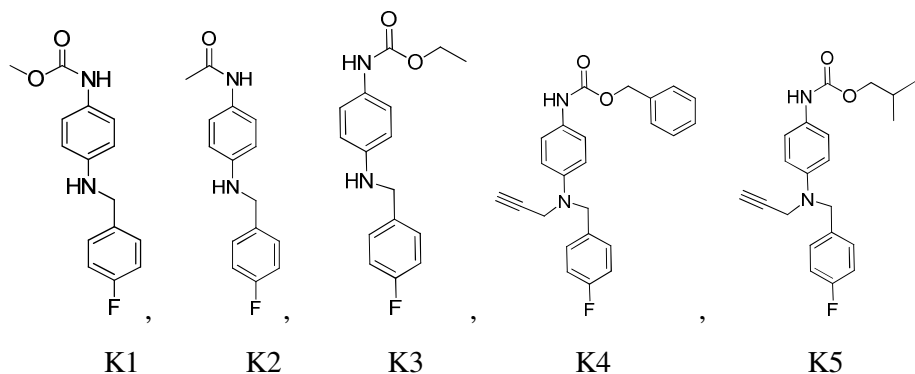
R₅ is a radical selected from the group consisting of H, halogen atom, amino and

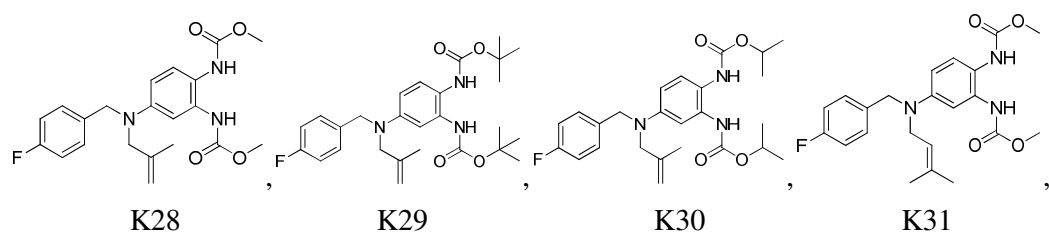
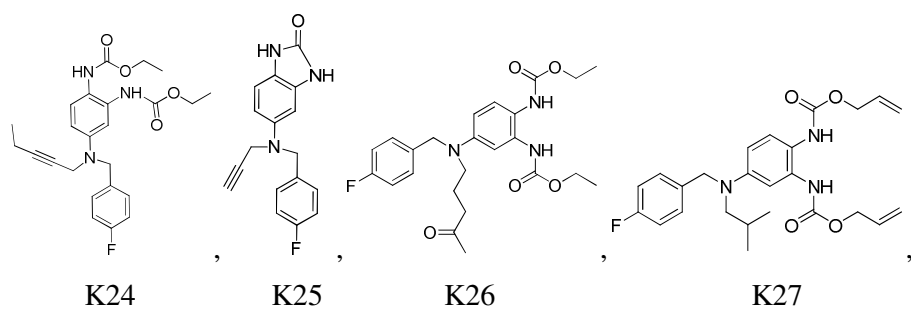
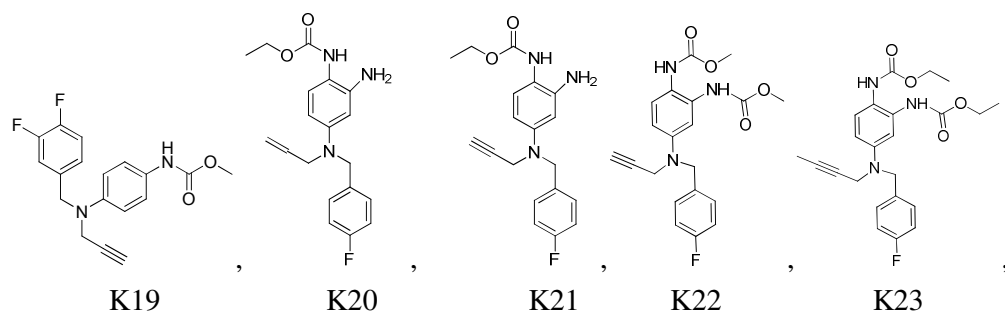
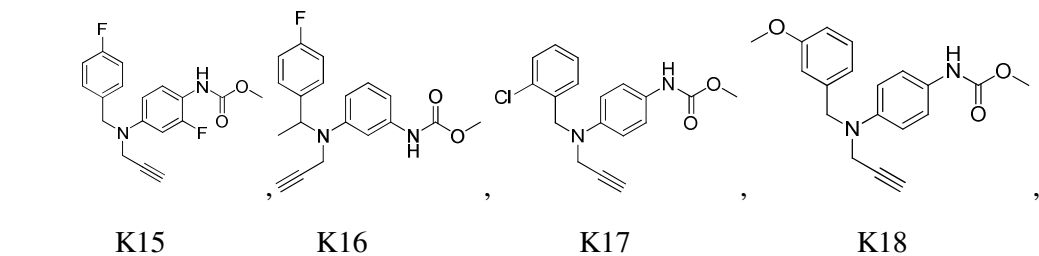
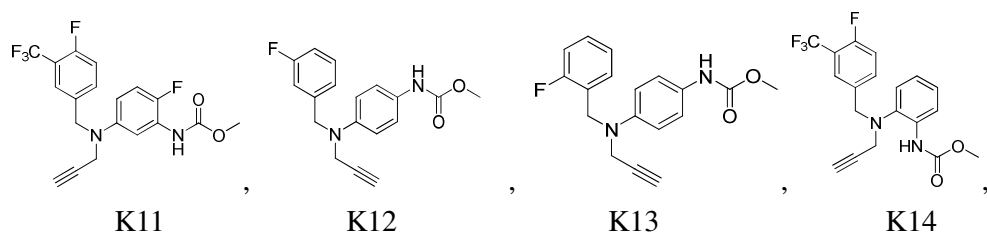
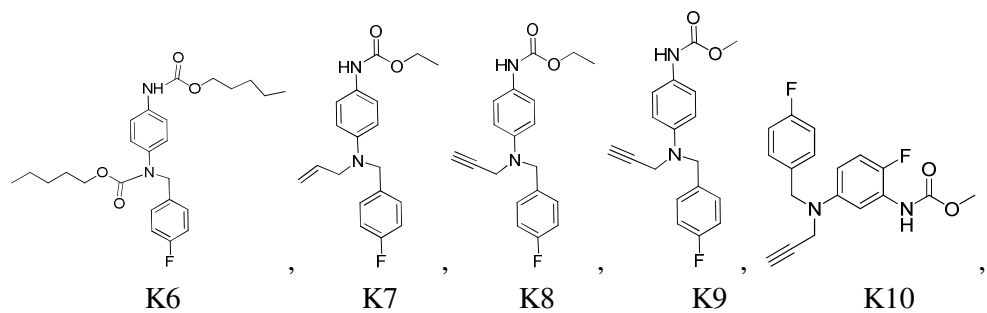
, or R₅, together with adjacent , forms a fused-ring structure of

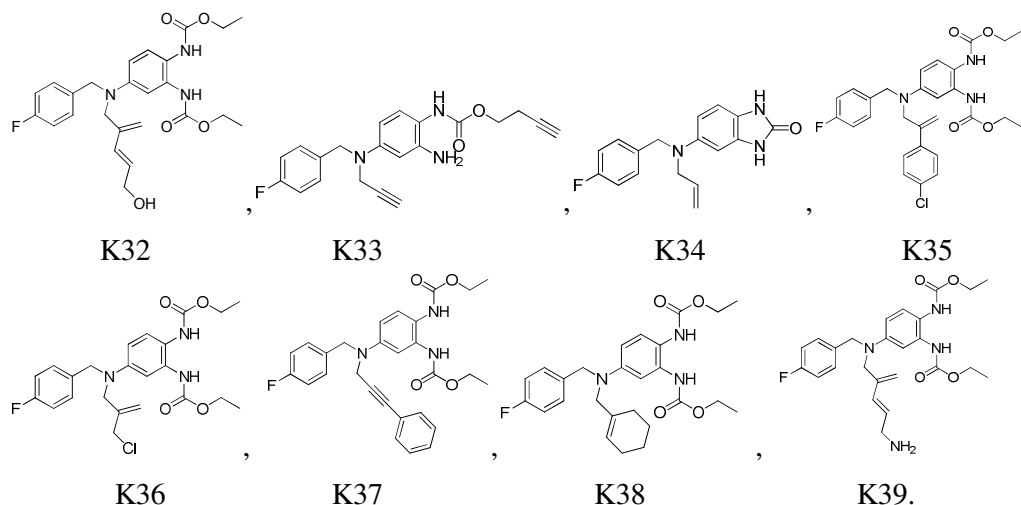


R₆ is H or C₁-C₆ alkyl.

According to the most preferable embodiments, parts of the representative compounds are listed as follows:







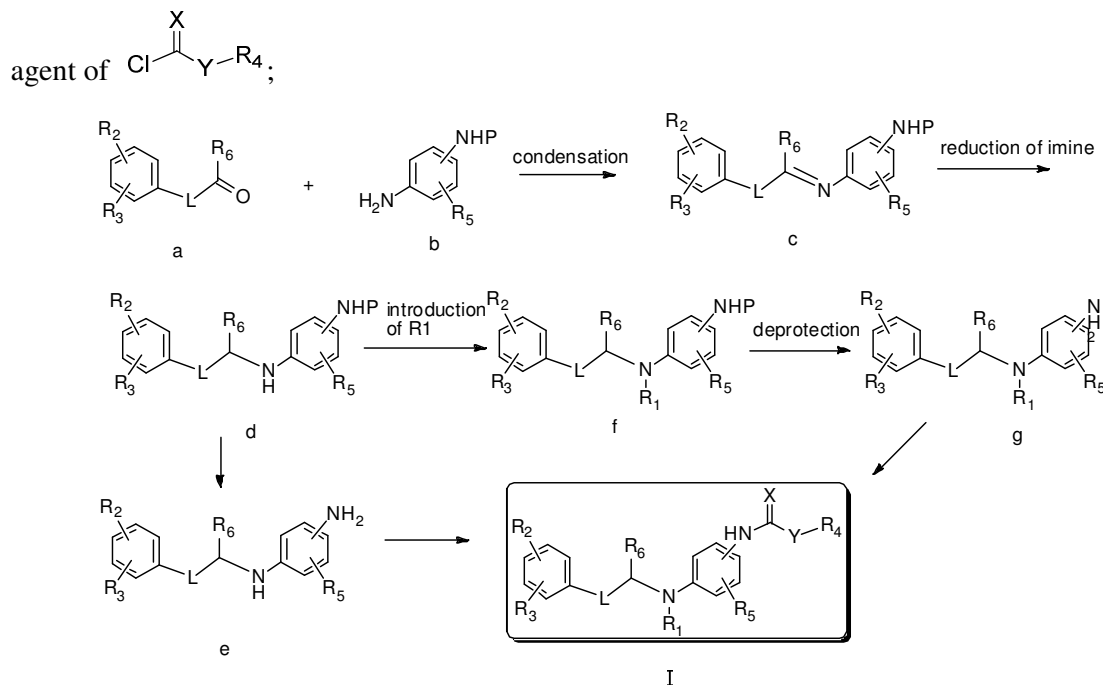
Pharmaceutically acceptable salts of the above compounds according to the present invention are salts formed by the above compounds with an acid, and the acid is selected from the group consisting of maleic acid, succinic acid, citric acid, tartaric acid, fumaric acid, formic acid, acetic acid, propanoic acid, propandioic acid, oxalic acid, benzoic acid, phthalic acid, methanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, naphthalenesulfonic acid, 1,5-naphthalenedisulfonic acid, camphoric acid, camphor sulfonic acid, salicylic acid, acetyl salicylic acid, aspartic acid, glutamic acid, lactic acid, gluconic acid, ascorbic acid, gallic acid, amygdalic acid, malic acid, sorbic acid, trifluoroacetic acid, taurine, homotaurine, isethionic acid, cinnamic acid, hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, nitric acid, phosphoric acid and perchloric acid.

In another object of the present invention, methods for preparing the above compounds or pharmaceutically acceptable salts thereof are provided, comprising the following schemes, wherein P is an optional protection group of amino. For particulars, Protective Groups in Organic Synthesis (Organic Chemical teaching and researching group of East China University of Science & Technology, East China University of Science & Technology press) can be referred to.

