COMPOUNDS FOR USE AS ANTI-VIRAL AGENTS

Technical Field

The present invention generally relates to novel derivative compounds and their use as anti-viral agents, and more specifically, to their use in medicine for treating patients suffering from diseases caused by flavivirus infection such as West Nile Virus infection and dengue virus infection.

Background

West Nile Virus (WNV) and dengue virus are flaviviruses that cause a large number of infections each year.

West Nile Virus

West Nile Virus is an arbovirus belonging to the genus *Flavivirus* within the family *Flaviviridae*. It is an RNA-type virus that is transmitted by a mosquito vector, and can be found in both tropical and temperate regions. WNV was first isolated from a feverish woman in the West Nile District of Uganda during research on yellow fever. WNV has since been detected in other parts of Africa, Europe, the Middle East, west and central Asia, Oceania, and in North America during the 1999 outbreak of encephalitis in New York City.

The clinical manifestation of WNV in human can vary significantly from being asymptomatic to developing a mild febrile syndrome termed "West Nile fever", or a neuroinvasive disease termed "West Nile meningitis or

encephalitis". Approximately 80% of WNV-infected patients are asymptomatic, 20% develop West Nile fever, and less than 1% develops severe neurological manifestations.

The symptoms of West Nile fever in human include an abrupt onset of fever, headache, fatigue, variable malaise, anorexia, nausea, myalgia, lymphadenopathy and a non-pruritic generalized maculopapular rash. Additional non-neurological manifestations that may develop in West Nile fever patients include hepatitis, pancreatitis, myocarditis, rhabdomyolysis, orchitis, and ocular manifestations. Neurological manifestations of WNV infection, on the other hand, include meningitis, encephalitis, acute flacid paralysis (AFP)/poliomyelitis, Parkinsonism, pharyngitis, conjunctivitis, coma, and seizure/status epilepticus. The duration of clinical manifestations may also vary significantly from a mild febrile illness that only lasts for several days to more debilitating symptoms that may last for weeks or months.

Other than humans, West Nile Virus infection may also cause severe neurological diseases and fatalities in other animal hosts, including birds, horses, dogs, cats, bats, chipmunks, skunks, squirrels and rabbits.

West Nile virions are spherical in shape and contain a 10.8kb single-stranded, positive sense RNA genome which is between 11,000 and 12,000 nucleotides long. The RNA genome encodes three structural proteins (which are the capsid (C), membrane (M) and envelope (E) proteins) and seven non-structural proteins (which are the NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 proteins). The WNV non-structural proteins are important because they are

responsible for viral RNA replication, viral assembly, and viral evasion of the host immune response.

Dengue Virus

Dengue is a mosquito-borne disease that is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. The clinical features of dengue fever vary according to the age of the patient and may range from non-specific febrile illness with rash in infants and young children to the abrupt onset of high fever, severe headache, pain behind the eyes, muscle and joint pains, and rash in older children and adults. Dengue haemorrhagic fever (DHF), on the other hand, is a potentially deadly complication characterized by high fever, haemorrhagic phenomena often with enlargement of the liver and in severe cases, circulatory failure.

At present, there is no commercial vaccine or causative treatment for the prevention or cure of dengue viral diseases, with careful clinical management and supportive therapy frequently being the main treatment options. Furthermore, vaccine development for dengue and DHF is complicated by the possibility of the disease being caused by any of four different serotypes of the virus, and by the potential increase in risk of more serious disease if protection is only acquired against one or two of the serotypes.

Dengue virus contains a single positive-stranded RNA genome, which is first translated into a polypeptide that is then processed by host cell proteases and the virus-encoded NS3 protease to generate three structural and

seven non-structural viral proteins. Activity of the NS3 protease has been shown to be markedly enhanced when coupled to the NS2B cofactor. Optimal activity of the NS3 protease has also been shown to be essential for maturation of the virus.

NS3 Protease and NS2B Cofactor

The NS3 protease and NS2B cofactor constitute the proteolytic complex that mediates the post-translational cleavage and processing of viral polypeptide precursors, and hence, play an essential role in viral replication in infected host cells. The NS3 protease is conserved amongst the flaviviruses. Hence, the NS3 protease is an ideal target for anti-WNV and anti-dengue drug discovery and design. If the activity of the NS3 protease can be effectively inhibited, the replication of the virus inside the host body will be prevented, thereby treating diseases caused by WNV and dengue virus.

Although research on WNV and dengue virus has been expanding rapidly over the past years, there is still no effective vaccine or anti-viral drug for WNV and dengue virus infection and related diseases.

Therefore, there is a need to provide an effective anti-viral treatment for treating WNV and dengue virus infections and related diseases in a patient.

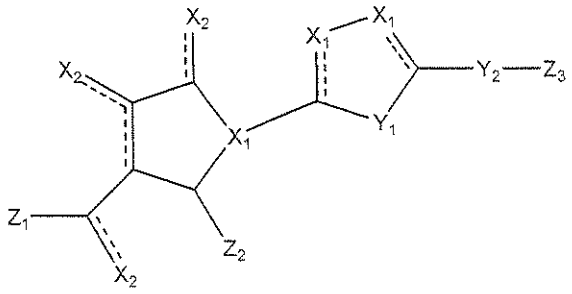
There is a need to provide a medicine for at least ameliorating the symptoms associated with WNV and dengue virus infections and related diseases in a patient.

