(51) International Patent Classification:
C07D 295/00 (2006.01) \textit{A61P} 3/00 (2006.01)
C07D 403/12 (2006.01) \textit{A61P} 5/00 (2006.01)
A61K 31/495 (2006.01) \textit{A61P} 11/00 (2006.01)
A61P 9/00 (2006.01) \textit{A61P} 25/00 (2006.01)
A61P 35/00 (2006.01) \textit{A61P} 31/00 (2006.01)
A61P 1/00 (2006.01) \textit{A61P} 27/00 (2006.01)

(52) International Application Number:
PCT/ CN2011/003784

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Published:
— with international search report (Art. 21(3))

(54) Title: PYRAZINE DERIVATIVES, PROCESS FOR MANUFACTURE AND USE THEREOF

\begin{equation}
\text{I}
\end{equation}

(57) Abstract: Pyrazine derivatives of formula (I) and pharmaceutically acceptable salts thereof are disclosed, wherein the designation of $R_1$, $R_2$, $R_3$, and $R_4$ are provided herein. Syntheses for preparation of such compounds are disclosed. Methods of use of these compounds and pharmaceutical compositions containing them for treatment and/or prevention of diseases and for manufacture of medicaments are disclosed. These compounds and pharmaceutical compositions have antioxidative and thrombolytic effects, and thus can be used for the treatment and/or prevention of cerebral stroke caused by ischemia, and used for manufacture of medicaments for the treatment and/or prevention of nervous system diseases caused by excessive amount of radicals and/or thrombosis, infectious diseases, metabolic system diseases, cardiovascular and cerebrovascular diseases, and age-related degenerative diseases.
PYRAZINE DERIVATIVES, PROCESS FOR MANUFACTURE AND USE THEREOF

FIELD OF THE INVENTION

The present invention relates to pyrazine derivatives and processes for preparation thereof and uses thereof in the manufacture of medicaments for treating and preventing diseases of the nervous system caused by excessive production of radicals and/or thrombosis, cardiovascular and cerebrovascular diseases, age-related degenerative diseases, and metabolic diseases.

BACKGROUND OF THE INVENTION

Oxygen is an essential element for the lives of human beings and animals. In the regular processes of metabolism, the human body produces different kinds of Reactive Oxygen Species (ROS), and also possesses many ways to inactivate ROS. Under normal conditions, the rate of ROS production will not exceed that of consumption via metabolism by tissues. However, in certain circumstances (for example, due to radiation, environmental factors, and overload of ferric ions), ROS may increase to a level exceeding that under the regulation of normal metabolism, or when something went wrong (e.g., caused by genetic defects) in the protective biological mechanism, the excessive amount of ROS may cause damage to cells and tissues, and thus induces some diseases and even causes death. Proteins, lipids and DNAs are substrates to which ROS may attack. In a human body, there are about $10^{12}$ oxygen molecules entering into the cells every day, 1/100 of which destroy proteins, and 1/200 of which destroy DNAs. Especially when the human body's capacity of natural resistance is low, ROS may become very harmful due to such destructions on DNAs, proteins and lipids.

In a regular condition, the anti-radical defence system of the human body is effective against the harm to the body caused by the radicals. In a pathological condition, however, some of the damaging radicals may escape from elimination, and these escaped radicals and their products may act directly upon cellular DNA, proteins and lipids, causing some damages on DNA and inducing overoxidation of cell membrane lipids. These results caused by ROS are called oxidative stress, and the oxidative stress can influence regular gene expression, cell differentiation and necrocytosis. Today, oxidative stress is considered a factor causing many
diseases.

Formation of cerebral atherosclerosis and thromboembolus may induce cerebral stroke. In the developed countries, the cerebrovascular diseases have been shown as the third leading cause for human death following heart diseases and tumors, and 5% of the elderly people over 65 years old suffer from cerebral stroke. In the United States, for example, more than 500,000 people are reported suffering from severe stroke, where 70-85% of the cerebral stroke is related to ischemic stroke, which has a mortality rate of 15-33%. The methods currently used for treatment of acute ischemic stroke include cytoprotection and thrombolysis, while cytoprotection is used to prevent cell death during ischemic reperfusion, and thrombolysis is used mainly to keep blood vessels clear using thrombolytic drugs during an early period of the disease. Despite much of the efforts, the cerebral stroke is still one of the most devastating diseases for medical treatments. One of the reasons that the current treatments for cerebral stroke are far from satisfactory is that so far no drugs have clearly demonstrated both thrombolytic and cytoprotective effects.

Parkinson's disease (PD) is a disease with clinical manifestations of resting tremor, myotonia, hypokinesia, and abnormal gait posture. It is currently known that the primary pathologic change of PD is of substantia nigra-striatum, which decreases the production of dopamine, and causes the above-mentioned clinical manifestations. The causes of substantia nigra degeneration are still unclear. Currently, most of the related studies suggested that oxidative stress plays an important role in the pathologic progress of PD.

Researchers found that many chemical substances show some radical-eliminating effects. Nitrones are a type of the compounds having a strong antioxidant activity and in vivo biological activity. The final products formed from the reactions of nitrones and radicals include hydroxylamine derivatives, aldehydes, amines, and nitrooxide radicals.

Phenyl-tert-Butyl Nitrones (PBN) can react with radicals to form nitrooxide radicals, while nitrooxide radicals are able to directly react with and thus eliminate other radicals, and also are able to oxidize reductive metals so as to inhibit the Fenton reduction and the metal-catalyzed Haber-Weiss reaction. When aging accelerated mice were intraperitoneally injected with PBN daily, an increase of 33% in the lifespan of the mice was indicated (Edamatsu et al, Biochem. Biophys. Res. Commun. 211:847, 1995). When 24-month-old rats were intraperitoneally
injected with 32 mg/kg of PBN daily for 9.5 months, it is indicated that lipid peroxidation reactions are diminished in two areas (cerebral cortex and globus pallidus) which are important for the cognitive function of the rats’ brains, and at the same time, the cognitive ability for older rats is enhanced. More importantly, when the experiment was run for 32 months, 7 of the 11 rats injected with PBN were still alive (Sack et al, Neurosci. Lett. 205:181, 1996). Unfortunately, however, PBN is still merely applied in research and has not yet been developed into any drugs.