Scheme 1:

Under catalysis of a weak acid, such as p-toluenesulfonic acid, carbonyl compound **a** reacts with phenylamine compound **b** for conducting a condensation reaction, thereby producing imine compound **C**. Imine compound **C** is reduced to secondary amine compound

d under a such as sodium borohydride. Substituent R_1 is introduced into secondary amine compound **d** to produce intermediate compound **f** by a substitution reaction with halogenated hydrocarbon, or an addition reaction or an acylation reaction with α,β -unsaturated carbonyl compound. The protection group of amino group on intermediate compound **d** or **f** is removed to produce intermediate amine compound **e** or **g** at conditions well known to a person skilled in the art, and a compound of formula **I** is obtained by reacting amine **e** or **g** with an acylating

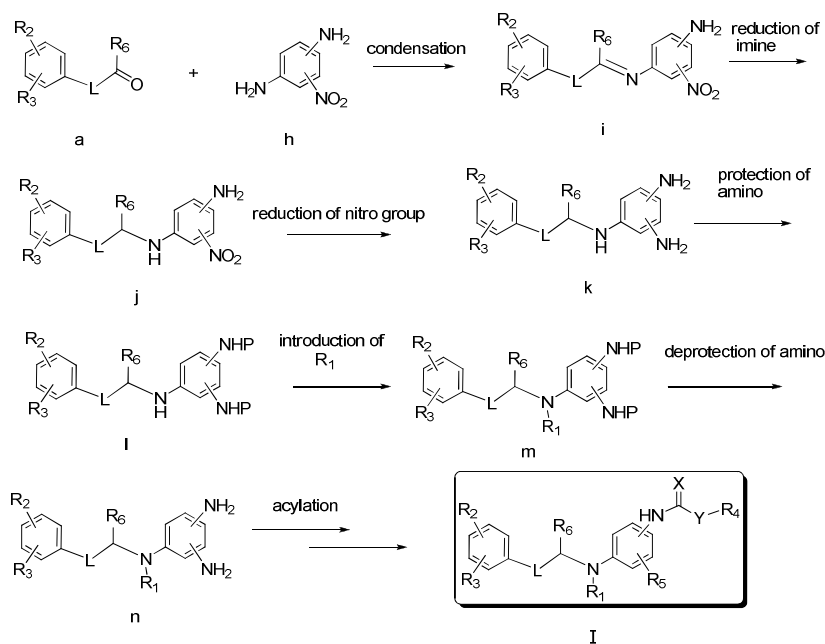


Scheme 1

Scheme 2

Under catalysis of a weak acid, such as p-toluenesulfonic acid, carbonyl compound **a** reacts with nitro-substituted benzene diamines compound **h** for conducting a condensation reaction, thereby producing imine compound **i**. Imine compound **i** is reduced to intermediate amine **j** under a reducing agent such as sodium borohydride. Diamino intermediate **k** is obtained by reducing the nitro on amine intermediate **j** in a manner well known to a person skilled in the art, such as catalyzing hydrogenation with Pd-C or reducing with SnCl_2 (stannous chloride). Intermediate **l** is obtained by introducing two protection groups **P** of amino group. Intermediate **m** is obtained by introducing substituent R_1 using a method similar to that of scheme 1. Intermediate **n** is obtained by removing two protection groups **P** to expose two amino groups. Finally, a compound of formula **I** is obtained by reacting intermediate **n** with an

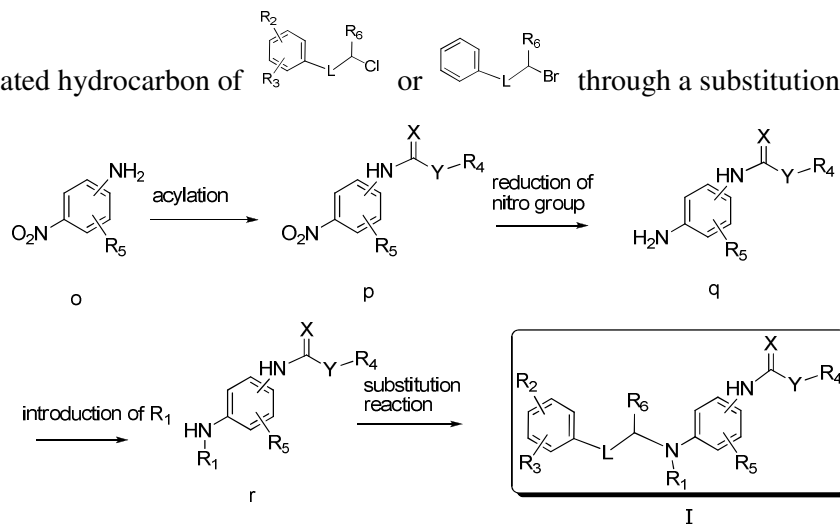
acylating agent of $\text{Cl}-\overset{\text{X}}{\underset{\text{Y}-\text{R}_4}{\text{C}}}=\text{O}$;



Scheme 2

Scheme 3:

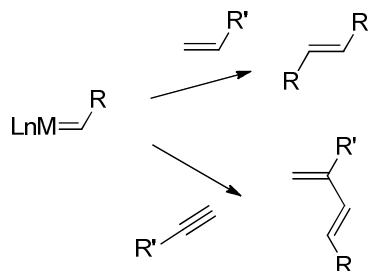
Intermediate compound **p** is obtained by reacting an acylating agent of $\text{Cl}-\overset{\text{X}}{\underset{\text{Y}-\text{R}_4}{\text{C}}}=\text{O}$ with nitro-substituted benzene amines compound **o**. Intermediate **r** is obtained by introducing substituent R_1 into amino compound **q** which is obtained by reducing nitro on intermediate compound **p**. Finally, a compound of formula I is obtained by reacting intermediate compound **r** with halogenated hydrocarbon of $\text{R}_2-\text{C}_6\text{H}_4-\text{L}-\text{CH}(\text{R}_6)-\text{X}$ or $\text{C}_6\text{H}_5-\text{L}-\text{CH}(\text{R}_6)-\text{X}$ through a substitution reaction:



Scheme 3

Scheme 4:

when substituent R_1 in the compound of general formula I is an alkenyl or alkynyl, other compounds of formula I according to the present invention can be obtained by olefin metathesis. The conventional olefin metathesis is a recombination reaction between olefins and olefins, or between olefins and alkynes under catalysis of metal ruthenium carbene complex (Grubbs catalyst). The scheme of the olefin metathesis is shown as follows:

**Scheme 5: preparation for salts of the compounds**

The compound of general formula I according to the present invention can be converted into pharmaceutically acceptable salts, such as hydrochloride, by adding a solution of acid into solution of the compound and completely removing solvent under reduced pressure.

In still another aspect of the present invention, a use of the above compounds, pharmaceutically acceptable salts or pharmaceutical composition thereof in preparing medicaments for the treatment of nervous system diseases, etc, is provided.

The nervous system diseases include epilepsy, convulsion, neuropathic pain, acute ischemic stroke or neurodegenerative diseases.

Advantageous Effects

Compared with existing medicaments, such as retigabine, the novel compounds described in the present invention are more stable and will not be readily oxidized and deteriorated.

Moreover, the compound provided by the present invention not only retains the high activity of activating potassium ion channels, which are the same as that of retigabine, but also exhibits a significant anti-epileptic action in vivo and high distribution concentration in brain tissue so as to achieve better therapeutic effects.

Best Mode for Carrying Out the Invention

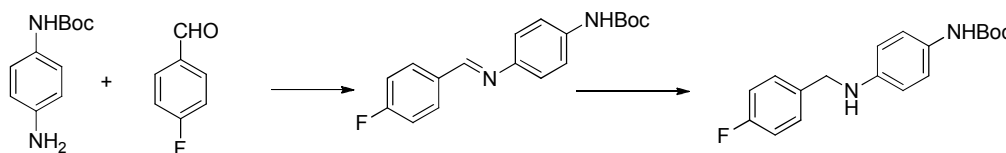
The present invention will be further illustrated based on the following examples, but the present invention will not be limited thereto.

I. Preparation Examples for compounds

In following preparation examples, NMR was conducted on a Mercury-Vx 300M instrument manufactured by Varian with calibration of δ H 7.26 ppm(CDCl_3), 2.50 ppm(DMSO-d_6) and 3.15 ppm (CD_3OD). The reagents were mainly provided by Shanghai Chemical Reagent Co. Ltd and the silicone plates (model HSGF 254) for TLC thin layer chromatography were manufactured by Huiyou Silica gel Development Co. Ltd, Yantai, Shandong. The products were purified by normal phase column chromatography with a silica gel (model zcx-11) of 200-300 mesh, wherein, the silica gel was manufactured by Branch of Qingdao Haiyang Chemical Co. Ltd.

Preparation example 1: synthesis of methyl 4-(N-parafluorobenzyl-imino) -aniline acid methyl ester (K1)

1.1. Synthesis of tert-butyl 4-(N-parafluorobenzyl-imino)-aniline formate

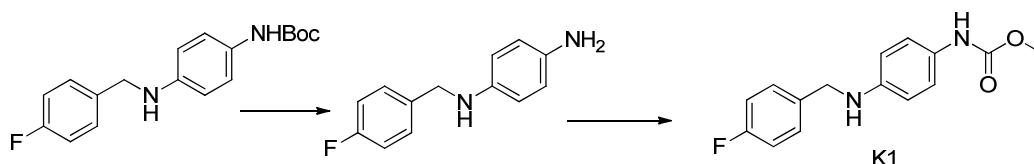


Mono-Boc-paraphenylenediamine (2.57g, 0.0123mol) and p-toluenesulfonic acid (62mg, 0.32mmol) were added in toluene (50mL), parafluorobenzaldehyde (1.68g, 0.0135mol) was then added to obtain a mixture. The mixture was heated to azeotropy for 12h so as to remove water thereto. Thereafter, the reaction solution was filtered while it was hot. A solid was precipitated when the filtrate was cooled down. The solid was filtered and dried, and the resulting crude product was obtained and was directly used for next step of reaction.

The above crude product (3.5g, 11.1mmol) was dissolved in a solution of dioxane/MeOH (30mL, the volume ratio of dioxane/methanol is 4:1). NaBH_4 (697mg, 0.018mol) was then further added in batches with stirring at room temperature until the reaction was completed. After quenching with water, the reaction solution was extracted with EtOAc (15mLx3). The

organic phases were washed with saturated saline solution, dried with anhydrous Na_2SO_4 and concentrated, a product of tert-butyl 4-(N-parafluorobenzyl-imino)-aniline formate (3.5g) was then obtained. ^1H NMR(300 MHz, CDCl_3): δ 7.31(t, $J=8.4\text{Hz}$, 2H), 7.14(d, $J=8.4\text{Hz}$, 2H), 7.01(t, $J=8.7\text{Hz}$, 2H), 6.56(d, $J=9.0\text{Hz}$, 2H), 6.22(s, 1H), 4.27(s, 2H), 1.50(s, 9H).

1.2. Synthesis of methyl 4-(N-parafluorobenzyl-imine)-aniline formate (K1)



tert-butyl 4-(N-parafluorobenzyl-imino)-aniline acid formate (100mg, 0.32mmol) obtained in the above step was dissolved in CH_2Cl_2 (2mL), trifluoroacetic acid (TFA, 1.5mL) was then added thereto. The resulting mixture was stirred for 1h at room temperature and then concentrated. The resulting compound was dissolved in dioxane (10mL), and diisopropyl ethylamine (120mg, 1.18mmol) was then added. Methyl chloroformate (45mg, 0.49mmol) was slowly added dropwise at 0°C , and the resulting mixture was stirred for 1h at room temperature. Thereafter, the reaction solution was diluted with water and extracted with EtOAc (10mLx3). The organic phases were washed with saturated saline solution, dried with anhydrous Na_2SO_4 , concentrated and then loaded on chromatography column (PE/EtOAc=4:1). A product of methyl 4-(N-parafluorobenzyl-imine)-aniline formate (K1) was obtained. ^1H NMR(300 MHz, CDCl_3): δ 7.13-7.26(m, 4H), 6.87-6.96(m, 4H), 6.61(s, 1H), 4.26(s, 2H), 3.77(s, 3H).

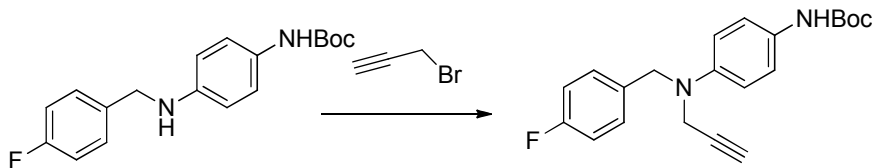
Following compounds were obtained by using a procedure similar to that of Preparation example 1:

Compound	Structural Formula	^1H NMR (CDCl_3 , 300 MHz) data, δ
K2		7.69(d, $J=7.8\text{Hz}$, 2H), 7.40-7.46(m, 6H), 7.24-7.34(m, 4H), 6.85-6.91(m, 4H), 6.61(s, 1H), 4.73(s, 2H), 4.38(s, 2H), 4.04-4.13(m, 1H), 1.52(s, 9H)
K3		7.29(t, $J=8.4\text{Hz}$, 2H), 7.03(t, $J=8.4\text{Hz}$, 2H), 6.95(d, $J=6.6\text{Hz}$, 2H), 6.88(d, $J=6.9\text{Hz}$, 2H), 4.55(s, 2H), 4.03(s, 2H), 2.59(q, $J=7.2\text{Hz}$, 2H), 2.25(s, 1H), 1.10(t, $J=7.5\text{Hz}$, 3H)

Preparation example 2: synthesis of methyl

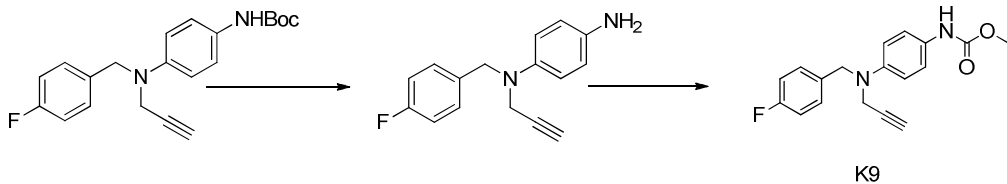
4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate (K9)

2.1. Synthesis of tert-butyl 4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate



Compound tert-butyl 4-(N-parafluorobenzyl-imino)-aniline formate (316mg, 1mmol) was dissolved in DMF (N,N-dimethylformamide) (5mL). Propargyl bromide (178mg, 1.5mmol) and diisopropyl ethylamine (i-Pr₂NEt) (258mg, 2mmol) were added dropwise. The resulting mixture was stirred for 2h at 80°C, cooled, diluted with water and extracted with EtOAc (10mLx3). The organic phases were washed with saturated saline solution, dried with anhydrous Na₂SO₄, concentrated and then loaded on chromatography column (the volume ratio of PE/EtOAc is 8:1). A product of tert-butyl 4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate (330mg, 93.2%) was then obtained. ¹H NMR(300 MHz, CDCl₃): δ 7.21-7.30(m, 4H), 7.00(t, *J*=8.4Hz, 2H), 6.86(d, *J*=9.0Hz, 2H), 6.32(s, 1H), 4.42(s, 2H), 3.92(d, *J*=2.4Hz, 2H), 2.20(t, *J*=2.4Hz, 1H), 1.50(s, 9H).