There is a need to provide active compounds for use in a medicament, which have sufficiently low toxicity such that they are not harmful to humans.

There is a need to provide active compounds which are capable of inhibiting the activity of the NS3 protease, in particular, the activity of the WNV and dengue virus NS3 protease.

Summary of Invention

According to a first aspect, there is provided a compound for medical use having the general formula (I)



(I)

and the pharmaceutically acceptable salts thereof, wherein

------ is an optional double bond;

X_1 and Y_1 are heteroatoms;

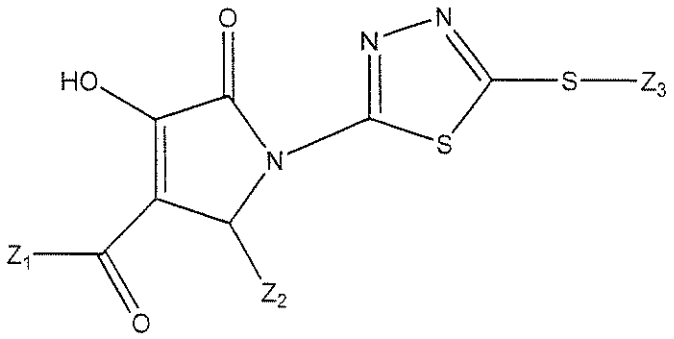
Y_2 is optional and is a heteroatom;

X_2 is independently selected from oxygen (O), nitrogen (N) and sulfur (S) when ------ is a double bond and is independently selected from a hydroxyl (-OH), thiol (-SH) when ------ is a single bond; and

Z_1, Z_2 and Z_3 are independently selected from the group consisting of optionally substituted aromatic ring group, heterocyclic group, straight or branched chain alkyl group, alkenyl group, alkynyl group, hydroxyl group, alcohols, straight or branched chain alkoxy group, cyano group, carboxyl group and ester group.

In one embodiment, the heteroatoms X_1 and Y_1 are independently selected from nitrogen (N), sulfur (S) and phosphorous (P).

In one embodiment, the compound has the general formula (II):



(II)

In one embodiment, the optionally substituted aromatic group of Z_1, Z_2 and Z_3 comprises an aryl group.

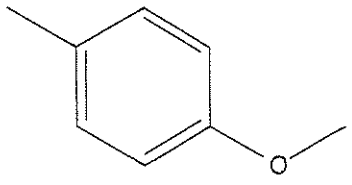
In one embodiment, the heterocyclic group of Z_1, Z_2 and Z_3 comprises a 4 to 6-membered ring.

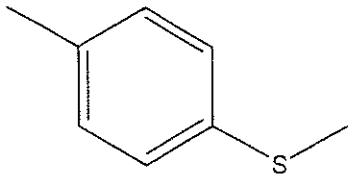
In one embodiment, the Z_1, Z_2 and Z_3 comprise a heterocyclic group fused to an aryl group.

In one embodiment, the Z_1, Z_2 and Z_3 comprise an aryl group substituted with at least one of an oxygen (O) atom, a sulfur (S) atom and a halide atom.

In one embodiment, the Z_1, Z_2 and Z_3 comprise two aryl groups linked by a heteroatom.

In one embodiment, Z_2 has one of the following structures:



; 

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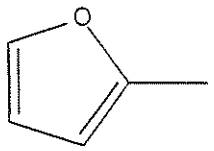

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In one embodiment, the Z_3 is a halide substituted aryl, an aryl substituted with an oxygen atom, an aryl substituted with an epoxide group, or a phenyl substituted with a fluoride (F) atom.

According to a second aspect, there is provided use of a compound as defined above in the manufacture of a medicament for the treatment, prevention, inhibition or amelioration of one or more diseases associated with West Nile Virus replication in a subject.

According to a third aspect, there is provided use of a compound as defined above in the manufacture of a medicament for the treatment, prevention, inhibition or amelioration of one or more diseases associated with dengue virus replication in a subject.

Definitions

The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention

to those terms alone, but are put forth for a better understanding of the following description.

Unless the context requires otherwise or specifically stated to the contrary, integers, steps, or elements of the invention recited herein as singular integers, steps or elements clearly encompass both singular and plural forms of the recited integers, steps or elements.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers, but not the exclusion of any other step or element or integer or group of elements or integers. Thus, in the context of this specification, the term "comprising" means "including principally, but not necessarily solely".

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The term "hydroxyl" as used herein refers to the functional group -OH.

The term "thiol" means -SH.

The term "halogen" or variants such as "halide" or "halo" as used herein refers to fluorine, chlorine, bromine and iodine.

The term "heterocyclic" as used herein, includes within its meaning monocyclic, bicyclic, polycyclic or fused hydrocarbon moieties having from 3 to 10 ring atoms wherein 1 to 5 ring atoms are heteroatoms selected from O, N, NH, or S. Examples include pyrrolidinyl, piperidinyl, quinuclidinyl, azetidinyl, morpholinyl, tetrahydrothiophenyl, tetrahydrofuranyl, tetrahydropyranyl, and the like.

The term "heteroatom" or variants such as "hetero-" as used herein refers to oxygen (O), nitrogen (N), phosphorus (P) and sulfur (S).