Tetramethylpyrazine (TMP, Chuxiongqin) is an active ingredient extracted from ligusticum wallichii (Chuanxiong), a traditional Chinese medicine. TMP has effects of radical elimination and thrombolysis/anticoagulation. TMP have been used clinically to treat cardiovascular and cerebrovascular diseases. It is founded that, however, the antioxidative effect of TMP is rather weak and its bioavailability is low. Clinically, multiple doses are needed for TMP to reach to an effective concentration.

Currently, there is no any effective curing method for the treatment of stroke, and the limited kinds of commercially available drugs are far from satisfactory due to inferior curative effects or toxic side effects. To be effective for any drugs to treat ischemic stroke, the following two functions are critical: thrombolysis and/or neuronal protection.

**SUMMARY OF THE INVENTION**

The present invention is directed to pyrazine derivatives and pharmaceutically acceptable salts thereof. These compounds and pharmaceutical compositions containing them showed strong effects of radical elimination and anticoagulation/thrombolysis, and strong capacity for neuroprotection.

The present invention is also directed to syntheses for preparation of the pyrazine derivatives and pharmaceutical compositions thereof.

The present invention is further directed to methods of use of the pyrazine derivatives and pharmaceutical compositions thereof for treatment of diseases and for manufacture of medicaments.

In one aspect of the invention, the pyrazine derivatives of the present invention are selected from compounds of formula 1:
or pharmaceutically acceptable salts thereof, wherein:

R\textsubscript{i} and R\textsubscript{2} are each independently hydrogen, hydroxyl, or a substituted or unsubstituted group selected from amino, carboxyl, alkyl, alkoxy, aryl, heteroaryl, esters, amines, carbamic acid ester and nitrones;

R\textsubscript{3} and R\textsubscript{4} are each independently hydrogen, hydroxyl, or a substituted or unsubstituted group selected from amino, carboxyl, alkyl, alkoxy, aryl, heteroaryl, esters, amines, carbamic acid ester, and nitrones; or R\textsubscript{3} and R\textsubscript{4} taken together with the carbon atom(s) they are attached form a substituted or unsubstituted fused ring.

R\textsubscript{i}, R\textsubscript{2}, R\textsubscript{3} and R\textsubscript{4} are not simultaneously hydrogen or methyl group;

If only one of R\textsubscript{i}, R\textsubscript{2}, R\textsubscript{3} and R\textsubscript{4} is a nitrone group, the nitrone group is not substituted by tertiary butyl.

According to some embodiments of the present invention, the compounds of formula I can be in a dimeric or polymeric structure through substitution of R\textsubscript{i} and/or R\textsubscript{4}.

In addition, the pyrazine derivative and pharmaceutically acceptable salts thereof provided in the invention can form a pharmaceutical composition, which includes a pyrazine derivative as a pharmaceutically active ingredient in a therapeutically effective amount, and a pharmaceutically acceptable carrier and excipient.

In another aspect of the invention, the methods for preparing the pyrazine derivatives include the steps of, for example, oxidizing a starting compound of pyrazine to form an aldehyde using active selenium dioxide, and refluxing the resulting aldehyde with a hydroxylamine to produce a pyrazine derivative with mono-substitution or multi-substitution of nitrone group. The method of preparation may also include the steps of reacting the starting pyrazine compound via bromination with NBS, and reacting with an active compound to produce a pyrazine composition.

In yet another aspect of the invention, the pyrazine derivatives can be used for treatment or prevention of the diseases induced by excessive production of ROS or thrombopoiesis and can be used to manufacture medicaments for the treatment or prevention of such diseases.

In comparison of the existing technologies, the present invention has the following advantages: The compounds of the present invention are novel in their structures and possess...
dual functions of thrombolysis and cytoprotection, and are capable of getting through blood-brain barrier, and further are safe and effective. These compounds are valuable candidates for new drugs for treating and/or preventing nervous system diseases caused by excessive radical production and/or thrombosis, infectious diseases, metabolic system diseases, cardiovascular and cerebrovascular diseases, age-related degenerative diseases, and cancers.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a synthetic process for the compound TN-2 according to an embodiment of the present invention.

FIG. 2 illustrates a synthetic process for the compound TN-2 according to another embodiment of the present invention.

FIG. 3 shows that the compound TN-2 has significant protective effects for PC12 cells induced by tert-butyl hydroperoxide (t-BHP).

FIG. 4 shows the protective effects of the compound TN-2 for the rats with ischemia caused by MCAo.

FIG. 5 shows the protective effects of the compound TN-2 for the damage of dopaminergic neuron induced by MPP⁺.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Definitions

When describing the pyrazine derivatives and uses thereof, unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings.

As used herein, the term "alkyl" refers to an unsubstituted or substituted straight-chain, branched-chain, or cyclic-chain having up to 15 carbon atoms; the straight-alkyl includes, for example, methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl and n-octyl, the cyclic alkyl ("cycloalkyl") includes, for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, and further the alkyl can be substituted by one or more substituents which include but not limited to NH₂, NO₂, N(CH₃)₂, ONO₂, F, Cl, Br, I, OH, OCH₃, C₂H, C₂CH₃, CN, aryl, and heteroaryl. The term "alkyl" may also refer to an unsubstituted or substituted straight-chain, branched-chain, or cyclic alkyl having up to 15 carbon atoms, which further
contains in the chain at least one heteroatom (e.g., nitrogen, oxygen, or sulfur); the above
straight-chain alkyl includes, for example, CH₂CH₂OCH₃, CH₂CH₂N(CH₃)₂, and CH₂CH₂SCH₃;
the branched-chain alkyl includes, for example, CH₂CH(OCH₃)CH₃, CH₂CH(N(CH₃)₂)CH₃
and CH₂CH(OCH₃)CH₃, the cyclic alkyl includes, for example, CH(CH₂CH₂)₂OH,
H(CH₂CH₂)₂NCH₃ and CH(CH₂CH₂)₂S, and further the alkyl can be substituted by one or
more substituents which include but not limited to NH₂, N(CH₃)₂, ONO₂, F, Cl, Br, I, OH,
OCH₃, C0₂H, C0₂CH₃, CN, aryl, and heteroaryl.