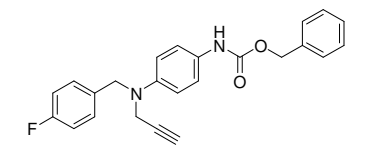
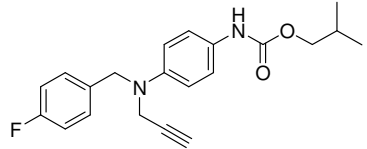
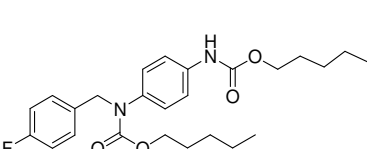
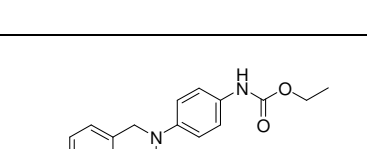
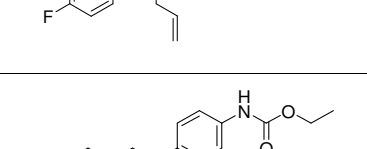
2.2. Synthesis of methyl 4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate



tert-butyl 4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate (100mg, 0.32mmol) obtained in the above step was dissolved in CH₂Cl₂ (2mL), trifluoroacetic acid (TFA, 1.5mL) was then added thereto. The resulting mixture was stirred for 1h at room temperature and then concentrated. The resulting compound was dissolved in dioxane (10mL), and diisopropyl ethylamine (109mg, 0.846mmol) was then added. Methyl chloroformate (40mg, 0.423mmol) was slowly added dropwise at 0°C, and the resulting mixture was stirred for 1h at room temperature. Thereafter, the reaction solution was diluted with water and extracted with EtOAc

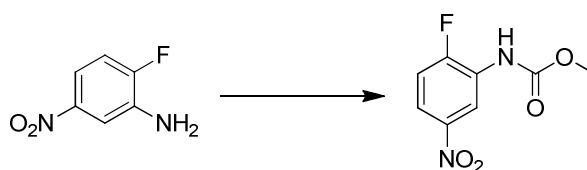
(10mLx3). The organic phases were washed with saturated saline solution, dried with anhydrous Na_2SO_4 , concentrated and then loaded on chromatography column (PE/EtOAc=4:1). A product of methyl 4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate (K9) was obtained. ^1H NMR(300 MHz, CDCl_3): δ 7.23-7.28(m, 4H), 6.99(t, J =8.4Hz, 2H), 6.83(d, J =8.7Hz, 2H), 4.41(s, 2H), 3.91(s, 2H), 3.72(s, 3H), 2.22(s, 1H).

Following compounds were obtained by using a procedure similar to that of Preparation example 2:

Compound	Structural Formula	^1H NMR (CDCl_3 , 300 MHz) data, δ
K4		7.35-7.39(m, 4H), 7.25-7.30(m, 5H), 7.03(d, J =8.7Hz, 2H), 6.85(d, J =8.7Hz, 2H), 5.18(s, 2H), 4.44(s, 2H), 3.94(s, 2H), 2.22(s, 1H)
K5		7.23-7.29(m, 4H), 6.99(t, J =8.7Hz, 2H), 6.83(d, J =9.3Hz, 2H), 6.67(s, 1H), 4.42(s, 2H), 3.93(s, 2H), 3.91(d, J =4.2Hz, 2H), 2.21(s, 1H), 1.92-1.97(m, 1H), 0.94(d, J =6.6Hz, 6H)
K6		7.30(d, J =8.7Hz, 2H), 7.16(t, J =8.7Hz, 2H), 6.97(d, J =8.4Hz, 3H), 6.74(s, 1H), 4.76(s, 2H), 4.07-4.16(m, 4H), 1.64-1.68(m, 2H), 1.53-1.56(m, 2H), 1.33-1.35(m, 4H), 1.23-1.27(m, 4H), 0.91(t, J =5.4Hz, 3H), 0.84(t, J =6.3Hz, 3H)
K7		7.15-7.23(m, 4H), 6.99(t, J =8.7Hz, 2H), 6.63(d, J =9.0Hz, 2H), 6.53(s, 1H), 5.80-5.89(m, 1H), 5.18(d, J =1.5Hz, 1H), 5.13(d, J =3.3Hz, 1H), 4.44(s, 2H), 4.18(q, J =6.6Hz, 2H), 3.93(d, J =4.8Hz, 2H), 1.26(t, J =6.6Hz, 3H)
K8		7.23-7.29(m, 4H), 7.00(t, J =8.4Hz, 2H), 6.85(d, J =9.0Hz, 2H), 6.59(s, 1H), 4.42(s, 2H), 4.18(q, J =7.2Hz, 2H), 3.92(d, J =2.1Hz, 2H), 2.21(t, J =2.4Hz, 1H), 1.28(t, J =7.2Hz, 3H)

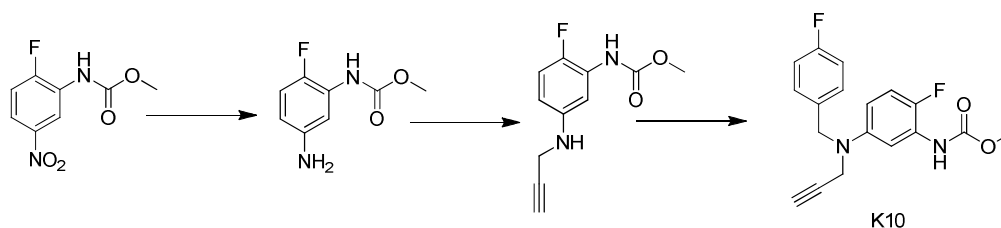
Preparation example 3: synthesis of methyl 2-fluoro-5-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate (K10)

3.1. Synthesis of methyl 2-fluoro-5-nitro aniline formate



2-fluoro-3-nitro group aniline (156mg, 1mmol) was dissolved in dried THF (5mL) under an atmosphere of nitrogen. The resulting solution was cooled to 0°C in an ice-bath and sodium hydride (40mg, 60%, 1mmol) was added thereto. Then the solution was warmed to room temperature with stirring for 60 min. Thereafter, the solution was cooled to 0°C and then methyl chloroformate (85.2μL, 1.1mmol) was added. After stirring for 10min, the mixture was diluted with water (10mL) and extracted with EtOAc (10mLx3). The combined organic phases were washed with saturated saline solution (15mLx3), dried with anhydrous Na₂SO₄, concentrated and then loaded on chromatography column (PE/EtOAc=10:1-5:1). A product of methyl 2-fluoro-5-nitro aniline formate (245mg, 84.5%) was obtained.

3.2. Synthesis of methyl 2-fluoro-5-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate (K10)



Compound methyl 2-fluoro-5-nitro aniline formate (95mg, 0.443mmol) obtained in the above step was dissolved in ethyl acetate (15mL) and the air therein was replaced with N₂. Pd-C (10%, 5mg) was rapidly added in the obtained solution and N₂ therein was replaced with H₂. The resulting solution was kept at room temperature for 6h, filtered and concentrated to produce a compound of methyl 2-fluoro-5-aminoaniline formate (72.5mg, 89.1%) in the form of colorless oil, which was directly used in the nest step;

under an atmosphere of nitrogen, compound methyl 2-fluoro-5-aminoaniline formate (72.5mg, 0.39mmol) was dissolved in dried DMF (5mL), then propargyl bromide (44μL, 0.59mmol) and DIPEA (140μL, 0.78mmol) were added. The resulting solution was stirred for 6h at 60°C, and diluted with water (10mL) and extracted with EtOAc (10mLx3). The combined organic phases were washed with saturated saline solution (15mLx3), dried with anhydrous Na₂SO₄, concentrated and then loaded on chromatography column (PE/EtOAc =6:1-4:1) to produce a product of methyl 2-fluoro-5-(N-propargyl-imino)-aniline formate (70.1mg, 81%);

under an atmosphere of nitrogen, methyl 2-fluoro-5-(N-propargyl-imino)-aniline formate (70mg, 0.32 mmol) was dissolved in dried DMF (5mL), then 4-fluorobenzyl bromide (81μL, 0.64mmol) and diisopropyl ethylamine (DIPEA) (115μL, 0.64mmol) were added. The resulting solution was stirred for 6h at 60°C, was diluted with water (10mL) and extracted with EtOAc (10mLx3). The combined organic phases were washed with saturated saline solution (15mLx3), dried with anhydrous Na₂SO₄, concentrated and then loaded on chromatography column (PE/EtOAc =6:1-4:1) to produce a product of methyl 2-fluoro-5-(N-parafluorobenzyl-N-propargyl- imino)-aniline formate (K10) (88.7mg, 84%). ¹H NMR(300 MHz, CDCl₃): δ 7.79(s, 1H), 7.30(m, 2H), 7.01(m, 2H), 6.93(dd, *J*=9.9 Hz, *J*=9.0 Hz, 2H), 6.83(s, 1H), 6.51(m, 1H), 7.01(m, 2H), 4.44(s, 2H), 3.94(d, *J*=2.4 Hz, 2H), 3.79(s, 3H), 2.24(t, *J*=2.4 Hz, 1H).

Following compounds were obtained by using a procedure similar to that of Preparation example 3:

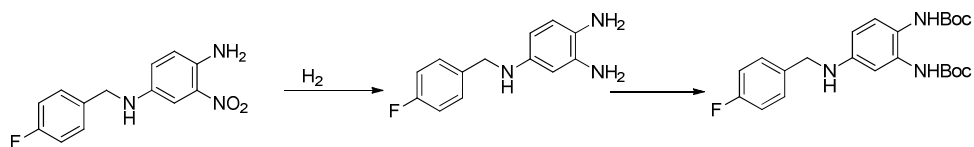
Compound	Structural Formula	¹ H NMR (CDCl ₃ , 300 MHz) data, δ
K11		7.80 (s, 1H), 7.59 (d, <i>J</i> =6.6Hz, 1H), 7.55 (m, 1H), 7.15 (t, <i>J</i> =8.1Hz, 2H), 6.95 (dd, <i>J</i> =9.0Hz, <i>J</i> =10.5 Hz, 2H), 6.84 (s, 1H), 6.48 (m, 1H), 4.48 (s, 2H), 3.97 (d, <i>J</i> =2.4Hz, 2H), 3.78 (s, 3H), 2.27 (t, <i>J</i> =2.1Hz, 1H)
K12		7.31 (t, <i>J</i> =8.7 Hz, <i>J</i> =5.7 Hz, 1H), 7.25 (m, 2H), 7.10 (d, <i>J</i> =7.2 Hz, 2H), 7.04 (d, <i>J</i> =9.9 Hz, 1H), 6.95 (td, <i>J</i> =8.4 Hz, <i>J</i> =2.1 Hz, 1H), 6.81 (d, <i>J</i> =10.8 Hz, 2H), 6.57 (s, 1H), 4.48 (s, 2H), 3.98 (d, <i>J</i> =2.1 Hz, 2H), 3.75 (s, 3H)
K13		7.32 (t, <i>J</i> =8.7 Hz, <i>J</i> =7.5 Hz, 1H), 7.24 (m, 3H), 7.10 (d, <i>J</i> =7.2 Hz, 1H), 7.06 (d, <i>J</i> =9.9 Hz, 1H), 6.80 (d, <i>J</i> =5.7 Hz, 2H), 6.64 (s, 1H), 4.56 (s, 2H), 4.10 (d, <i>J</i> =2.4 Hz, 2H), 3.75 (s, 3H), 2.25 (t, <i>J</i> =2.4, 1H)
K14		8.08 (d, <i>J</i> =8.1Hz, 1H), 7.78 (s, 1H), 7.56 (d, <i>J</i> =6.6Hz, 1H), 7.44 (m, 1H), 7.36 (dd, <i>J</i> =6.6Hz, <i>J</i> =1.2Hz, 1H), 7.19 (t, <i>J</i> =7.5Hz, 1H), 7.12 (t, <i>J</i> =9.0Hz, 1H), 7.01 (td, <i>J</i> =7.5Hz, <i>J</i> =1.5Hz, 1H), 4.16 (s, 2H), 3.78 (s, 3H), 3.59 (d, <i>J</i> =2.1Hz, 2H), 2.36 (t, <i>J</i> =2.1Hz, 1H)