The term "aromatic group", or variants such as "aryl" or "arylene" as used herein refers to single, polynuclear, conjugated and fused residues of aromatic hydrocarbons having from 6 to 10 carbon atoms. Exemplary aryl groups include, but are not limited to phenyl, naphthyl, pyridyl, quinoyl, tetrahydronaphthyl, furanyl, indanyl, indenyl, indoyl, and the like.

The term "epoxide" as used herein refers to an organic three-membered cyclic ether, wherein at least one oxygen bridges a carbon-carbon bond. In general, the carbon atoms of the carbon-carbon bond are adjacent and the compound may be optionally substituted with atoms other than carbon and oxygen atoms, such as hydrogen atoms and halogens. Exemplary epoxides include ethylene oxide, propylene oxide, glycidol and epichlorohydrin.

The term "optionally substituted" as used herein means the group to which this term refers may be

unsubstituted, or may be substituted with one or more groups independently selected from hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

The term "cyano" means -CN.

The term "nitro" means -NO_2.

As used herein, the term "alkyl group" includes within its meaning straight chain or branched chain saturated aliphatic groups having from 1 to 10 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. For example, the term alkyl includes, but is not limited to, methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, isobutyl, tert-butyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-

dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, and the like.

The term "lower alkyl" refers to a straight or branched saturated hydrocarbon chain having 1, 2, 3, 4, 5, or 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, t-butyl, n-hexyl, and the like.

The term "alkenyl group" includes within its meaning straight or branched chain unsaturated aliphatic hydrocarbon groups having from 2 to 10 carbon atoms, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms and having at least one double bond, of either E, Z, cis or trans stereochemistry where applicable, anywhere in the alkyl chain. Examples of alkenyl groups include but are not limited to ethenyl, vinyl, allyl, 1-methylvinyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, 3-butentyl, 1,3-butadienyl, 1-pentenyl, 2-pententyl, 3-pentenyl, 4-pentenyl, 1,3-pentadienyl, 2,4-pentadienyl, 1,4-pentadienyl, 3-methyl-2-butenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 2-methylpentenyl, 1-heptenyl, 2-heptentyl, 3-heptenyl, 1-octenyl, 1-nonenyl, 1-decenyl, and the like. The term "lower alkenyl" refers to a straight or branched chain hydrocarbon unsaturated aliphatic hydrocarbon group having at least one double bond and from 2 to 7 carbon atoms.

The term "alkynyl group" as used herein includes within its meaning straight or branched chain unsaturated aliphatic hydrocarbon groups having from 2 to 10 carbon atoms and having at least one triple bond anywhere in the carbon chain. Examples of alkynyl groups include but are not limited to ethynyl, 1-propynyl, 1-butynyl, 2-butynyl, 1-methyl-2-butynyl, 3-methyl-1-butynyl, 1-pentynyl, 1-hexynyl, methylpentynyl, 1-heptynyl, 2-heptynyl, 1-octynyl, 2-octynyl, 1-nonyl, 1-decynyl, and the like. The term "lower alkynyl" refers to a straight or branched chain unsaturated aliphatic hydrocarbon having at least one triple bond and from 2 to 7 carbon atoms.

The term "alcohol" as used herein refers to any straight, branched, or cyclic; substituted or unsubstituted alkyl, alkenyl, or alkynyl groups attached to at least one hydroxyl moiety. Non-limiting examples of alcohols include ethanol, methanol, isopropanol, 1-propanol, 1-butanol, 2-butanol, tert-butanol, isobutanol, and pentanol.

The term "cycloalkyl" as used herein refers to cyclic saturated aliphatic groups and includes within its meaning monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon moieties having from 3 to 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. Examples of cycloalkyl groups include but are not limited to cyclopropyl, 2-methylcyclopropyl, cyclobutyl, cyclopentyl, 2-methylcyclopentyl, 3-methylcyclopentyl, cyclohexyl, and the like.

The term "cycloalkenyl" as used herein, refers to cyclic unsaturated aliphatic groups and includes within its meaning monocyclic, bicyclic, polycyclic or fused

polycyclic hydrocarbon moieties having from 3 to 10 carbon atoms and having at least one double bond, of either E, Z, cis or trans stereochemistry where applicable, anywhere in the alkyl chain. Examples of cycloalkenyl groups include but are not limited to cyclopropenyl, cyclopentenyl, cyclohexenyl, and the like.

The term "heterocycloalkenyl" as used herein, includes within its meaning monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon moieties having from 3 to 10 ring atoms and having at least 1 double bond, wherein from 1 to 5 ring atoms are heteroatoms selected from O, N, NH or S.

The term "heteroaromatic group" and variants such as "heteroaryl" or "heteroarylene" as used herein, includes within its meaning single, polynuclear, conjugated and fused aromatic moieties having 5 to 20 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N, NH and S. Examples of such groups include pyridyl, 2,2'-bipyridyl, phenanthrolinyl, quinolinyl, thiophenyl, furanyl and pyrrolic moieties and the like.

The term "alkoxy" as used herein refers to straight chain or branched alkyloxy groups. Examples include methoxy, ethoxy, n-propoxy, isopropoxy, tert-butoxy, and the like.

The term "lower alkoxy" refers to a straight chain or branched alkyloxy group containing from 1 to 6 carbon atoms.

The term "ester" refers to a compound of formula -COO-alkyl or -COO-aryl, wherein "alkyl" and "aryl" are as defined above.

The term "carboxyl" refers to a compound having the functional group -C(O)OH.