As used herein, the term "aryl" refers to an unsubstituted or substituted aromatic group,
carbocyclic group and heteroaryl. The aryl can be either a monocyclic group or a fused
polycyclic group; for example, phenyl is a monocyclic aryl, and naphthyl is a fused polycyclic
aryl. The aryl can be substituted by one or more substituents, which include but not limited to
NH₂, N(CH₃)₂, ONO₂, F, Cl, Br, I, OH, OCH₃, C0₂H, C0₂CH₃, CN, aryl, and heteroaryl.

The heteroaryl relates to substituted or an unsubstituted monocyclic or polycyclic group,
where the ring contains at least one heteroatom, such as nitrogen, oxygen and sulfur. For
example, a typical heteroaryl includes one or more nitrogen atoms such as in tetrazolyl,
pyrrolyl, pyridyl (e.g., pyrid-4-yl, pyrid-3-yl, pyrid-2-yl), pyridazinyl, indyl, quinolyl (e.g.,
quinol-2-yl, quinol-3-yl), imidazolyl, isoquinolyl, pyrazolyl, pyrazinyl, pyrimidinyl, pyridonyl
and pyridazinyl; a typical heteroaryl includes at least one oxygen atom such as in fur-2-yl,
fur-3-yl and benzofuryl; a typical heteroaryl includes at least one sulfur atom such as in thiynyl
and benzothienyl; a typical heteroaryl containing more than one kind of heteroatoms includes
furoazetidinyl, oxazolyl, isoxazolyl, thiazolyl and phenothioxinyl. The heteroaryl can be
substituted by one or more substituents which include but not limited to NH₂, N(CH₃)₂, O-alkyl,
NH-alkyl, N(alkyl)₂, NHC(0)-alkyl, ONO₂, F, Cl, Br, I, OH, OCF₃, OSO₂CH₃, C0₂H,
C0₂-alkyl, CN, aryl, and polyaryl. Furthermore, the heteroaryl also includes those with a
heteroatom in the ring being oxidized, for example, to form N-oxide, ketone, or sulfone.

The phrase "pharmaceutically acceptable," as used herein, means that there is no
unacceptable toxicity in a salt or excipient. The pharmaceutically acceptable salts include
inorganic anions such as those of chloride, bromide, iodide, sulfate, sulfite, nitrate, nitrite, and
phosphate and phosphite, and organic anions such as those of acetate, propionate, cinnamate,
tosylate, citrate, lactate and gluconate. In adding to the pharmaceutically acceptable excipients
described herebelow, see also: E. W. Martin, in Remington's Pharmaceutical Sciences Mack
In one aspect, the present invention provides pyrazine derivatives of formula I:

\[
\begin{array}{c}
R_3 \\
\text{N} \\
R_2 \\
\text{N} \\
R_1 \\
\end{array}
\]

and pharmaceutically acceptable salts thereof, wherein:

- \( R_1 \) and \( R_2 \) are each independently hydrogen, hydroxyl, or a substituted or unsubstituted group selected from amino, carboxyl, alkyl, alkoxy, aryl, heteroaryl, esters, amines, carbamic acid ester, and nitrone;
- \( R_3 \) and \( R_4 \) are each independently hydrogen, hydroxyl, or a substituted or unsubstituted group selected from amino, carboxyl, alkyl, alkoxy, aryl, heteroaryl, esters, amines, carbamic acid ester, and nitrone group; or \( R_3 \) and \( R_4 \) taken together with the carbon atom(s) they are attached form a substituted or unsubstituted fused ring.

- \( R_i, R_2, R_3 \) and \( R_4 \) are not simultaneously hydrogen or methyl group;
- If only one of \( R_i, R_2, R_3 \) and \( R_4 \) is a nitrone group, the nitrone group is not substituted by tertiary butyl.

In some embodiments, \( R_i \) and \( R_3 \) in formula I are each independently a substituted or unsubstituted nitrone group, \( R_2 \) and \( R_4 \) are alkyls, and the pyrazine derivatives have a structure of formula II:

\[
\begin{array}{c}
\Theta \\
\text{N} \\
R_6 \\
\text{N} \\
R_4 \\
\text{N} \\
R_5 \\
\end{array}
\]

wherein, \( R_3 \) and \( R_6 \) are each independently a substituted or unsubstituted straight-chain alkyl, branched-chain alkyl, or cycloalkyl.

In a preferred exemplary embodiment, \( R_2 \) and \( R_4 \) in formula II are methyl, and \( R_5 \) and \( R_6 \) are tert-butyl. Thus, the pyrazine derivative has a structure of TN-2 as follows:
In another preferred exemplary embodiment, R_2 and R_4 in formula II are methyl, and R_5
and R_6 are cyclohexyl. Thus, the pyrazine derivative has a structure of TN-3 as follows:

![TN-3](image)

In addition, in different embodiments of the compounds of formula I, R_3 and R_4 taken
together with the carbon atom(s) they are attached form a substituted fused ring, while the
substitute on the ring can be an alkyl, or one or more of nitrate groups. In some exemplary
embodiments, the pyrazine derivatives have a structure of TN-4 or TN-5 as follows:

![TN-4](image) ![TN-5](image)

In other embodiments, the pyrazine derivatives of formula I have a dimeric structure of
formula III, or even a polymeric structure, through substitution on R_9 and/or R_4:

![III](image)

wherein, R_7, R_8 and R_9 are each independently hydrogen, or a substituted or unsubstituted
group of hydroxyl, alkyl, or nitrone group; X is C, O, N or S, attached to an adjacent carbon
atom to form a hydrocarbon, ether, ammonia or sulfhydryl, or attached to an adjacent carbon
atom that can be optionally further oxidized into carbonyl, to form a ketone, acyloxy, or
acylamino.