K15		7.80 (brs, 1H), 7.55 (d, $J=6.3$ Hz, 1H), 7.49 (m, 1H), 7.17 (t, $J=9.6$ Hz, 1H), 6.64-6.59 (m, 2H), 6.53 (s, 1H), 4.49 (s, 2H), $J=3.97$ (d, $J=2.7$ Hz, 2H), 3.77 (s, 3H), 2.26 (t, $J=2.7$ Hz, 1H)
K16		7.37-7.29(m, 2H), 7.25(d, $J=8.4$ Hz, 2H), 7.06-6.93(m, 4H), 6.43(brs, 1H), 4.90(m, 1H), 3.78(d, $J=2.4$ Hz, 2H), 3.75(s, 3H), 2.16(t, $J=2.4$ Hz, 1H), 1.54-1.47(m, 3H)
K17		7.41-7.30(m, 2H), 7.25-7.19(m, 4H), 6.46(brs, 1H), 4.59(s, 2H), 4.06(d, $J=2.4$ Hz, 2H), 3.75(s, 3H), 2.25(t, $J=2.4$ Hz, 1H)
K18		7.26-7.22(m, 4H), 6.90-6.85(m, 4H), 6.48(d, $J=8.4$ Hz, 2H), 6.43(brs, 1H), 4.42(s, 2H), 3.93(d, $J=2.1$ Hz, 2H), 3.80(s, 3H), 3.75(s, 3H), 2.21(t, $J=2.4$ Hz, 1H)
K19		7.26(d, $J=8.7$ Hz, 2H), 7.18-7.05(m, 3H), 6.85(d, $J=8.7$ Hz, 1H), 6.47(brs, 1H), 4.42(s, 2H), 3.96(d, $J=2.1$ Hz, 2H), 3.69(s, 3H), 2.23(t, $J=2.4$ Hz, 1H)

Preparation example4: synthesis of ethyl

2-amino-4-(N-parafluorobenzyl-N-allyl-imino) -aniline formate (K20)

4.1. Synthesis of tert-butyl 2-(tert-butoxycarbonyl imino)-4-(N-parafluorobenzyl-imino)-aniline formate

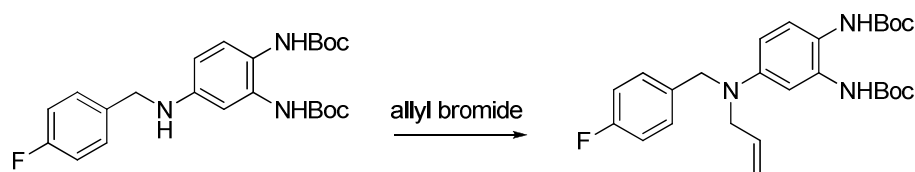


According to a procedure similar to that of Preparation example 1, intermediate 2-nitro-4-(N-parafluorobenzyl-imino)-aniline was obtained by using o-nitro paraphenylene diamine and parafluorobenzaldehyde as starting materials. 2-nitro-4-(N-parafluorobenzyl-imino)-aniline (2.61g, 0.01mol) was dissolved in THF (30mL) and air therein was replaced with N_2 . Then Pd-C (10%, 261mg) was rapidly added and N_2 therein was replaced with H_2 . The hydrogenation

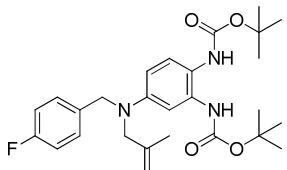
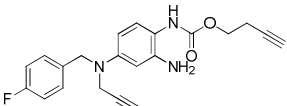
reaction was conducted overnight, and the resulting reaction solution was filtered and concentrated to produce a product of 2-amino-4-(N-parafluorobenzyl-imino)-aniline (2.3g, 99.6%, colorless oil, instable, prone to be oxidized and deteriorated), which was rapidly used in next step. ^1H NMR(300 MHz, CDCl_3): δ 7.32(t, $J=8.7\text{Hz}$, 2H), 7.02(t, $J=8.7\text{Hz}$, 2H), 6.58(d, $J=8.7\text{Hz}$, 1H), 6.01-6.08(m, 2H), 4.22(s, 2H).

2-amino-4-(N-parafluorobenzyl-imino)-aniline (1g, 4.32mmol) obtained in the above step was dissolved in THF/ H_2O (40mL, 1:1), di-tert-butoxy carbonic anhydride (Boc_2O) (2.83g, 12.96mmol) and sodium bicarbonate (1.16g, 12.96mmol) were added thereto. The resulting solution was stirred overnight at room temperature, diluted with water, and extracted with EtOAc (30mLx3). The combined organic phases were washed with saturated saline solution (15mLx3), dried with anhydrous Na_2SO_4 . The pooled organic phase was concentrated under reduced pressure to remove solvent, and the residue was purified by silica gel chromatography column (PE/EtOAc=5:1) to produce an intermediate tert-butyl 2-(tert-butoxycarbonyl imino)-4-(N-parafluorobenzyl-imino)-aniline formate (1.67g, 89.5%, colorless oil). ^1H NMR(300 MHz, CDCl_3): δ 7.28(t, $J=8.7\text{Hz}$, 2H), 7.00(t, $J=8.7\text{Hz}$, 3H), 6.89(s, 1H), 6.26(d, $J=8.7\text{Hz}$, 1H), 4.25(s, 2H), 1.48(s, 18H).

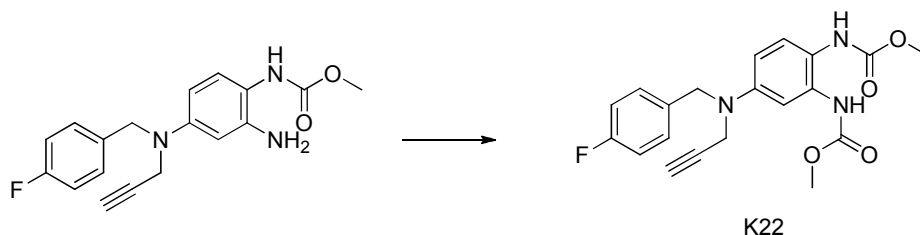
4.2. Synthesis of tert-butyl 2-(tert-butoxycarbonyl imino)-4-(N-parafluorobenzyl-N-allyl-imino)-aniline formate



Intermediate tert-butyl 2-(tert-butoxycarbonyl imino)-4-(N-parafluorobenzyl-imino)-aniline formate (150mg, 0.348mmol) was dissolved in DMF (5mL). Allyl bromide (55mg, 0.452mmol) and $i\text{-Pr}_2\text{NEt}$ (99mg, 0.766mmol) were added dropwise. The resulting solution was stirred for 2h at 50°C , and then was cooled down, diluted with water (10mL) and extracted with EtOAc (10mLx3). The organic phases were washed with saturated saline solution, dried with anhydrous Na_2SO_4 , concentrated and then loaded on chromatography column (PE/EtOAc=4:1) to produce a compound of tert-butyl

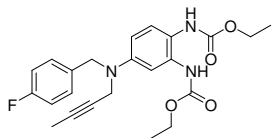
K29		7.16(dd, $J=7.5$ Hz, $J=5.7$ Hz, 2H), 7.06(d, $J=8.7$ Hz, 1H), 6.98(t, $J=8.4$ Hz, 2H), 6.91(brs, 1H), 6.74(s, 1H), 6.37(s, 1H), 6.37(d, $J=8.7$ Hz, 2H), 4.87(s, 1H), 4.80(s, 1H), 4.49(s, 2H), 3.83(s, 2H), 1.72(s, 3H), 1.48(s, 18H)
K33		7.25(t, $J=8.4$ Hz, 2H), 7.15(s, 1H), 7.01(t, $J=8.7$ Hz, 2H), 6.40(d, $J=8.4$ Hz, 2H), 4.46(s, 2H), 4.29(t, $J=4.5$ Hz, 2H), 3.96(s, 2H), 3.67(s, 8H), 3.80(t, $J=9.0$ Hz, 2H), 2.23(s, 1H)

Preparation example 5: synthesis of methyl 2-(methoxy carbonyl-imino)-4-(N-parafluoro benzyl-N-propargyl-imino)-aniline formate (K22)



Compound methyl 2-amino-4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate was obtained by using a procedure similar to that of Preparation example 4. The resulting compound (80mg, 0.24mmol) was dissolved in dioxane (10mL), DIPEA (100mg, 0.98mmol) was further added thereto, and a solution of methyl chloroformate (50mg, 0.53mmol) in dioxane (2mL) was slowly added dropwise at 0°C. The mixture was stirred at the room temperature until the reaction was completed. The resulting solution was diluted with water and extracted with EtOAc (10mLx3). The organic phases were washed with saturated saline solution, dried with anhydrous Na₂SO₄, concentrated and then loaded on chromatography column (PE/EtOAc=4:1-2:1) to produce a product of methyl 2-(methoxy carbonyl-imino)-4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate (K22) (68mg, 72%). ¹H NMR(300 MHz, CDCl₃): δ 7.25(t, $J=8.4$ Hz, 2H), 7.12(d, $J=8.7$ Hz, 2H), 6.99(t, $J=8.7$ Hz, 2H), 6.57(d, $J=8.7$ Hz, 1H), 4.45(s, 2H), 3.94(s, 2H), 3.71(s, 6H), 2.22(s, 1H).

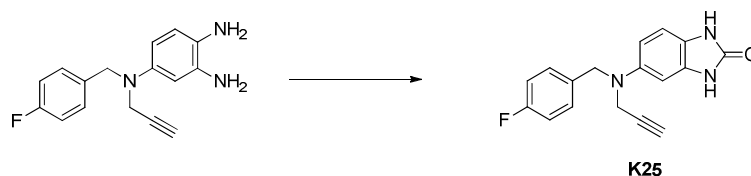
Following compounds were obtained by using a procedure similar to that of Preparation example 5:

Compound	Structural Formula	¹ H NMR (CDCl ₃ , 300 MHz) data, δ
K23		7.25(t, $J=8.4$ Hz, 2H), 7.09-7.15(m, 2H), 7.01(t, $J=8.7$ Hz, 2H), 6.59(d, $J=6.0$ Hz, 1H), 4.47(s, 2H), 4.20(q, $J=6.3$ Hz, 4H), 3.91(s, 2H), 1.79(s, 3H), 1.28(t, $J=7.2$ Hz, 6H)

K24		7.26(t, $J=9.0$ Hz, 2H), 7.15(d, $J=8.4$ Hz, 2H), 7.03(t, $J=8.7$ Hz, 2H), 6.60(d, $J=8.7$ Hz, 1H), 4.49(s, 2H), 4.20(q, $J=6.9$ Hz, 4H), 3.95(s, 2H), 2.17(q, $J=5.7$ Hz, 2H), 1.29(t, $J=6.9$ Hz, 6H), 1.10(t, $J=7.5$ Hz, 3H)
K26		7.14 (dd, $J=8.7$ Hz, $J=5.4$ Hz, 2H), 7.07 (d, $J=9.0$ Hz, 1H), 7.00 (t, $J=8.4$ Hz, 2H), 6.41 (brs, 1H), 6.41 (dd, $J=8.7$ Hz, $J=2.4$, 2H), 4.47 (s, 2H), 4.19 (m, 4H), 3.35 (t, $J=7.2$ Hz, 2H), 2.47 (t, $J=6.9$ Hz, 2H), 2.11 (s, 3H), 1.90 (m, 2H), 1.29 (t, $J=6.9$ Hz, 6H)。
K27		7.17 (dd, $J=8.4$ Hz, $J=5.4$ Hz, 4H), 7.03 (d, $J=9.0$ Hz, 1H), 6.97 (t, $J=9.0$ Hz, 4H), 6.72 (brs, 2H) 6.35 (dd, $J=9.0$ Hz, $J=2.7$ Hz, 2H), 4.50 (s, 4H), 4.22 (t, $J=6.9$ Hz, 4H), 4.13 (t, $J=7.2$ Hz, 4H), 3.99 (s, 4H), 1.67 (s, 6H), 1.28 (m, 12H)
K28		7.21 (dd, $J=8.7$ Hz, $J=5.7$ Hz, 2H), 7.09 (d, $J=9.0$ Hz, 1H), 7.03 (t, $J=8.7$ Hz, 2H), 6.41 (s, 1H), 6.41 (d, $J=8.7$, 2H), 4.92 (s, 1H), 4.85 (s, 1H), 3.88 (s, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 1.77 (s, 3H)
K30		7.17 (dd, $J=8.7$ Hz, $J=5.4$ Hz, 2H), 7.06 (d, $J=9.0$ Hz, 1H), 6.99 (t, $J=8.7$ Hz, 2H), 6.86 (s, 1H), 6.38 (s, 1H), 6.38 (dd, $J=8.7$ Hz, $J=2.4$ Hz, 2H), 4.97 (m, $J=6.3$ Hz, 2H), 4.88 (s, 1H), 4.81 (s, 1H), 4.50 (s, 2H), 3.84 (s, 2H), 561.73 (s, 3H), 1.27 (m,)
K31		7.19 (dd, $J=8.4$ Hz, $J=5.7$ Hz, 2H), 7.06 (d, $J=8.4$ Hz, 2H), 6.96 (t, $J=8.4$ Hz, 3H), 6.42 (s, 1H), 6.42 (dd, $J=8.7$ Hz, $J=2.4$ Hz, 2H), 5.23 (t, $J=6.3$ Hz, 2H), 4.43 (s, 2H), 3.91 (d, $J=6.3$ Hz, 2H), 3.74 (s, 3H), 3.73 (s, 3H), 1.71 (s, 3H), 1.63 (s, 3H)
K35		7.34-7.26 (m, 4H), 7.19-7.14 (dd, $J=8.4$ Hz, $J=8.1$ Hz, 4H), 7.07 (d, $J=8.7$ Hz, 1H), 6.98 (t, $J=8.7$ Hz, 4H), 6.54 (s, 1H), 6.44 (d, $J=7.5$ Hz, 1H), 5.44 (s, 1H), 5.16 (s, 1H), 4.55 (s, 2H), 4.29 (s, 2H), 4.19 (m, 4H), 1.28 (t, $J=7.2$ Hz, 6H)
K36		7.17 (dd, $J=8.4$ Hz, $J=5.4$ Hz, 2H), 7.08 (d, $J=9.0$ Hz, 2H), 6.99 (t, $J=9.0$ Hz, 2H), 6.94 (s, 1H), 6.44 (dd, $J=9.0$ Hz, $J=2.7$ Hz, 1H), 6.44 (brs, 1H), 5.29 (s, 1H), 5.11 (s, 1H), 4.54 (s, 2H), 4.18 (m, 4H), 4.07 (s, 2H), 1.28 (m, 6H)