The term "amino" as used herein refers to groups of the form -NRaRb wherein Ra and Rb are individually selected from the group including but not limited to hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl groups.

The term "aralkyl" as used herein, includes within its meaning single, polynuclear, conjugated and fused aromatic hydrocarbon moieties attached to straight or branched chain alkyl or alkenyl groups.

The term "heteroaralkyl" as used herein, includes within its meaning single, polynuclear, conjugated and fused aromatic hydrocarbon moieties attached to straight or branched chain alkyl or alkenyl groups.

The present invention includes within its scope all isomeric forms of the compounds disclosed herein, including all diastereomeric isomers, racemates and enantiomers. Thus, formulae (I) to (IV) should be understood to include, for example, E, Z, cis, trans, (R), (S), (L), (D), (+), and/or (-) forms of the compounds, as appropriate in each case.

The term "patient" refers to patients of human or other mammal and includes any individual it is desired to examine or treat using the methods of the invention. However, it will be understood that "patient" does not imply that symptoms are present. Suitable mammals that fall within the scope of the invention include, but are not restricted to, primates, livestock animals (eg. sheep, cows, horses, donkeys, pigs), laboratory test

animals (e.g. rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g. cats, dogs) and captive wild animals (e.g. foxes, deer, dingoes). "Mammal" refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, and pet companion animals such as a household pet and other domesticated animal such as, but not limited to, cattle, sheep, ferrets, swine, horses, poultry, rabbits, goats, dogs, cats and the like. Preferred companion animals are dogs and cats. Preferably, the mammal is human.

In the context of this invention, the term "administering" and variations of that term including "administer" and "administration", includes contacting, applying, delivering or providing a compound or composition of the invention to an organism, or a surface by any appropriate means.

In the context of this invention, the term "treatment" refers to any and all uses which remedy a disease state or symptoms, prevent the establishment of disease, or otherwise prevent, hinder, retard, or reverse the progression of disease or other undesirable symptoms in any way whatsoever.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which retain the biological effectiveness and properties of the active compound of the anti-viral composition, which are not otherwise undesirable. A thorough discussion of pharmaceutically acceptable salts is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

In the context of this invention, the terms "therapeutically effective amount" and "diagnostically effective amount" include within their meanings a sufficient but non-toxic amount of a compound or composition of the invention to provide the desired therapeutic or diagnostic effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered, the mode of administration, and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

"Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the individual to be treated; each unit containing a predetermined quantity of compound(s) is calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The compound(s) may be formulated for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in an acceptable dosage unit. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the ingredients.

As used herein, the term "West Nile Virus" (or "WNV") includes "West Nile-like viruses". Likewise, the term "dengue virus" includes "dengue-like viruses". The

terms further include the naturally-occurring form of the pathogens (e.g. wild-type); naturally-occurring variants of the pathogen; and variants generated in the laboratory, including variants generated by selection, variants generated by chemical modification, and variants generated by genetic modification (e.g. pathogens modified in a laboratory by recombinant DNA methods).

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3,

from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

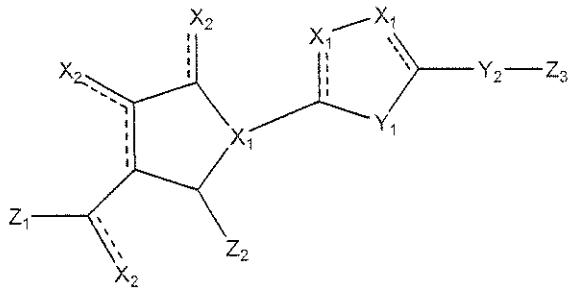
Detailed Disclosure of Embodiments

Exemplary, non-limiting embodiments of a composition comprising novel derivative compounds capable of inhibiting or treating the symptoms associated with WNV and dengue virus infection and disease in a patient will now be disclosed.

The inventors have surprisingly found that the active compounds as disclosed herein exhibit inhibitive activity to WNV and dengue virus NS3 protease *in vitro*. Accordingly, the active compounds disclosed herein can be used as medicinal agents in the treatment of patients infected with the WNV or dengue virus, and/or afflicted with WNV or dengue virus related diseases.

The Active Compounds

The active compounds may be represented by a compound having the general formula (I)



(I)

and the pharmaceutically acceptable salts thereof, wherein

$\overset{\text{------}}{\text{———}}$ is an optional double bond;

X_1 and Y_1 are heteroatoms;

Y_2 is optional and is a heteroatom;

X_2 is independently selected from oxygen (O), nitrogen (N) and sulfur (S) when $\overset{\text{------}}{\text{———}}$ is a double bond and is independently selected from a hydroxyl (-OH), thiol (-SH) when $\overset{\text{------}}{\text{———}}$ is a single bond; and

Z_1, Z_2 and Z_3 are independently selected from the group consisting of an aromatic ring group, a heterocyclic group, a straight or branched chain alkyl group, an alkenyl group, an alkynyl group, a hydroxyl group, an alcohol, a straight or branched chain alkoxy group, a cyano group, a carboxyl group and an ester group, each optionally substituted by one or more groups selected from hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphonyl, phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

Suitable heteroatoms include, without limitation, oxygen (O), sulfur (S), and nitrogen (N) and phosphorous

(P). In one embodiment, the heteroatoms X_1 and Y_1 are independently selected from nitrogen (N), sulfur (S) and phosphorous (P).