In some embodiments, the pyrazine derivatives have the above-described dimeric
structure, wherein X is N-\text{tBu} for example, and thus have a structure of formula IV:
Further, the pyrazine derivatives, having the dimeric structure of formula IV, can be selected from at least one of the compounds of TN-6 through TN-14 as follows:

- **TN-6**: $R_{1}, R_{2}, R_{3}, R_{7}, R_{8}, R_{9} = \text{CH}_3$;
- **TN-7**: $R_{1} = \text{HC=NC} + (0 - )t\text{Bu}, R_{2}, R_{3}, R_{7}, R_{8}, R_{9} = \text{CH}_3$;
- **TN-8**: $R_{1}, R_{9} = \text{HC=NC} + (0 - )t\text{Bu}, R_{2}, R_{3}, R_{7}, R_{8} = \text{CH}_3$;
- **TN-9**: $R_{1}, R_{8} = \text{HC=NC} + (0 - )t\text{Bu}, R_{2}, R_{3}, R_{7}, R_{9} = \text{CH}_3$;
- **TN-10**: $R_{1}, R_{3}, R_{8} = \text{HC=NC} + (0 - )t\text{Bu}, R_{2}, R_{7}, R_{9} = \text{CH}_3$;
- **TN-11**: $R_{1}, R_{7}, R_{8} = \text{HC=NC} + (0 - )t\text{Bu}, R_{2}, R_{3}, R_{9} = \text{CH}_3$;
- **TN-12**: $R_{1}, R_{2}, R_{7}, R_{8} = \text{HC=NC} + (0 - )t\text{Bu}, R_{3}, R_{9} = \text{CH}_3$;
- **TN-13**: $R_{1}, R_{2}, R_{7}, R_{8} = \text{HC=NC} + (0 - )t\text{Bu}, R_{3}, R_{9} = \text{CH}_3$;
- **TN-14**: $R_{1}, R_{2}, R_{3}, R_{7}, R_{8}, R_{9} = \text{HC=NC} + (0 - )t\text{Bu}$.

In addition, in exemplary embodiments, the pyrazine derivatives with the dimeric structure of formula IV can be TN-15 or TN-16 as follows:

![Diagram of TN-15 and TN-16](image)

Further, in some other embodiments, the pyrazine derivatives of formula I can have a structure of formula V:

![Diagram of V](image)

wherein, $R_{10}$ is a substituted or unsubstituted straight-chain alkyl, branched-chain alkyl, cycloalkyl, or biologically active small molecule moiety including, lipoate or cysteine for example.

Furthermore, a pharmaceutical composition can be formed from the pyrazine derivative or pharmaceutically acceptable salts thereof. The pharmaceutical composition may include the pyrazine derivative, as a pharmaceutical active ingredient in a therapeutically effective amount, and a pharmaceutically acceptable carrier and excipient.
In the other aspect, methods of preparation of the pyrazine derivatives as described herein are provided in the invention. The methods, for example, can include the steps of oxidizing a starting compound of pyrazine into an aldehyde using active selenium dioxide, and refluxing the resulting aldehyde with an appropriate hydroxylamine for 3 hours to give a pyrazine derivative with a mono-substituted or multi-substituted nitrone.

In some other embodiments, a method of preparation of the pyrazine derivative includes the steps of reacting a starting compound of pyrazine via bromination with NBS, and further reacting with an active compound to form a pyrazine composition. Wherein, the active compound can be selected from, for example, the following ones:

\[
\begin{align*}
\text{OH} & \quad \text{OH} & \quad \text{H} \\
\end{align*}
\]

In additional embodiments, a method of preparation of the pyrazine derivative includes the steps of reacting 3,6-dimethyl-2,5-pyrazine dicarboxaldehyde with tert-butyl hydroxylamine; or reacting 3,6-dimethyl -2,5-dibromo methylpyrazine with tert-butyl hydroxylamine, and then oxidizing with sodium tungstate and hydrogen peroxide.

The novel compounds as described herein include compositions of pyrazine derivatives with nitrones and pyrazine derivative with other biologically active moieties, both of the compositions are antioxidants with antithrombotic activity. On one hand, these compounds are able to eliminate the radicals in the blood and tissues of human body including superoxide anion \((\text{O}_2^-)^+)\), peroxynitrite nitrate (ONO^-)\) and hydroxyl radical (\(\cdot\text{OH}\)); and on the other hand, these compounds are able to dissolve thrombus in blood vessels. Therefore, these compounds can be used for treating and/or preventing the diseases caused by excessive production of free radicals and/or thrombosis. These diseases include but not limited to nervous system diseases, such as hypoxic-ischemic herebral damage, stroke, cerebral trauma, Alzheimer's disease, epilepsy, Parkinson disease, Huntington's disease, amyotrophic lateral sclerosis, AIDS dementia, multiple sclerosis, chronic pain, priapism, cystic fibrosis, schizophrenia, depression, premenstrual syndrome, anxiety, addiction, and migraines; also include cardiovascular diseases, such as cardiopulmonary lateral flow, respiratory distress syndrome, heart ischemia-reperfusion, heart ischemia-reperfusion, toxic shock syndrome, adult respiratory distress syndrome, cachexia, myocarditis, atherosclerosis, coronary heart disease,
and heart attack; also include inflammatory infectious diseases, such as inflammatory bowel disease, diabetes mellitus, rheumatoid arthritis, asthma, hepatic cirrhosis, allograft rejection, encephalomyelitis, meningitis, pancreatitis, peritonitis, vasculitis, lymphocytic choriomeningitis, glomerulonephritis, systemic lupus erythematosus, stomach bowel disorders, obesity, hunger disease, hepatitis, and renal failure; also include eye disorders, such as diabetic retinopathy, uveitis, glaucoma, blepharitis, chalazion, allergic ocular disease, corneal ulcers, keratitis, cataract, senile macular degeneration, and optic neuritis; and further these compounds can be used for treating and/or preventing cancers, such as neuroblastoma.