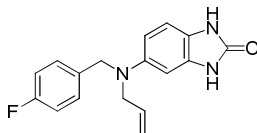
K37		7.39-7.37 (m, 2H), 7.34-7.26 (m, 5H), 7.18 (d, $J=8.7$ Hz, 1H), 7.02 (t, $J=8.7$ Hz, 2H), 6.66 (dd, $J=8.7$ Hz, $J=2.1$ Hz, 1H), 6.52 (brs, 1H), 4.56 (s, 2H), 4.20 (m, 6H), 1.29 (m, 6H)
K38		7.16 (dd, $J=8.4$ Hz, $J=5.4$ Hz, 2H), 7.05 (d, $J=8.7$ Hz, 1H), 6.98 (t, $J=9.0$ Hz, 4H), 6.88 (brs, 2H), 6.39 (dd, $J=9.0$ Hz, $J=2.7$ Hz, 2H), 5.50 (s, 1H), 4.47 (s, 4H), 4.20 (m, 4H), 3.80 (s, 2H), 2.04 (m, 2H), 1.99 (m, 2H), 1.60 (m, 4H), 1.28 (m, 6H)

**Preparation example 6: synthesis of
5-(N-parafluorobenzyl-N-propargyl-imino)-benzimidazole-2-one (K25)**

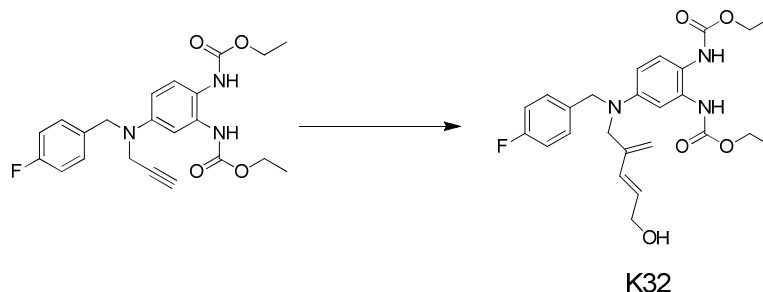


2-amino-4-(N-parafluorobenzyl-N-propargyl-imino)-aniline was obtained by using method of Preparation example 4. The resulting compound (27mg, 0.1mmol) was dissolved in THF (5mL). A solution of solid Triphogene (30mg, 0.1mmol) in THF (2mL) and Et₃N (30mg, 0.3mmol) were added dropwise thereto. The resulting solution was stirred for 2h at room temperature for 2h, and concentrated and loaded on column (PE/EtOAc/MeOH=10:10:1-5:5:1) to produce a product of 5-(N-parafluorobenzyl-N-propargyl-imino)-benzimidazole-2-one (K25) (25mg, 84.5%). ¹H NMR(300 MHz, CD₃OD): δ 7.34(t, $J=8.7$ Hz, 2H), 7.03(t, $J=8.7$ Hz, 2H), 6.89(d, $J=8.7$ Hz, 1H), 6.72(d, $J=8.7$ Hz, 2H), 4.42(s, 2H), 3.95(s, 2H), 2.60(s, 1H).

Compound 5-(N-parafluorobenzyl-N-allyl-imino)-benzimidazole-2-one (K34) was obtained by using a method similar to that of Preparation example 6. ¹H NMR(300 MHz, DMSO-*d*₆): δ 10.22(s, 1H), 10.14(s, 1H), 7.26(t, $J=7.8$ Hz, 2H), 7.13(d, $J=8.1$ Hz, 1H), 6.69(d, $J=8.4$ Hz, 1H), 6.32(d, $J=8.4$ Hz, 1H), 6.28(s, 1H).



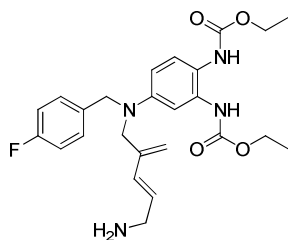
Preparation example7: synthesis of ethyl 2-(ethoxy carbonyl-imino)-4-[N-parafluorobenzyl
-N-1-(6-hydroxyl-2-alkenyl-3-hexylene)-imino]-aniline formate (K32)



Compound ethyl 2-(ethoxy carbonyl-imino)-4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate was obtained by using a method similar to that of Preparation example 5. Under an atmosphere of argon, the resulting compound (15mg, 0.036mmol) and allyl alcohol (21mg, 0.36mmol) were dissolved in anhydrous dichloromethane (3ml), and oxygen therein was removed by insufflation of argon for 15min. Grubbs II catalyst was rapidly added, in, the atmosphere was changed for 3 times and the resulting reaction mixture was reflux overnight. The reaction mixture was concentrated at reduced pressure to remove solvent, and the residue was purified on silica gel chromatography column (PE/EtOAc=4:1-2:1) to produce a product of ethyl 2-(ethoxy carbonyl-imino)-4-[N-parafluorobenzyl-N-1-(6-hydroxyl-2-alkenyl-3-hexylene) -imino]-aniline formate K32(15mg, 85%) in a form of black oil. ¹H NMR(300 MHz, CDCl₃): δ 7.18(dd, *J*=8.7 Hz,*J*=8.4 Hz,2H),7.07 -6.96(m,5H),6.40(dd,*J*=8.7 Hz,*J*=2.7 Hz,1H),6.36(s,1H),5.88-5.79(dt,*J*=16.2 Hz,*J*=5.15 Hz,1H),5.03(s,1H),4.52(s,1H),4.18(m,4H), 4.10(s,2H),1.28(m,6H).

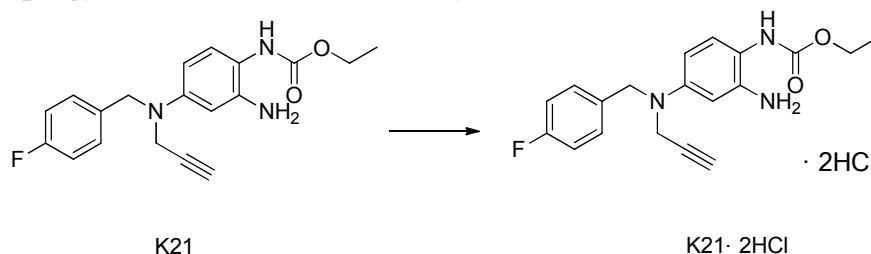
Compound ethyl 2-(ethoxycarbonyl-imino)-4-[N-parafluorobenzyl-N-1-(6-amino-2-alkenyl -3-hexylene)-imino]-aniline formate (K39) was obtained by using a method similar to that of Preparation example 7. ¹H NMR (CDCl₃, 300 MHz);δ 7.17(dd, *J*=8.4 Hz,*J*=5.4 Hz,2H),7.07(d,*J*=9.0 Hz,2H),6.98(t,*J*=8.7 Hz,2H),6.59(s,1H),6.35(dd,*J*=9.0 Hz,*J*=2.7

Hz,1H),6.29(d, $J=15.9$ Hz,1H),5.76(d, $j=15.9$ Hz,1H),5.11(s,1H),4.99(s,1H),4.49(s,2H),4.17(m,4H),4.08(s,2H),1.25(m,6H).



K39

Preparation example 8: preparation of ethyl 2-amino-4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate dihydrochloride (K21·2HCl)



511mg (1.5mmol) of compound ethyl 2-amino-4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate (K21) was dissolved in dichloromethane (5mL). A solution (1mL) of HCl (5N) in ethyl acetate was added, the resulting solution was stirred for 10 min and the solvent was removed under reduced pressure, thereby obtaining a compound of ethyl 2-amino-4-(N-parafluorobenzyl-N-propargyl-imino)-aniline formate dihydrochloride (K21·2HCl) (624mg).

The hydrochlorides of compound K3 (K3·HCl), compound K17 (K17·HCl) and compound K18 (K18·HCl) as well as dihydrochloride of compound K20 (K20·2HCl) were obtained by using a method similar to that of Preparation example 8.

II. Electrophysiological experiment example: electrophysiological experiment of the present compounds on Chinese hamster oocytes (CHO)

1. Cell culture and transfection

The formulation of culture fluid for Chinese hamster oocytes (CHO) (Culture Collection of Chinese Academy of Sciences): 50/50 DMEM/F-12(Cellgro, Mamassas, VA), 10% fetal

bovine serum (FBS) (Gibco, Australia) and 2mM L-glutamic acid (Invitrogen). Expression and mutation of KCNQ channels: 24h before transfection, oocytes were lysed with trypsin (Sigma, China) and plated on a culture dish with diameter of 60mm. Lipofectamine2000TM agent (Invitrogen) was used for Transfection according to the protocol thereof. 24h after the transfection, cells were lysed and replated on slides immersed with poly-L-lysine (Sigma). After cotransfection with GFP (green fluorescent protein), transfected cells could be determined under a fluorescence microscopy.

2. Electrophysiological recordings in CHO cells

Whole-cell voltage-clamp recording was performed by using Axopatch-200B amplifier (Molecular Devices, Sunnyvale, CA) at room temperature. Borosilicate glass capillary tubes (World Precision Instruments, Sarasota, FL) were drawn into electrodes. The electric resistance of the electrodes filled with an intracellular fluid is 3 to 5 M Ω . The formulation of the intracellular fluid was as follows: 145mM KCl, 1 mM MgCl₂, 5 mM EGTA, 10 mM HEPES and 5mM MgATP (the pH value of the intracellular fluid was adjusted to 7.3 with KOH). During the recording, an extracellular fluid was continuously perfused by using a BPS perfusion system (ALA Scientific Instruments, Westburg, NY). The formulation of the extracellular fluid was as follows: 140 mM NaCl, 5mM KCl, 2 mM CaCl₂, 1.5 mM MgCl₂, 10 mM HEPES and 10 mM glucose (the pH value of the extracellular fluid was adjusted to 7.4 with NaOH). Electric signal was filtered at 1kHz and further converted into digital signal by using pClamp 9.2 software (Molecular Devices, Sunnyvale, CA) in DigiData 1322A. Resistors were connected in series to compensate 60 to 80% of signal loss. At present, multivoltage scheme was generally adopted, wherein the clamp voltage was set at -100mV. Current was induced in cells by a series of 2000 ms stimulating voltage (from -90mV up to +50mV, with interval of 10mV).

3. Experimental results

In the following table, $V_{1/2}$ is the voltage at which 50% of cells were activated, $\Delta V_{1/2}$ is amount of a left-ward shift of $V_{1/2}$, negative sign (-) represents a left-ward shift of the current activation curve and positive sign (+) represents a right-ward shift of the curve. I/I_0 is the

activation multiple, wherein, I_0 is the maximum induced current produced under stimulation of -10mV testing voltage, I is the maximum induced current produced under stimulation of -10mV testing voltage after administration (10 μ M), $I/I_0 > 1$ represents activating activity, and $I/I_0 < 1$ represents inhibiting activity. N is the number of tested cells. NT represents non-tested.

Compound	$\Delta V_{1/2}$ (mV)	I/I_0	N
K1	-8.1 $\pm$ 3.1	2.84 $\pm$ 0.08	3
K2	-3.0 $\pm$ 2.0	1.50 $\pm$ 0.08	4
K3·HCl	-13.9 $\pm$ 2.5	2.41 $\pm$ 0.20	3
K4	-11.5 $\pm$ 2.0	2.09 $\pm$ 0.25	3
K5	-14.3 $\pm$ 3.3	1.23 $\pm$ 0.08	4
K6	-1.6 $\pm$ 3.0	1.24 $\pm$ 0.02	3
K7	-4.7 $\pm$ 2.0	1.35 $\pm$ 0.27	2
K8	-7.3 $\pm$ 2.5	1.99 $\pm$ 0.37	3
K9	-24.21 $\pm$ 2.4	3.27 $\pm$ 0.10	3
K10	-18.7 $\pm$ 2.6	2.75 $\pm$ 0.31	3
K11	-15.7 $\pm$ 1.0	2.24 $\pm$ 0.36	3
K12	-21.1 $\pm$ 1.9	2.33 $\pm$ 0.17	3
K13	-7.6 $\pm$ 1.9	2.31 $\pm$ 0.36	3
K14	-14.7 $\pm$ 2.1	1.43 $\pm$ 0.04	4
K15	-22.0 $\pm$ 1.5	2.09 $\pm$ 0.31	3
K16	-20.6 $\pm$ 4.5	1.47 $\pm$ 0.10	3
K17·HCl	-16.5 $\pm$ 2.4	2.29 $\pm$ 0.38	3
K18·HCl	-12.5 $\pm$ 3.2	2.72 $\pm$ 0.22	3
K19	-14.9 $\pm$ 2.7	2.52 $\pm$ 0.23	3
K20	-49.7 $\pm$ 3.4	1.75 $\pm$ 0.12	3
K21	-47.9 $\pm$ 3.5	1.53 $\pm$ 0.15	3
K22	-27.31 $\pm$ 3.9	2.10 $\pm$ 0.35	3
K23	-15.34 $\pm$ 3.8	1.44 $\pm$ 0.14	3
K24	-1.87 $\pm$ 2.5	1.2 $\pm$ 0.1	3
K25	-5.8 $\pm$ 1.7	1.8 $\pm$ 0.1	3
K26	NT	1.25 $\pm$ 0.07	3
K27	NT	1.52 $\pm$ 0.17	3
K28	NT	1.43 $\pm$ 0.05	3
K29	NT	1.24 $\pm$ 0.10	3
K30	NT	1.57 $\pm$ 0.25	3
K31	NT	1.36 $\pm$ 0.13	3
K32	-6.0 $\pm$ 1.5	1.76 $\pm$ 0.09	3
K33	-36.8 $\pm$ 3.3	NT	3
K34	NT	1.44 $\pm$ 0.16	3
K35	NT	NT	
K36	NT	NT	

K37	NT	NT	
K38	NT	0.16±0.02	3
K39	NT	1.03±0.11	3

III. Examples for evaluation on pharmacodynamics effects of compounds *in vivo*

***In vivo* pharmacodynamics effects example 1: therapeutic effects of compounds K9 and K1 on convulsion induced by pentylenetetrazole (PTZ)**

1. Object of experiment

Comparison of the anti-convulsive effects of compounds K9 and K1 with that of retigabine (prepared in the lab according to procedure disclosed in US5384330).