In a preferred embodiment, X_1 is nitrogen (N).

In a preferred embodiment, Y_1 is sulfur (S).

In one embodiment, both X_1 and Y_1 are nitrogen (N).

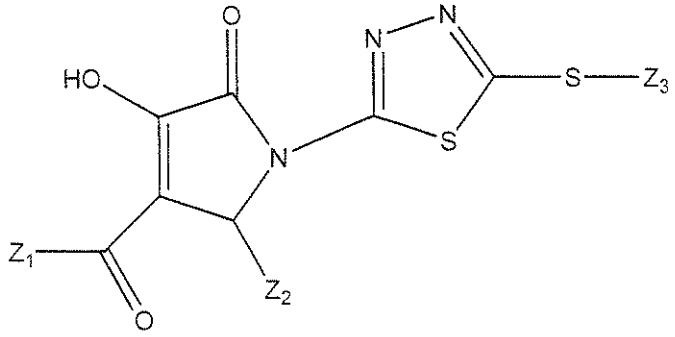
In one embodiment, both Y_1 and Y_2 are sulfur (S).

In a preferred embodiment, X_1 is nitrogen (N) and Y_1 is sulfur (S). In another preferred embodiment, X_1 is sulfur (S) and Y_1 is nitrogen (N).

In one embodiment, where ------ is a double bond, X_2 is oxygen (O).

In one embodiment, where ------ is a single bond, X_2 is a hydroxyl (-OH).

In one embodiment, the compound has the general formula (II):

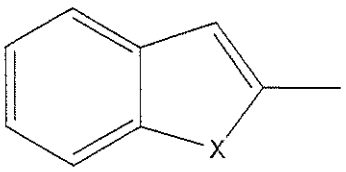


(II)

In one embodiment, the optionally substituted aromatic group of Z_1, Z_2 and Z_3 comprises an aryl group. Preferably, Z_1, Z_2 and Z_3 are independently a 5 to 7-membered ring aryl group. Preferably, the aryl group of Z_1, Z_2 and Z_3 comprises a phenyl ring or naphthalene ring.

The heterocyclic group of Z_1, Z_2 and Z_3 may comprise a 4 to 6-membered ring. Preferably, the heterocyclic group of Z_1, Z_2 and Z_3 is a 5-membered ring comprising an oxygen atom.

In one embodiment, Z_1, Z_2 and Z_3 comprise a heterocyclic group fused to an aryl group. In one preferred embodiment, Z_1 comprises the heterocyclic group fused to the aryl group having one of the following structures:



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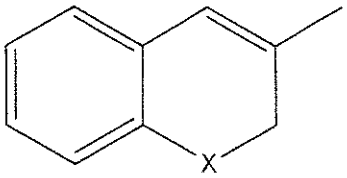
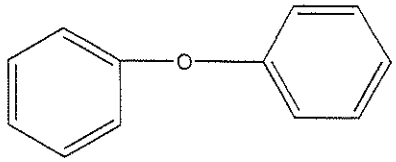


wherein X is O, S or NH.

In a preferred embodiment, X is oxygen (O).

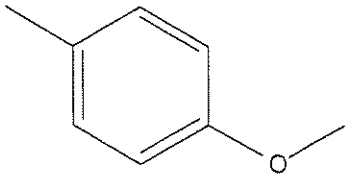
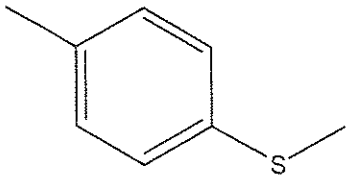
In one embodiment, Z_1, Z_2 and Z_3 comprise an aryl group substituted with at least one of an oxygen (O) atom, a sulfur (S) atom and a halide atom. Suitable halide atoms include fluoride, chloride, bromide and iodide. Preferably, the halide atom is a fluoride or a chloride. In one embodiment, Z_2 is substituted with at least one of a fluoride (F) and a chloride (Cl) atom. In another embodiment, Z_2 is substituted with two or more halides selected from the group of fluoride (F) and chloride (Cl).

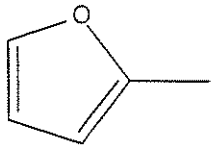
In one embodiment, Z_1, Z_2 and Z_3 comprise two aryl groups linked by a heteroatom. In a preferred embodiment, Z_2 has the structure (III):



(III)

In other embodiments, Z_2 has one of the following structures:

 ;  ; and



In one embodiment, Z_3 is a halide substituted aryl. In a preferred embodiment, Z_3 is an aryl substituted with an oxygen atom. In another preferred embodiment, Z_3 is an aryl substituted with an epoxide group. In yet another preferred embodiment, Z_3 is a phenyl substituted with a fluoride (F) atom.

In one embodiment, Z_3 is a straight or branched chain alkyl group. In a preferred embodiment, the alkyl group is a lower alkyl having 1 to 6 carbon atoms. In yet another preferred embodiment, Z_3 is selected from the group consisting of $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH_2CH_2CH_2CH_3$, $-CH_2CH_2CH_2CH_2CH_3$, $-CH_2CH_2CH_2CH_2CH_2CH_3$ and $-CH(CH_3)_2$.

In one embodiment, Z_3 is an alkenyl group. In a preferred embodiment, Z_3 is selected from the group

consisting of $-CH_2CH_2$, $-CH_2CH_2CH_2$, $-CH_2CHCH_3$, $-CHCH_2CH_3$, and $-CH(CH_2)_4$.