The embodiments of the invention provide compositions of pyrazine derivatives with nitrones and pyrazine derivatives with biologically active moieties, and these compositions can be used to patients in the form of a pharmaceutical acceptable salts or complex drugs. A certain component needs to be mixed with an appropriate carrier or excipient to form a pharmaceutical composition to reach a desirable therapeutically effective amount. "Therapeutically effective amount" is intended to include a necessary amount of a compound described herein, or of a combination of compounds described herein when the compound or the combination of the compounds are used to attain a therapeutic effect for treating and/or preventing a disease, such as the effects of restraining excessive amount of radicals, and mitigating cell damages caused by stroke, heart attack or infectious disease.

The compounds as described herein can be prepared in different dosage forms, which include solid, semi-solid, liquid, and aerosol (Remington's Pharmaceutical Sciences, Mack Publishing Company (1995), Philadelphia, PA, 19th ed). These dosage forms can be further divided into more specific forms, including tablet, pill, sugar lozenge, granule, gel, paste, solution, suppository, injection, inhalant and spray. These dosage forms can be used for local or systemic administration and for immediate-release or sustained release. There are many routes of administration of these drugs, which include oral, buccal, rectal, peritoneal, intraperitoneal, transdermal administration, subcutaneous and endotracheal administrations.

When the compound or composition as described herein is applied in a dosage form of injection, the compound or composition can be prepared, by using a water-soluble or lipid-soluble solvent, into a solution, suspension or emulsion. The lipid-soluble solvent can be, for example, plant oil, synthetic fatty acid glyceride, higher fatty acid ester and/or proylene...
glycol. The compounds as described herein are more readily dissolved in Hank's solution, Ringer's solution or physiological saline.

When applied through oral administration, the compound or composition as described herein can be prepared through certain common techniques into a complex by adding a pharmaceutical acceptable excipient. Such excipients can be used to prepare these compounds into different dosage forms, such as tablet, pill, suspension, and gel. There are many ways for oral preparation, for example, by mixing the compound and the solid excipient, grinding fully the resulting mixture, adding appropriate auxiliary agents, and processing the mixture into particles. The auxiliary agents, which can be used for oral preparation, include, for example, sugars such as lactose, sucrose, mannitol, or sorbitol; celluloses such as corn starch, wheat starch, potato starch, gelatin, gummi tragacantae, methyl cellulose, hydroxyproylmethyl-cellulose, sodium carboxymethyl cellulose, and polyethylene pyrrole ketones.

The compounds as described herein can be prepared also in the form of spray, which can be achieved by using a pressurizer and a sprayer or dry powder inhaling device. Suitable spray agents used for spraying include, for example, dichlorodifluoromethane, fluorine chloroform, dichloro-tetrafluoroethane, carbon dioxide, and dimethyl ether. The amount of spray delivered from a sprayer can be controlled by the adjustment of the injecting valve of the sprayer.

The dosage forms as described herein are all related to the therapeutically effective amount of the compounds of the invention. The therapeutically effective amount of the compounds as described herein may depend on specific conditions of patients under the treatment. To determine the appropriate dose, various factors much be taken into account, for example, the route of administration to be used, weight and conditions of the patient to be treated, and observation and subjective judgment made by the prescribing physician. The therapeutically effective amount is usually determined by an experienced prescribing physician.

Examples

The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way.
Example 1. Synthesis of TN-2 (FIG. 1)

In a 500 mL three-necked flask was placed methanol (200 mL), and 3,6-dimethyl-2,5-pyrazine dicarboxaldehyde (2.0 g, 0.012 mol) was added, then tert-butyl hydroxylamine (4.3 g, 0.048 mol) was added, and the reaction was heated under reflux for 3 hrs. The obtained mixture was separated by column chromatography (ethyl acetate 100%) to obtain a light yellow solid compound TN-2 (1.0 g). Yield: 26.8%, mp: 198-201°C. \(^1\)HNMR (CDCl\(_3\)): 1.61 (s, 18H), 2.48 (s, 3H), 2.50 (s, 3H), 7.83 (s, 2H); ESI-MS: 307 [M+H]\(^+\), 329 [M+Na]\(^+\); Anal. (C\(_2\)H\(_9\)N\(_3\)O) C, H, N; found C 62.52%, H 8.73%, N 18.19%; requires: C, 65.13; H, 8.65; N, 18.99.

\[ \text{TN-2} \]

Example 2. Synthesis of TN-2 (FIG. 2)

In a 250 mL round-bottomed flask was placed 2,5-di-tert-butylamine methyl-3,6-dimethyl pyrazine (5.6 g, 0.02 mol) was added, an appropriate amount of methanol was added, then Na\(_2\)WO\(_4\)2H\(_2\)O (1.64 g, 0.005 mol) and 30% H\(_2\)O\(_2\) (10 mL) were added, and the reaction was stirred at room temperature for 2 hrs. The resultant mixture was filtered and evaporated to remove methanol, saturated Na\(_2\)S\(_2\)O\(_3\) was added, extracted with ethyl acetate, evaporated to remove most of the ethyl acetate. The product was separated by using column chromatography (ethyl acetate, 100%) to obtain a white solid TN-2 (1.97 g), in a yield of 32%. The analytical data are the same as above in Example 1.

Example 3. Synthesis of TN-4

In a 250 mL three-necked flask was added 2-methyl-quinoxaline (2.88 g, 0.02 mol), benzoyl peroxide (20 mg) was added, CC\(_4\) (80 mL) was added, and the reaction was refluxed at 70°C for 10 hrs. The reaction was cooled and then filtered to obtain a crude 2-bromomethyl quinoxaline, the compound obtained was not separated and to the resulting material was added an excess amount of tert-butyl amine, and the reaction was stirred at room temperature for 3 hrs to obtain 5-methyl tert-butylamine quinoxaline (1.25 mg), in a yield of 29.1%.
To the above-obtained compound (670 mg, 0.006 mol) were added methanol (60 mL), Na2WO4·2H2O (0.18 g) and 30%H2O2 (1.75 mL), the reaction was run at room temperature for 2.5 hrs. The product was separated by column chromatography (ethyl acetate : petroleum ether = 4:1) to obtain a light yellow compound TN-3 (460 mg) in a yield of 35.9%. 1HNMR (CDCl3): 1.70 (s, 9H), 7.77 (m, 2H), 8.03 (m, 2H), 8.14 (s, 1H), 10.49 (s, 1H); ESI-MS: 230 [M+H]+;
Anal. (C13H15N30) C, H, N; found C 67.80%, H 6.90%, N 17.86%; requires: C, 68.10; H, 6.59; N, 18.33.