2. Treatment of samples

Retigabine: in the form of light grey powder, it can be easily dissolved in water to produce a colorless and transparent solution.

Compound K9: in the form of yellow granule, it can not be easily dissolved in water. The compound was added into a solution of 0.2% CMC, and ultrasonic treatment was performed for 30 min, thereby obtaining a homogeneous suspension.

Compound K1: in the form of light yellow granule, it can not be easily dissolved in water. The compound was added into a solution of 0.2% CMC, and ultrasonic treatment was performed for 30 min, thereby obtaining a homogeneous suspension.

3. Laboratory animals

C57BL/6J mice (body weight: 16g to 18g) were purchased from Laboratory Animal Center, Chinese Academy of Sciences.

4. Experimental procedure

The compounds to be tested were administrated to animals by gastric lavage (30mg/kg); one hour later, pentylenetetrazole was administrated by subcutaneous injection (PTZ 80mg/kg); and response of animals were observed within a period of 60min.

5. Experimental results:

Compound	Dosage, Administration route, period of observation	Number	Incidence rate of convulsion	Latency period of clonus (s)	Latency period of whole-body tonic seizures(s)	Mortality rate
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K9		10	4/10	351±84.3	1382±339	4/10
K1	1h after administration by gastric lavage	10	4/10	250±114	1785±408	3/10
Retigabine	(30mg/kg)	10	5/10	No typical clonic seizures were observed	1472±961	4/10
Control of PTZ		10	9/10	134±51.5	921±248	8/10

Animals treated with retigabine were quite and less active. After 20 min, animals returned to normal. After 1 h, no significant abnormal response was observed. For the animals treated with compounds K9 and K1 respectively, the convulsion incidence rates decreased significantly, which were manifested by for prolonged latency period of clonic seizures in mice with acute convulsion induced by PTZ, prolonged latency period of whole-body tonic seizures, and reduced mortality. The above results indicated that compounds of K9, K11 and retigabine had a comparable anti-convulsive effect.

***In vivo* pharmacodynamics effect example 2: therapeutic effects of compounds K21 administrated by intraperitoneal injection on animal models induced by PTZ (pentylenetetrazole) and MES (maximum electroshock)**

1. Object of experiments

The anti-convulsive effect of compound K21 was compared with that of retigabine as a positive control.

2. Treatment of samples

Retigabine: in the form of light grey powder, it can be easily dissolved in water to produce a colorless and transparent solution.

Compound K21: in the form of cyan lamellar granule, it can not be easily dissolved in water. The compound was dissolved in 50 ul of DMSO, and 0.5% of HEC was added to produce a homogeneous suspension.

3. Laboratory animals

KM mice (male, body weight: 22±2 g) were purchased from Laboratory Animal Center, Chinese Academy of Sciences.

4. Experimental procedure

(1) Test with PTZ

Compounds to be tested were administrated by intraperitoneal injection (10 mg/kg, 5mg/kg, 2.5mg/kg, 0.1ml/10g). 30min later, pentylenetetrazole (PTZ,100mg/kg) was administrated by subcutaneous injection. Response of animals were observed immediately and recorded within a period of 1h.

(2) Test with MES

YLS-9A model physiological pharmaceutical electronic stimulator was used with configuration 8, and the stimulating voltage was set as 160V, wave number as 90, i.e., the period of stimulation was 5.4 sec. One time of electrical stimulation was applied to animals through ear clip electrodes, and a convulsion characterized by a tonic hind limb extension was observed. One day before the test, animals were screened in the above procedure to record the values of MES. On test, the compounds to be tested were administrated to animals by intraperitoneal injection (10 mg/kg, 0.1ml/10g), and 30min later, MES was applied according to parameters set the last day, and response of animals were observed and recorded immediately.

5. Experimental results:

(1) results of PTZ test

Compound	Dosage	Administration route, period of observation	Incidence rate of convulsion	Latency period of clonus (s)	Incidence of tonic fore limb extension	Incidence of tonic hind limb extension	Latency period of tonic hind limb(sec)	Mortality rate
K21	10mg/kg	Intraperitoneal injection, observation for 1h	12/12	226±51 ^{**}		0/12 ^{***}		0/12 ^{***}
K21	5mg/kg		10/10	238±54 ^{***}	3/10	0/10 ^{***}		1/10 ^{**}
K21	2.5mg/kg		9/10	184±42	2/10	0/10 ^{***}		1/10 ^{**}
Retigabine	10mg/kg		10/10	239±113 ^{**}		4/12 [*]	1100±319	2/12 ^{**}
Retigabine	5mg/kg		10/10	184±40	4/10	2/10 [*]	883±151	5/10
Retigabine	2.5mg/kg		10/10	179±60	5/10	1/10 ^{**}	827	7/10
Physiological saline	0.2ml		11/11	137±24	3/11	8/11	1033±364	9/11

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

(2) results of MES test

Compound	Dosage, administration route	Incidence of convulsion	Mortality rate
K21		2/10 ^{***}	0
Retigabine	10mg/kg, administration by intraperitoneal injection	4/11 ^{**}	0
Physiological saline		10/10	10%

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

6. Experimental conclusion

Compound K21 completely avoided the grand mal seizure in MES test, significantly reduced the incidence rate of convulsion and mortality rate in PTZ test, and prolonged the latency period of clonic seizures in mice with acute convulsion with a dose-response relationship to a certain degree. At the same dosage, the anti-convulsive effect of compound K21 was slightly stronger than that of retigabine as a positive control, and both of them exhibited some sedative effect.

***In vivo* pharmacodynamics effect example 3: therapeutic effects of compounds K21 orally administrated on animal models induced by PTZ and MES**

1. Object of experiments

The anti-convulsive effect of compound K21 orally administrated was compared with that of retigabine as an positive control.

2. Treatment of samples

Retigabine: in the form of light grey powder, it can be easily dissolved in water to produce a colorless and transparent solution.

Compound K21: in the form of cyan lamellar granule, it can not be easily dissolved in water. The compound was dissolved in 50ul of DMSO, the resulting solution added into 0.2% of CMC solution, and ultrasonic treatment was performed for 20 min to produce a homogeneous suspension.

3. Laboratory animals

KM mice (male, body weight: 22 ± 2 g for PTZ test; 18 g for MES test) were purchased from Laboratory Animal Center, Chinese Academy of Sciences.

4. Experimental procedure

(1) Test with PTZ

Animals were fasted for 12h, and then compounds CF341 and retigabine (30mg/kg, 0.2ml/10g) were administrated by gastric lavage. 1h later, pentylenetetrazole (PTZ, 100mg/kg) was administrated by subcutaneous injection. Response of animals were observed immediately and recorded within a period of 1h.

(2) Test with MES

YLS-9A model physiological pharmaceutical electronic stimulator was used with configuration 8, and the stimulating voltage was set as 160V, wave number as 90, i.e., the period of stimulation was 5.4 sec. One time of electrical stimulation was applied to animals through ear clip electrodes, and a convulsion characterized by a tonic hind limb extension was observed. One day before the test, animals were screened in the above procedure to record the values of MES.

Animals were fasted for 12h, the compounds to be tested (30mg/kg, 0.2ml/10g) were then administrated by gastric lavage. 1 hour later, MES was applied according to parameters set the last day, and response of animals were observed and recorded immediately.

5. Experimental results

(1) results of PTZ test

Compound	Dosage	Administration route, period of observation	Incidence rate of clonus	Latency period of clonus(sec)	Incidence of tonic fore limb extension	Incidence of tonic hind limb extension	Latency period of fore limb extension (sec)	Mortality rate
K21	30mg/kg	Administrated by gastric lavage, observation for 1h	12/12	$148\pm 37^*$	5/12	0/12**	1561 ± 690	3/12
Retigabine	30mg/kg		12/12	177 ± 70	3/12**	0/12**	1063 ± 284	0/12**
Physiological saline	0.4ml		12/12	104 ± 18	9/12	7/12	964 ± 174	7/12

When compared with negative control, * represents $p<0.05$ and ** represents $p<0.01$.

(2) results of MES test

Compound	Dosage, administration route	Incidence rate of convulsion	Protection rate of compounds
K21	30mg/kg,	2/13 ^{***}	84.6% ^{***}
retigabine	administrated by	5/13 ^{***}	61.5% ^{***}
NS	gastric lavage	13/13	0%

When compared with negative control, *** represents $p<0.001$.

7. Experimental conclusion

Both compound K21 and retigabine exhibited similar effect in significantly inhibiting the incidence of the grand mal seizure in MES test. In PTZ test, retigabine could obviously reduce the incidence rate of convulsion and mortality rate of animals. Compound K21 could significantly inhibit tonic seizures of animals, although the mortality rate of K21 group is less than that of control, there was no significant differences between them.

IV. Pharmacokinetics Examples of compounds

Pharmacokinetics example 1: research on distribution of K9 and retigabine in mice's brain tissue

1. Dosage regimen

Before test, 108 of healthy KM mice (male, body weight: 18g to 20g) were fasted for 12 h with access to water *ad libitum*. Two hours after administration, animal were fed uniformly.

Specific arrangement was as following table.

Group	number of animals	Compound	Administrati on manner	Administration dosage (mg/kg)	Administration volume (ml/kg)	Sampling time point(h)
1	27	K9	Gastric lavage	20	10	0.25, 0.5, 1, 2, 3, 4, 6, 8 and 10 h
2	27	K9	Intravenous injection	20	10	5 min, 0.25, 0.5, 1, 2, 4, 6, 8 and 10 h
3	27	retigabine	Gastric lavage	20	10	0.25, 0.5, 1, 2, 3, 4, 6, 8 and 10 h
4	27	retigabine	Intravenous injection	20	10	5 min, 0.25, 0.5, 1, 2, 4, 6, 8 and 10 h

Before test, animals were fasted for 12h with access to water *ad libitum*. Two hours after administration, animal were fed uniformly.

After administration by gastric lavage or intravenous injection, mice were sacrificed by cutting abdominal aorta at time points set as the above with 3 mice for each time point. 0.5 mL of whole blood was collected for each animal, placed into a heparinized test tube and subjected to centrifugation at 3000 rpm to separate plasma, which was then cryopreserved in a -20°C refrigerator. After the animals were sacrificed, their whole brain was collected, washed with ice-cooled physiological saline to remove residual blood. Then the residual liquid was absorbed to dryness. The treated brain was labeled and cryopreserved in a -20°C refrigerator.

2. Experimental results

The concentrations of K9 and retigabine in plasma and brain tissue were determined by LC-MS.

2.1. K9

After mice were administrated with compound K9 (20 mg/kg) by gastric lavage, the maximum concentrations (C_{max}) in plasma and brain tissue were 2197 ng/ml and 4421 ng/g respectively. The corresponding areas under the concentration-time curve (AUC_{0-t}) were 1865 ng · h/ml and 3565 ng · h/g respectively. The exposure amount in brain tissue was 1.9 times of that in plasma. The elimination half-life of K9 was about 1.2 h.

After mice were administrated with compound K9 (20 mg/kg) by intravenous injection, the corresponding areas under the concentration-time curve (AUC_{0-t}) of plasma and brain tissue were 7185 ng · h/ml and 10694 ng · h/g respectively. Plasma clearance rate was 2.78 L/h/kg. The exposure amount in brain of compound K9 was 1.5 times of that in plasma.

Upon dose correction, the absolute bioavailability following administration with compound K9 by gastric lavage (20 mg/kg) in mice was 26% according to AUC_{0-t} of plasma.

2.2 Retigabine

After mice were administrated with retigabine (20 mg/kg) by gastric lavage, the maximum concentrations (C_{max}) in plasma and brain tissue were 2788 ng/ml and 849 ng/g respectively.

The corresponding areas under the concentration-time curve (AUC_{0-t}) were 21088 ng · h/ml and 3460 ng · h/g respectively. The exposure amount in brain tissue was 16% of that in plasma. The elimination half-life of retigabine was about 7h.