In one embodiment, Z_3 is an alkynyl group. In a preferred embodiment, Z_3 is selected from the group consisting of $-CCCH_3$, $-CH_2CCH$, $-CH_2CH_2CCH$, $-CH_2CH_2CH_2CCH$, $-CH_2CH_2CH_2CH_2CCH$, and $-CH_2CCCH_3$.

In one embodiment, Z_3 is a hydroxyl group. In a preferred embodiment, Z_3 is selected from the group consisting of $-OH$, $-CH_2OH$, $-CH_2CH_2OH$, $-CH_2CH_2CH_2OH$, and $-CH_2CH_2CH_2CH_2OH$.

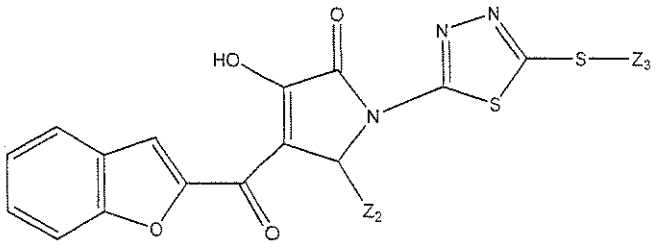
In one embodiment, Z_3 is a straight or branched chain alkoxy group. In a preferred embodiment, the alkoxy group is a lower alkoxy having 1 to 6 carbon atoms. In yet another preferred embodiment, Z_3 is selected from the group consisting of $-CH_2OCH_3$, $-CH_2CH_2OCH_3$, $-CH_2CH_2CH_2OCH_3$, $-CH_2CH_2CH_2CH_2OCH_3$, $-CH_2OCH_2CH_3$, $-CH_2OCH_2CH_2CH_3$, and $-CH_2OCH_2CH_2CH_2CH_3$.

In one embodiment, Z_3 is a cyano group. In a preferred embodiment, Z_3 is selected from the group consisting of $-CN$, $-CH_2CN$, $-CH_2CH_2CN$, $-CH_2CH_2CH_2CN$ and $-CH_2CH_2CH_2CH_2CN$.

In one embodiment, Z_3 is a carboxyl group. In a preferred embodiment, Z_3 is selected from the group consisting of $-COOH$, $-CH_2COOH$, $-CH_2CH_2COOH$, $-CH_2CH_2CH_2COOH$ and $-CH_2CH_2CH_2CH_2COOH$.

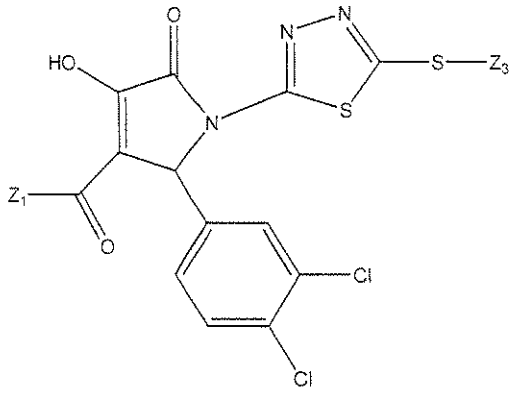
In one embodiment, Z_3 is an ester group. In a preferred embodiment, Z_3 is selected from the group consisting of $-CH_2COOCH_3$, $-CH_2CH_2COOCH_3$, $-CH_2CH_2CH_2COOCH_3$, $-CH_2CH_2CH_2CH_2COOCH_3$, $-CH_2COOCH_2CH_3$, $-CH_2COOCH_2CH_2CH_3$, and $-CH_2COOCH_2CH_2CH_2CH_3$.

In one embodiment, the active compound is represented by the general formula (IV):



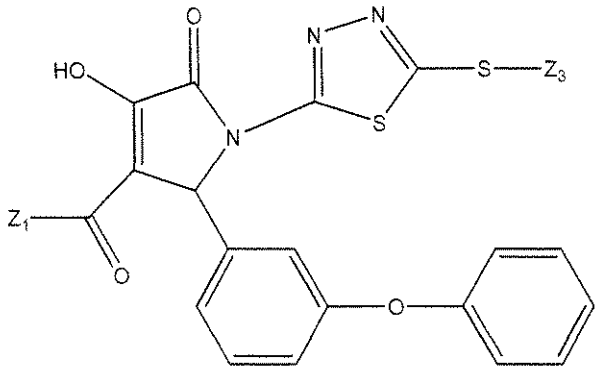
(IV)

In one embodiment, the active compound is represented by the general formula (V):



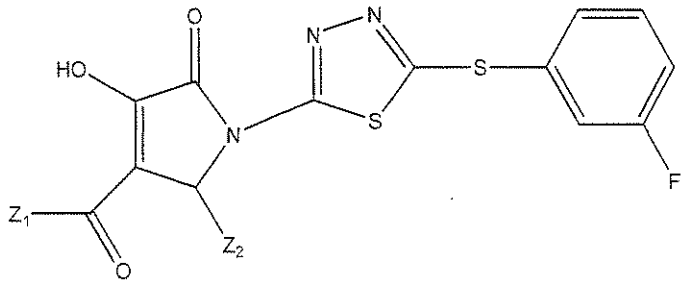
(V)

In one embodiment, the active compound is represented by the general formula (VI):