![TN-4](image)


In a 250 mL three-necked bottle was placed 5-methyl quinoxaline (2.88 g, 0.02 mol), benzoyl peroxide(20 mg) was added, CC14 (80 mL) was added, then the reaction was refluxed at 70 °C for 10 hrs. The product was cooled and filtered to obtain a crude 2-bromomethyl quinoxaline, the compound was not separated, an excess amount of tert-butyl amine was added, and the reaction was stirred at room temperature for 3 hrs to obtain 5-methyl tert-butylamine quinoxaline (670 mg) in a yield of 15.6%.

The above-obtained compound (670 mg, 0.003 mol) were added methanol (60 mL), Na2WO4·2H2O (0.18 g) and 30%H2O2 (3.5 mL), the reaction was proceeded at room temperature for 2.5 hrs. The product was separated by column chromatography (ethyl acetate : petroleum ether = 2:1) to obtain a light yellow compound TN-4 (154 mg) in a yield of 21.5%. 1HNMR (CDCl3): 1.69 (s, 9H), 7.83 (dd, IH), 8.10 (dd, IH), 8.80 (d, IH), 8.87 (d, IH), 9.19 (s, IH), 9.96 (dd, IH); ESI-MS: 230 [M+H]+; Anal. (C13H15N30) C, H, N; found C 68.04%, H 6.95%, N 18.0%; requires: C, 68.10; H, 6.59; N, 18.33.

![TN-5](image)

Example 5. Synthesis of TN-6
In a 250 mL three-necked bottle was placed 3,5,6-trimethyl-2-bromide methylpyrazine (4.28 g, 0.02 mol), then an appropriate amount of tert-butyl amine was added dropwise, and the reaction was stirred at room temperature for 12 hrs, filtered and evaporated to dryness, the crude product was separated by column chromatography (petroleum ether : ethyl acetate = 5:1) to obtain a white powder solid (1.57 g), in a yield of 25%. $^1$H NMR (CDCl$_3$): 1.25 (s, 9H), 2.30 (s, 6H), 2.35 (s, 6H), 2.39 (s, 6H), 3.86 (s, 4H); ESI-MS: 342 [M+H]$^+$, 364 [M+Na]$^+$; Anal. (C$_{12}$H$_9$N$_3$O) C, H, N; found C 62.52%, H 8.73%, N 18.19%; requires: C, 65.13; H, 8.65; N, 18.99.

Example 6. Synthesis of TN-7

To the compound TN-6 (0.682 g, 0.002 mol) obtained in Example 5 was added 1,4-dioxane (100 mL), then an active selenium dioxide (330 mg, 0.003 mol) was added, the reaction was heated under reflux at 107°C for 3 hrs, a light yellow color was indicated by using 2,4-dinitrophenylhydrazine. The product was cooled to room temperature, evaporated to remove 1,4-dioxane, and separated by column chromatography (petroleum ether : ethyl acetate = 2:1) to obtain a solid (237.8 mg) in a yield of 33.5%.

To ethanol (100 mL) was added the above-obtained solid (237.8 mg) was added, and then tert-butyl hydroxylamine (0.12 g) was added. The reaction was refluxed at 84°C for 3 hrs, then cooled to room temperature, and evaporated to remove ethanol. The product was separated by column chromatography (petroleum ether : ethyl acetate = 5:1) to obtain a light yellow solid (183.5 mg), in a yield of 64.3%, ESI-MS: 427 [M+H]$^+$, 449 [M+Na]$^+$.

Example 7. Synthesis of TN-15

In an appropriate amount of THF was dissolved 2-hydroxymethyl-3,5,6-trimethyl
pyrazine (3.04 g, 0.02 mol), NaOH (2 g, 0.05 mol) was added, and then 3,5,6-trimethyl-2-
bromide methylpyrazine (5.35 g, 0.025 mol) was added at stirring at room temperature. The
product was filtered and the filtrate evaporated to dryness, the resultant crude material was
separated by column chromatography (petroleum ether : ethyl acetate = 3:1) to obtain a white
powder solid (4.8 g), in a yield of 84%. ESI-MS: 287 [M+H]+, 309 [M+Na]+.

Example 8. Synthesis of TN-16

In an appropriate amount of THF was dissolved 2-formic acid-3,5,6-trimethyl pyrazine
(3.32 g, 0.02 mol), then K₂C₀₃ (6.90 g, 0.05 mol) was added, and 3,5,6-trimethyl-2-bromide
methylpyrazine (5.35 g, 0.025 mol) was added at stirring at room temperature. The product
was filtered and the filtrate was evaporated to dryness, and the resultant crude material was
separated by column chromatography (petroleum ether : ethyl acetate = 3:1) to obtain a white
powder solid (4.5 g) in a yield of 75%. ESI-MS: 301 [M+H]+, 323 [M+Na]+.

Example 9. Synthesis of TN-17

Biotin (4.88 g, 0.02 mol) was dissolved in DMF (100 mL). Triethylamine (2.9 mL, 0.02
mol) and 3,5,6-trimethyl-2-bromide methylpyrazine (5.35 g, 0.025 mol) were added dropwise at
stirring. The reaction was stirred at room temperature for 5 hrs. The completion of the reaction
was detected by TLC. Water (80 mL) was added for dilution, and the mixture was extracted
with chloroform (100 mL x 2), the combined organic phases were washed with water (100 mL
x 2), dried with anhydrous Na₂S₀₄. The product was separated by column chromatography to
obtain a white powder solid (5.2 g), in a yield of 68.8%. ESI-MS: 379 [M+H]+, 401 [M+Na]+.
Example 10. Tests on cytoprotection of brain cells of rats by using TBN (FIG. 3)

PC12 cells were inoculated in a 96-well plate of strong adsorption with 90 μL per well, cultured for 36 hrs in an incubator set at 37°C and 5% CO₂. After 36 hrs, each of the drugs was added in four concentration gradients. Cultured for half an hour in an incubator set at 37°C and 5% CO₂, replaced with serum-free medium, t-BHP (10 mL, final concentration of 200 μM) was added in each of the wells except the well of the control. Then the material was placed and cultured in an incubator for 24 hrs. The cells were cultured for 24 hrs, and MTT (15 μL, 5 mg/mL) was added in each of the wells. The material was cultured in an incubator for 4 hrs, and then DMSO (150 mL) was added in each of the wells to be further incubated for at least half an hour to ensure the crystals were completely dissolved. The absorbance (A value) was measured by using a micro-plate reader at 570 nm. The results showed significant effect of cytoprotection of TN-2 towards the t-BHP-induced cell damage; the intensity of the effect was significantly higher than that of TMP, (FIG. 3). As shown in FIG. 3, *P<0.05 as compared with the t-BHP group; the difference is significant.