After mice were administrated with retigabine (20 mg/kg) by intravenous injection, the corresponding areas under the concentration-time curve (AUC_{0-t}) of plasma and brain tissue were 59987 ng · h/ml and 8661 ng · h/g respectively. Plasma clearance rate was 0.322 L/h/kg. The exposure amount in brain of retigabine was 14% of that in plasma.

Upon dose correction, the absolute bioavailability following administration with retigabine by gastric lavage (20 mg/kg) in mice was 35.2% according to AUC_{0-t} of plasma.

Pharmacokinetics example 2: research on distribution of K20·2HCl and K21·2HCl in mice's brain tissue

1. Dosage regimen

Before test, 54 healthy KM mice (male, body weight: 18g to 20g) were fasted for 12h with access to water *ad libitum*. Two hours after administration, animal were fed uniformly.

Specific arrangement was as following table.

Group	number of animals	Compound	Administration manner	Administration dosage (mg/kg)	Administration volume (ml/kg)	Sampling time point(h)
1	27	K20·2HCl	gastric lavage	20	10	0.25, 0.5, 1, 2, 3, 5, 7, 9 and 24 h
2	27	K21·2HCl	gastric lavage	5*	10	0.25, 0.5, 1, 2, 3, 5, 7, 9 and 24 h

As for “*”, mice administrated with compounds K21·2HCl by gastric lavage (20 mg/kg) were obviously restless, and one of them died, therefore, the administration dosage was decreased to 5 mg/kg.

Before test, animals were fasted for 12h with access to water *ad libitum*. Two hours after administration, animal were fed uniformly.

After administration by gastric lavage, mice were sacrificed by cutting abdominal aorta at time points set as the above with 3 mice for each time point. 0.5 mL of whole blood was collected for each animal, placed into a heparinized test tube and subjected to centrifugation at 11000 rpm for 10 min to separate plasma, which was then cryopreserved in a -20°C refrigerator.

After the animals were sacrificed, their whole brain was dissected and collected, washed with ice-cooled physiological saline to remove residual blood. Then the residual liquid was absorbed to dryness. The treated brain was labeled and cryopreserved in a -20°C refrigerator.

2. Experimental results

2.1. K20·2HCl

After mice were administrated with compound K20·2HCl (20 mg/kg) by gastric lavage, time to reach the maximum concentrations (T_{max}) for prototype drug K20·2HCl and its metabolite retigabine in plasma or brain tissue was 0.25h. The exposure amounts of metabolite retigabine in plasma and brain tissue were 2.9% and 1.8% of those of prototype drug K20·2HCl, respectively. The concentration of prototype drug K20·2HCl in brain tissue was 2.3 times of that in plasma, and for metabolite retigabine, 1.4 times.

2.2. K21·2HCl

After mice were administrated with compound K21·2HCl (5 mg/kg) by gastric lavage, time to reach the maximum concentrations (T_{max}) for prototype drugs K21·2HCl and its metabolite retigabine in plasma or brain tissue was 0.25h. The exposure amounts of metabolite retigabine in plasma and brain tissue were 5.4% and 4.6% of those of prototype drug K21·2HCl, respectively. The concentration of prototype drug K21·2HCl in brain tissue was 2.4 times of that in plasma, and for metabolite retigabine, 2.0 times.

As can be seen from the above experimental results, the compounds provided by the present invention not only maintain retigabine's activating activities on potassium ion channels, but also exhibit a significant anti-epilepsy effect *in vivo*.

1. In male KM mice model, compound K21·2HC administrated by intraperitoneal injection exhibits superior anti-convulsive effect to that of compound retigabine as a positive control.

2. Similarly, in male KM mice model, compound K21·2HC and retigabine administrated by gastric lavage (10mg/kg) show comparable effect in MES test, in other words, both of them can significantly prevent grand mal seizures. In PTZ test, retigabine can significantly reduce the incidence rate of convulsion and mortality rate of animals, while compound K21·2HCl can

obviously inhibit the tonic seizures of animals.

Pharmacokinetics research showed that the compounds of the present invention have better concentration distribution in brain tissue, compared with retigabine.

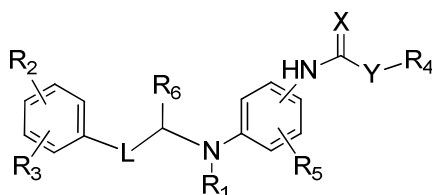
1. After mice are administrated with compound K9 by gastric lavage (20 mg/kg), the exposure amount thereof in brain tissue is 1.9 times of that in plasma. After mice are administrated with compound K9 by intravenous injection (20 mg/kg), the exposure amount thereof in brain is 1.5 times of that in plasma. Under the same condition, as for retigabine administrated by gastric lavage and intravenous injection, the exposure amounts in brain are 16% and 14% of those in plasma.

2. After mice are administrated with compound K20 by gastric lavage (20 mg/kg), the concentration thereof in brain tissue is 2.3 times of that in plasma.

3. After mice are administrated with compound K21·2HCl by gastric lavage (5 mg/kg), the concentration thereof in brain tissue is 2.4 times of that in plasma.

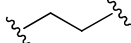
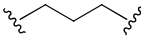
CLAIMS

1. A compound of the following general formula I or a pharmaceutically acceptable salt thereof:



I

wherein,

L is not present, or L is a linking radical selected from the group consisting of , , and , optionally substituted by C₁-C₄ alkyl;

R₁ is a radical selected from the group consisting of H, C₁-C₆ alkyl, C₃-C₆ cycloalkyl, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, C₁-C₆ alkylamino carbonyl, C₂-C₈ alkenyl, C₅-C₇ cycloalkenyl, C₂-C₈ alkynyl and C₆-C₁₀ aryl; wherein, the C₁-C₆ alkyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkoxy, C₁-C₄ alkylcarbonyl, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl; the C₂-C₈ alkenyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, C₁-C₄ alkoxy, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl; and the C₂-C₈ alkynyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, C₁-C₄ alkoxy, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl;

R₂ is a radical selected from the group consisting of halogen atom and C₁-C₄ alkoxy;

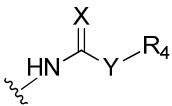
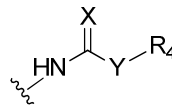
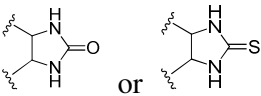
R₃ is a radical selected from the group consisting of H, halogen atom, trifluoromethyl, cyano, nitro, C₁-C₄ alkylcarbonyl and C₁-C₄ alkoxy carbonyl;

X is O, S or NH;

Y is not present, or Y is O or NR₇, wherein R₇ is a radical selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl and C₃-C₈ cycloalkyl;

R₄ is a radical selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl and C₆-C₁₀ aryl;

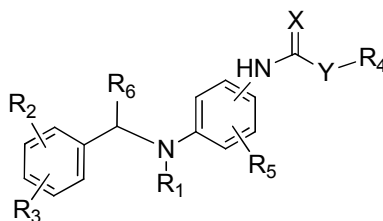
R₅ is a radical selected from the group consisting of H, halogen atom, amino, C₁-C₆

alkylamino and , or R₅, together with adjacent , forms a fused-ring structure of  or  ;

R₆ is H, C₁-C₆ alkyl or C₃-C₆ cycloalkyl; and

in the above said definitions of substituents, the halogen atom or the halogen atom in halogenated group is F, Cl or Br.

2. The compound or the pharmaceutically acceptable salt thereof according to claim 1, wherein the compound or the pharmaceutically acceptable salt has a structure of general formula II:



II

wherein,

R₁ is a radical selected from the group consisting of H, C₁-C₆ alkyl, C₃-C₆ cycloalkyl, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, C₁-C₆ alkylaminocarbonyl, C₂-C₈ alkenyl, C₅-C₇ cycloalkenyl, C₂-C₈ alkynyl and C₆-C₁₀ aryl; wherein, the C₁-C₆ alkyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl; the C₂-C₈ alkenyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, C₁-C₄ alkoxy, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl; the C₂-C₈ alkynyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, C₁-C₄ alkoxy, halogen atom, C₆-C₁₀ aryl or halogenated C₆-C₁₀ aryl;

R₂ is a radical selected from the group consisting of halogen atom and C₁-C₄ alkoxy;

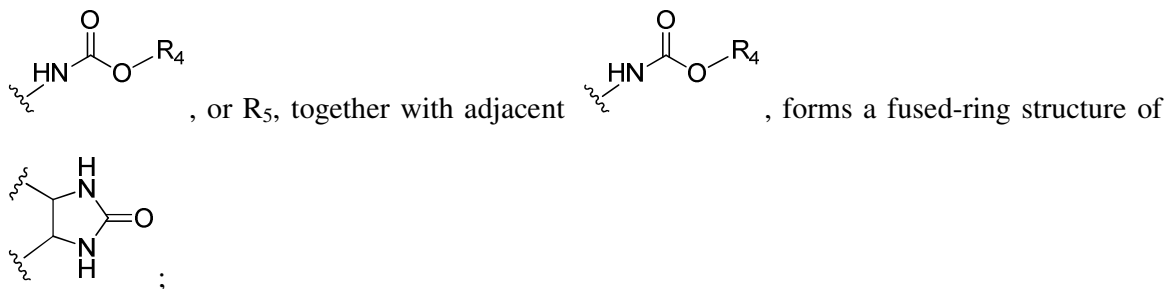
R₃ is a radical selected from the group consisting of H, halogen atom, trifluoromethyl and nitro;

X is O;

Y is not present or Y is O;

R₄ is a radical selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl and C₆-C₁₀ aryl;

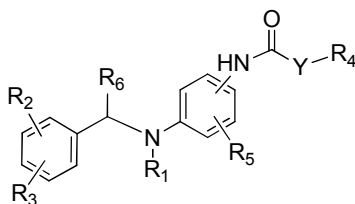
R₅ is a radical selected from the group consisting of H, halogen atom, amino and



R₆ is H, C₁-C₆ alkyl or C₃-C₆ cycloalkyl;

in the above said definitions of substituents, the halogen atom or the halogen atom in halogenated group is F, Cl or Br.

3. The compound or the pharmaceutically acceptable salt thereof according to claim 1, wherein the compound has a structure of general formula III:



III

wherein,

R₁ is a radical selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₈ alkenyl, C₅-C₇ cycloalkenyl and C₂-C₈ alkynyl; wherein, the C₁-C₆ alkyl is unsubstituted or optionally substituted by hydroxyl, amino, C₁-C₄ alkylcarbonyl, halogen atom, phenyl or halogenated phenyl; the C₂-C₈ alkenyl is unsubstituted or optionally substituted by hydroxyl, amino, halogen atom, phenyl or halogenated phenyl; the C₂-C₈ alkynyl is unsubstituted or optionally substituted by hydroxyl, amino, halogen atom, phenyl or halogenated phenyl;

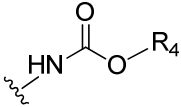
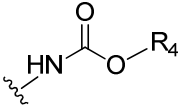
R₂ is a radical selected from the group consisting of F, Cl and methoxyl;

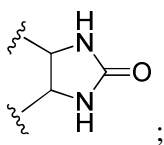
R₃ is a radical selected from the group consisting of H, halogen atom and trifluoromethyl;

Y is not present or Y is O;

R₄ is a radical selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl and C₆-C₁₀ aryl;

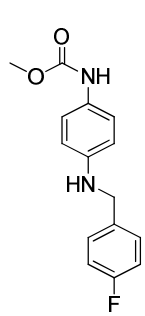
R₅ is a radical selected from the group consisting of H, halogen atom, amino and

, or R₅, together with adjacent , forms a fused-ring structure of

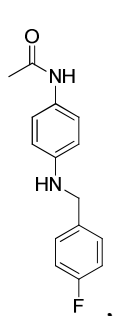


R₆ is H or C₁-C₆ alkyl.

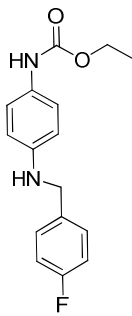
4. The compound or the pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is one selected from compounds K1 to K39:



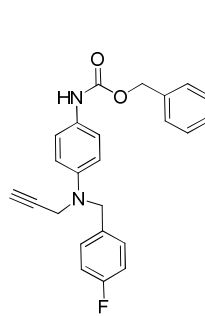
K1



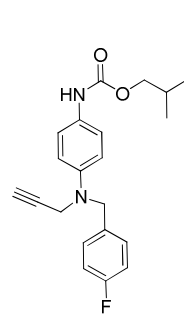
K2



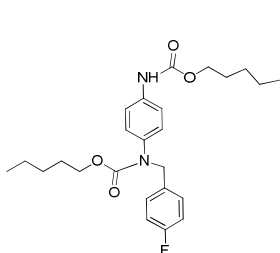
K3



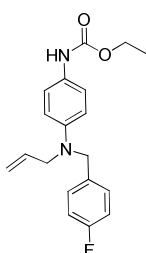
K4



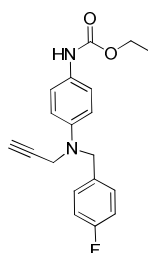
K5



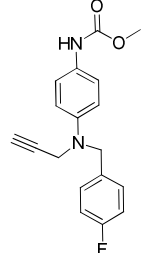
K6



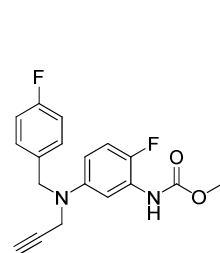
K7



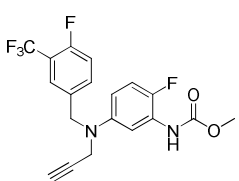
K8



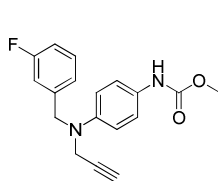
K9



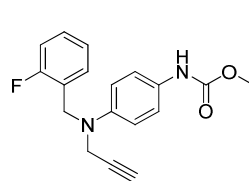
K10



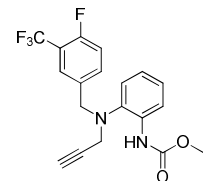
K11



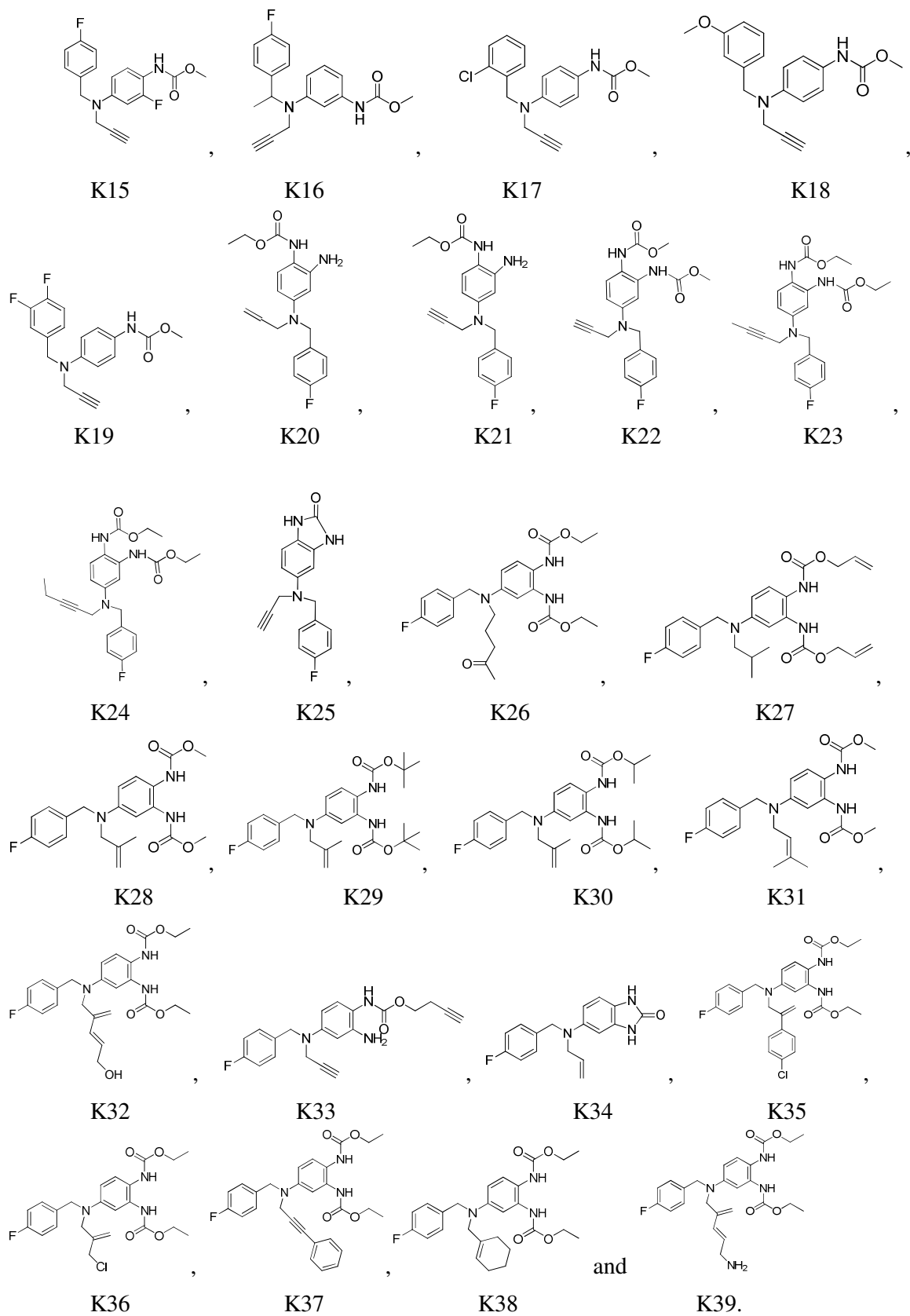
K12



K13



K14



5. The compound or the pharmaceutically acceptable salt thereof according to claim 1,

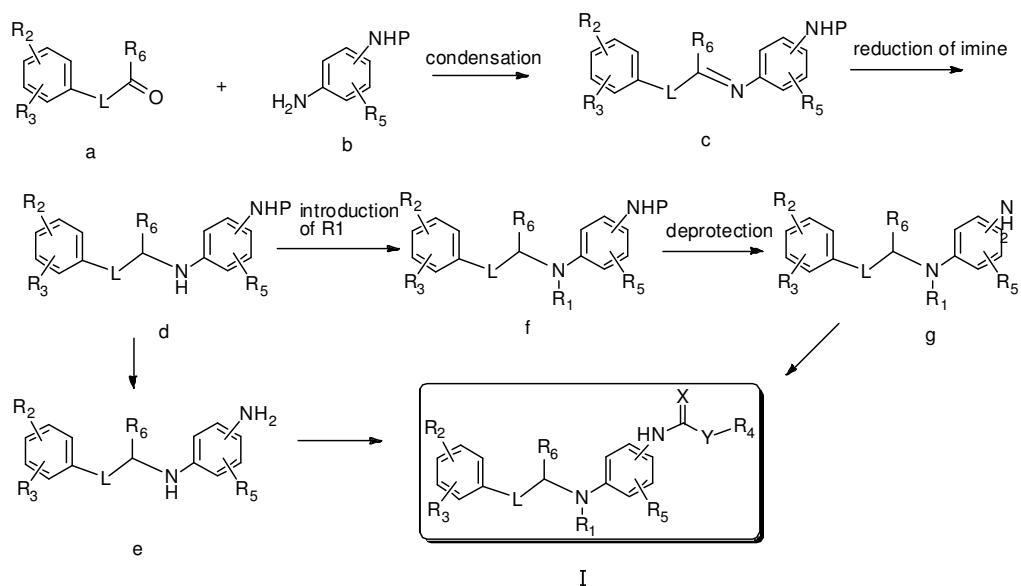
wherein the pharmaceutically acceptable salt of the compound is a salt formed by the compound with an acid, and the acid is selected from the group consisting of maleic acid, succinic acid, citric acid, tartaric acid, fumaric acid, formic acid, acetic acid, propanoic acid, propandioic acid, oxalic acid, benzoic acid, phthalic acid, methanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, naphthalenesulfonic acid, 1,5-naphthalenedisulfonic acid, camphoric acid, camphor sulfonic acid, salicylic acid, acetyl salicylic acid, aspartic acid, glutamic acid, lactic acid, gluconic acid, ascorbic acid, gallic acid, amygdalic acid, malic acid, sorbic acid, trifluoroacetic acid, taurine, homotaurine, isethionic acid, cinnamic acid, hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, nitric acid, phosphoric acid and perchloric acid.

6. A process for the preparation of the compound or a pharmaceutically acceptable salt thereof according to claim 1, wherein the process is anyone of schemes 1 to 4:

scheme 1:

Under catalysis of weak acid, p-toluenesulfonic acid, carbonyl compound a reacts with phenylamine compound b for conducting a condensation reaction, thereby producing imine compound c; imine compound c is reduced to secondary amine compound d under a reducing agent sodium borohydride; substituent R1 is introduced into secondary amine compound d to produce intermediate compound f by a substitution reaction with halogenated hydrocarbon, or an addition reaction or an acylation reaction with α,β -unsaturated carbonyl compound; the protection group P of amino group on intermediate compound d or f is removed to produce intermediate amine compound f or g, and a compound of formula I is obtained by reacting

amine f or g with an acylating agent of $\text{Cl}-\overset{\text{X}}{\underset{\text{Y}-\text{R}_4}{\text{C}}}=\text{O}$;

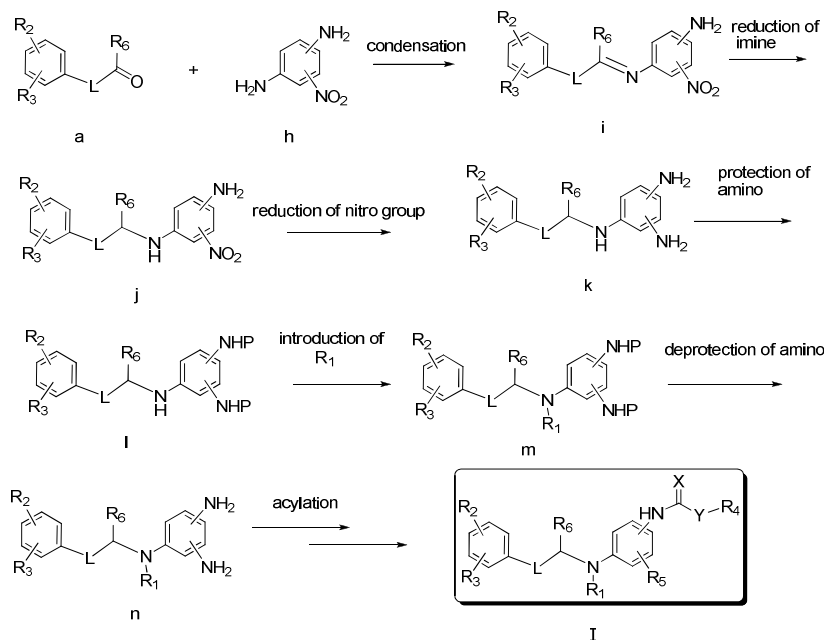


Scheme 1

scheme 2

Under catalysis of a weak acid, p-toluenesulfonic acid, carbonyl compound **a** reacts with nitro-substituted benzene diamines compound **h** for conducting a condensation reaction, thereby producing imine compound **i**; imine compound **i** is reduced to amine intermediate **j** under a reducing agent sodium borohydride; diamino intermediate **k** is obtained by reducing the nitro on amine intermediate **j**; then intermediate **l** is obtained by introducing two protection groups **P** of amino group into diamino intermediate **k**; intermediate **m** is obtained by introducing substituent R_1 using a method similar to that of acheme 1; intermediate **n** is obtained by removing two protection groups **P** to expose two amino groups; finally, a compound of formula **I** is obtained by reacting intermediate **n** with an acylating agent of

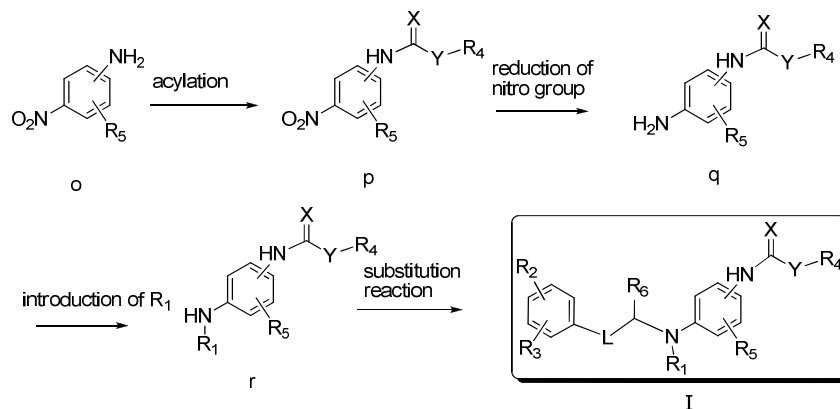
$\text{Cl}-\overset{\text{X}}{\underset{\text{Y}}{\text{C}}}-\text{R}_4$; wherein the reaction for reducing nitro is conducted by using catalyzing hydrogenation with Pd-C or SnCl_2 ;



Scheme 3:

intermediate compound **p** is obtained by reacting an acylating agent of $\text{Cl}-\text{C}(=\text{X})-\text{Y}-\text{R}_4$ with nitro-substituted benzene amines compound **o**; intermediate **r** is obtained by introducing substituent R_1 into amino compound **q** which is obtained by reducing nitro on intermediate compound **p**; finally a compound of formula I is obtained by reacting intermediate compound **r**

with halogenated hydrocarbon of $\text{R}_2-\text{C}_6\text{H}_3(\text{R}_3)-\text{CH}(\text{R}_6)-\text{Cl}$ or $\text{C}_6\text{H}_5-\text{CH}(\text{R}_6)-\text{Br}$ through a substitution reaction:



Scheme 4:

when substituent R_1 in the compound of general formula I is an alkenyl or alkynyl, other

compounds of formula I according to the present invention can be obtained by olefin metathesis;

Scheme 5: preparation for salts of the compounds

The compound of general formula I according to the present invention can be converted into pharmaceutically acceptable salts by adding a solution of acid into solution of the compound and completely removing solvent under reduced pressure.

7. A pharmaceutical composition, wherein the pharmaceutical composition comprises the compound or the pharmaceutically acceptable salt thereof according to claim 1 as an active ingredient and pharmaceutical acceptable auxiliary substances.

8. Use of the compound or the pharmaceutically acceptable salt thereof according to claim 1 in preparing medicaments for treating nervous system diseases.

9. The use according to claim 8, wherein the nervous system diseases include epilepsy, convulsion, neuropathic pain, acute ischemic stroke or neurodegenerative diseases.