Example 11. Tests on cytoprotection of rats having cerebral ischemia caused by MCAO by using TN-2 (FIG. 4)

Anesthesia was performed on rats (female SD rats, in body weight of 260-300 g) with intraperitoneal injection of 10% hydrated chloral hydrate in a dose of 400 mg/kg or with inhalation of 3.5% halothane. At the entrance of MCAO (Middle Cerebral Artery Occlusion Ischemia) was blocked with a nylon wire to cause cerebral ischemia. After ischemia occurred for 1 hr, the rats were respectively intravenously injected with EDA (63 mg/kg), TBN (80 mg/kg), TN-2 (65 mg/kg), and saline (control group). There were 6 rats in each group. After ischemia occurred for 2 hrs, the nylon wires were removed, and reperfusion was performed for 24 hrs. Brain tissues were taken and the cerebellums were removed. The material was rinsed in PBS solution, and put in a freezer set at -20°C for a moment, and the brain tissues were cut.
into slices with a thickness of about 2 mm, and immediately placed in a solution of 0.5% triphenyltetrazolium chloride (TTC). Incubation was performed at 37°C for 30 minutes. The extent of cerebral infarction was evaluated. The results showed significant treatment effects of TN-2 on stroke of the rats (FIG. 4). In FIG. 4, the data were examined by one-tailed t-test; the mark "*" refers to the data in comparison with the control; drug dosage: TBN (80 mg/kg), and Edaravone (63 mg/kg); and each of the drugs were used in equal molarity.

Example 12. Tests on cytoprotection on injuries of dopaminergic neurons induced by MPP+ using TN-2 (FIG. 6)

Dopaminergic neurons were cultured for 5 days, and L-Deprenyl, TMP and TN-2 were added in a concentration gradient of 500 μM, 50 μM and 5 μM respectively. Two hours later, MPP+ (with a final concentration of 10 μM) was added into each of the wells except those of the control. As shown in FIG. 5, the results indicated that the effects of cytoprotection by TN-2 to the MPP+-induced cell damage were significant.

In the foregoing description of the embodiments, it is indicated that the present invention, through the novel compounds and their uses described herein, provided useful and unique approaches for the treatment or prevention of the diseases, such as neurological disorder, cardiovascular disease, inflammation and cancer, caused by excess amount of radicals. While certain specific embodiments have been described in detail, many details have been set forth for purposes of illustration and are not intended to limit the scope of the claims attached hereafter. It should be understood that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably with different substitutions, changes and modifications without deviating from the basic principles of the invention defined by the claims attached herein and their equivalents.
1. A pyrazine derivative of formula I
\[
\begin{align*}
\hat{\theta} \\
\hat{\theta}
\end{align*}
\]
and pharmaceutical acceptable salts thereof, wherein:

R\textsubscript{i} and R\textsubscript{2} are each independently hydrogen, hydroxyl, or a substituted or unsubstituted group selected from amino, carboxyl, alkyl, alkoxy, aryl, heteroaryl, esters, amines, carbamic acid ester, and nitrone;

R\textsubscript{3} and R\textsubscript{4} are each independently hydrogen, hydroxyl, or a substituted or unsubstituted group selected from amino, carboxyl, alkyl, alkoxy, aryl, heteroaryl, esters, amines, carbamic acid ester, and nitrone group; or R\textsubscript{3} and R\textsubscript{4} taken together with carbon atom(s) they are attached form a substituted or unsubstituted fused ring;

R\textsubscript{i}, R\textsubscript{2}, R\textsubscript{3} and R\textsubscript{4} are not simultaneously hydrogen or methyl group;

if only one of R\textsubscript{i}, R\textsubscript{2}, R\textsubscript{3} and R\textsubscript{4} is a nitrone group, the nitrone group is not substituted by tertiary butyl.

2. The pyrazine derivative according to claim 1, wherein R\textsubscript{i} and R\textsubscript{3} are each independently a substituted or unsubstituted nitrone group, R\textsubscript{2} and R\textsubscript{4} are alkyl, the pyrazine derivative having a structure of formula II:
\[
\begin{align*}
\hat{\theta} \\
\hat{\theta}
\end{align*}
\]
wherein, R\textsubscript{5} and R\textsubscript{6} are each independently a substituted or unsubstituted straight-chain alkyl, branched-chain alkyl, or cycloalkyl.

3. The pyrazine derivative according to claim 2, wherein R\textsubscript{2} and R\textsubscript{4} are methyl, R\textsubscript{5} and R\textsubscript{6} are tert-butyl, the pyrazine derivative having a structure of TN-2:
4. The pyrazine derivative according to claim 2, wherein $R_2$ and $R_4$ are methyl, $R_5$ and $R_6$ are cyclohexyl, the pyrazine derivative having a structure of TN-3:

5. The pyrazine derivative according to claim 1, wherein $R_3$ and $R_4$ taken together with carbon atom(s) they are attached form a substituted fused ring, wherein substitute on the ring includes an alkyl, or one or more nitrate groups.

6. The pyrazine derivative according to claim 5, wherein the pyrazine derivative has a structure of TN-4 or TN-5:

7. The pyrazine derivative according to claim 1, wherein the pyrazine derivative has a dimeric or polymeric structure through substitution on $R_4$ and/or $R_i$.

8. The pyrazine derivative according to claim 7, wherein the pyrazine derivative has a dimeric structure through substitution on $R_4$, and thus is of formula III:
wherein, \( R_7, R_8 \) and \( R_9 \) are each independently hydrogen, or a substituted or unsubstituted group of hydroxyl, alkyl, or nitrone group; \( X \) is C, O, N, or S, attached to an adjacent carbon atom to form a hydrocarbon, ether, ammonia or sulfhydryl, or attached to an adjacent carbon atom that can be optionally further oxidized into carbonyl, to form a ketone, acyloxy, or acylamino.

9. The pyrazine derivative according to claim 8, wherein \( X \) is N-\( t \)Bu, the pyrazine derivative having a structure of formula IV:

\[
\begin{align*}
\text{TN-6: } & R_i, R_2, R_3, R_7, R_8, R_9 = \text{CH}_3; \\
\text{TN-7: } & R_i = \text{HC}=\text{N}^+ (0-) \text{ } t\text{Bu}, R_2, R_3, R_7, R_8, R_9 = \text{CH}_3; \\
\text{TN-8: } & R_i, R_8 = \text{HC=NN}^+ (0-) \text{ } t\text{Bu}, R_2, R_3, R_7, R_8 = \text{CH}_3; \\
\text{TN-9: } & R_8 = \text{HC=NN}^+ (0-) \text{ } t\text{Bu}, R_2, R_3, R_7, R_9 = \text{CH}_3; \\
\text{TN-10: } & R_i, R_3, R_8 = \text{HC=NN}^+ (0-) \text{ } t\text{Bu}, R_2, R_7, R_9 = \text{CH}_3; \\
\text{TN-11: } & R_i, R_7, R_8 = \text{HC=NN}^+ (0-) \text{ } t\text{Bu}, R_2, R_3, R_9 = \text{CH}_3; \\
\text{TN-12: } & R_i, R_2, R_7, R_8 = \text{HC=NN}^+ (0-) \text{ } t\text{Bu}, R_3, R_9 = \text{CH}_3; \\
\text{TN-13: } & R_i, R_2, R_7, R_8 = \text{HC=NN}^+ (0-) \text{ } t\text{Bu}, R_3, R_9 = \text{CH}_3; \\
\text{TN-14: } & R_i, R_2, R_3, R_7, R_8, R_9 = \text{HC=NN}^+ (0-) \text{ } t\text{Bu}.
\end{align*}
\]

10. The pyrazine derivative according to claim 8, having a structure of TN-15 or TN-16:

11. The pyrazine derivative according to claim 1, having a structure of formula V:
wherein, R is a substituted or unsubstituted straight-chain alkyl, branched-chain alkyl, cycloalkyl, or biologically active small molecule moiety including lipoate or cysteine.

12. A pharmaceutical composition, comprising the pyrazine derivative of any one of claims 1-11 as a pharmaceutical active ingredient in a therapeutically effective amount, and a pharmaceutically acceptable carrier and excipient.

13. A method of preparation of the pyrazine derivative of claim 1, comprising the steps of oxidizing a starting compound of pyrazine into an aldehyde using active selenium dioxide, and refluxing the aldehyde with an appropriate hydroxylamine for 3 hours to give a pyrazine derivative with a mono-substituted or multi-substituted nitrone.

14. A method of preparation of the pyrazine derivative of claim 1, comprising the steps of reacting a starting compound of pyrazine via bromination with NBS, and further reacting with an active compound to form a pyrazine composition, wherein, the active compound is selected from the group consisting of:

15. A method of preparation of the pyrazine derivative of claim 8, comprising the steps of reacting 3,6-dimethyl-2,5-pyrazine dicarboxaldehyde with tert-butyl hydroxylamine; or reacting 3,6-dimethyl -2,5-dibromo methylpyrazine with tert-butyl hydroxylamine, and oxidizing with sodium tungstate and hydrogen peroxide.

16. A use of the pyrazine derivative of any one of claims 1-11 or a pharmaceutical composition thereof in manufacture of a medicament for treatment of a nervous system disease, cardiovascular disease, inflammatory infectious disease, eye disorder, and/or cancer.
17. The use of the pyrazine derivative of claim 16, wherein, the nervous system disease is selected from the group consisting of stroke, cerebral trauma, epilepsy, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease, hypoxic-ischemic brain damage, AIDS, dementia, multiple sclerosis, and chronic pain disorder.

18. The use of the pyrazine derivative of claim 16, wherein, the cardiovascular diseases is selected from the group consisting of cardiopulmonary lateral flow, respiratory distress syndrome, heart ischemia-reperfusion, heart ischemia-reperfusion, toxic shock syndrome, adult respiratory distress syndrome, cachexia, myocarditis, atherosclerosis, coronary heart disease, and heart attack.

19. The use of the pyrazine derivative of claim 16, wherein the inflammatory infectious disease is selected from the group consisting of inflammatory bowel disease, diabetes mellitus, rheumatoid arthritis, asthma, hepatic cirrhosis, allograft rejection, encephalomyelitis, meningitis, pancreatitis, peritonitis, vasculitis, lymphocytic choriomeningitis, glomerulonephritis, systemic lupus erythematosus, stomach bowel disorders, obesity, hunger disease, hepatitis, and renal failure.

20. The use of the pyrazine derivative of claim 16, wherein, the eye disorder is selected from the group consisting of diabetic retinopathy, uveitis, glaucoma, blepharitis, chalazion, allergic ocular disease, corneal ulcers, keratitis, cataract, senile macular degeneration, and optic neuritis.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C. See patent family annex.

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Date of the actual completion of the international search 20 Sep. 2011 (20.09.2011)

Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451

Authorized officer HE, Xiaoping Telephone No. (86-10)62084365

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INTERNATIONAL SEARCH REPORT

INTERNATIONAL application No.
PCT/CN2011/076756

CLASSIFICATION OF SUBJECT MATTER

C07D295/00 (2006.01)
C07D403/12 (2006.01)
A61K31/495 (2006.01)
A61P9/00 (2006.01)
A61P35/00 (2006.01)
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