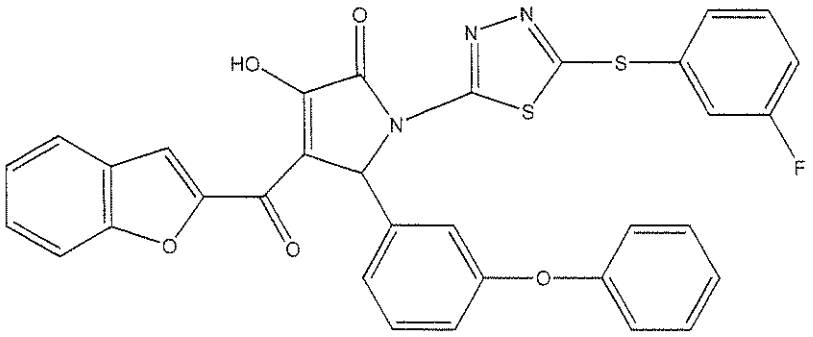
(VI)

In one embodiment, the active compound is represented by the general formula (VII):



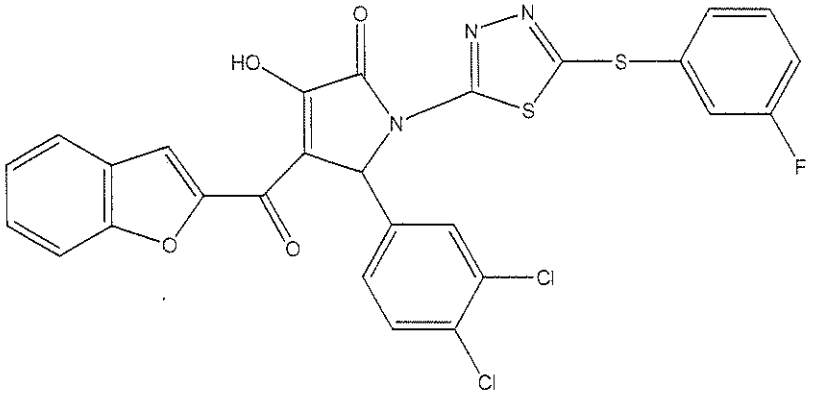
(VII)

In a preferred embodiment, the active compound is represented by the general formula (VIII):



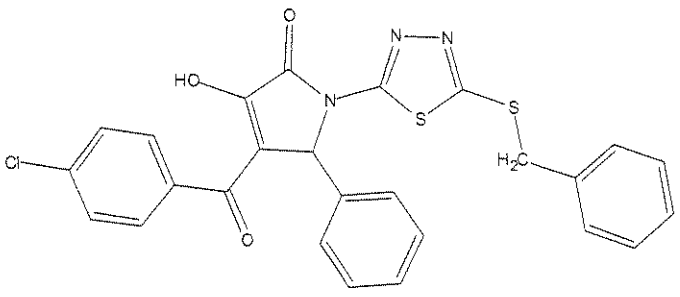
(VIII)

In another preferred embodiment, the active compound is represented by the general formula (IX):



(IX)

Other exemplary active compounds include:



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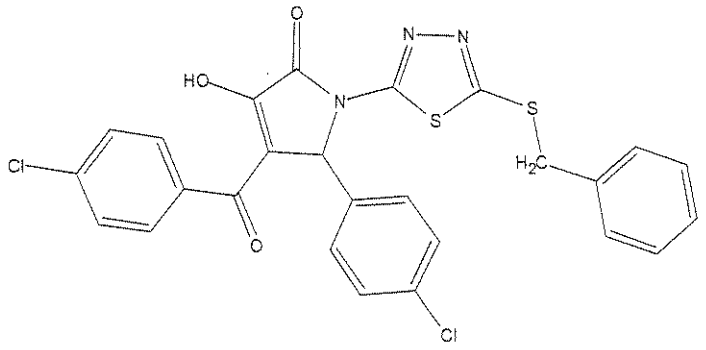
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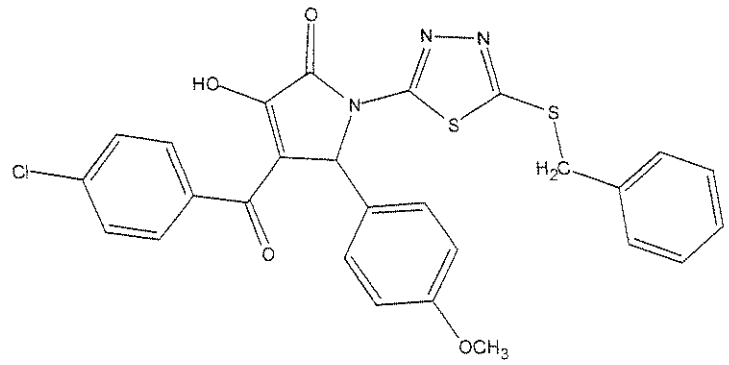
HO
N
N
N
S
S
H2C
Cl
O
Cl
;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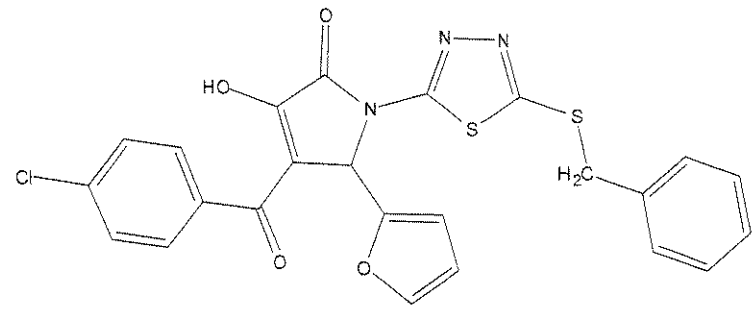
N
N
N
S
S
H2C
Cl
O
OCH3
;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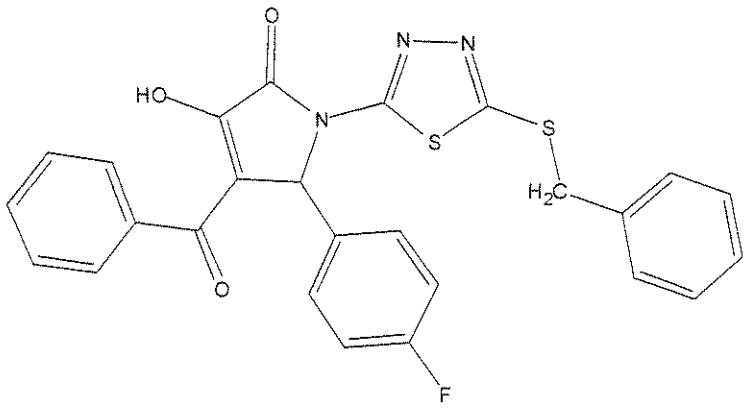
HO
N
N
N
S
S
H2C
Cl
O
O
;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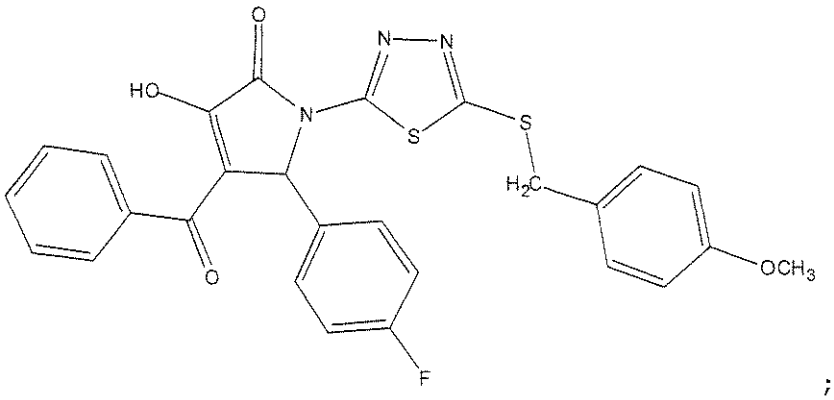
HO
N
N
N
S
S
H2C
O
F
;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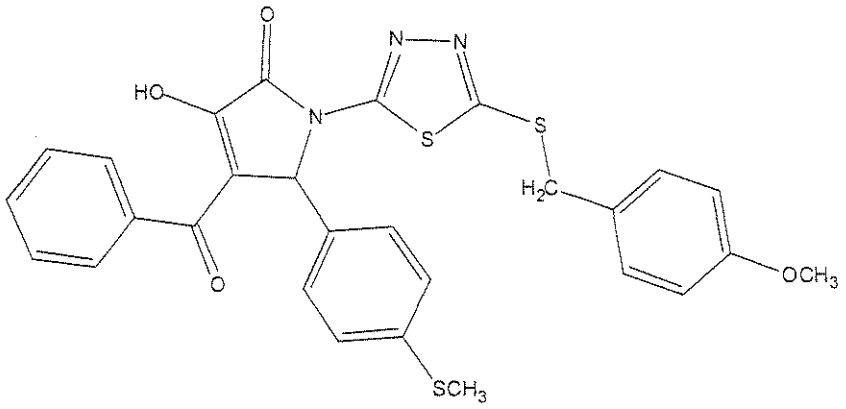
HO
N
N
N
S
S
H2C
OCH3
O
F

;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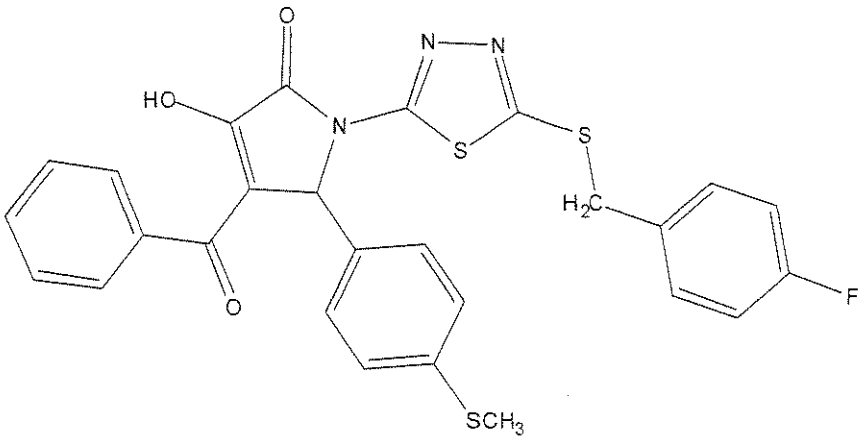
N
N
N
S
S
H2C
OCH3
O
SCH3

;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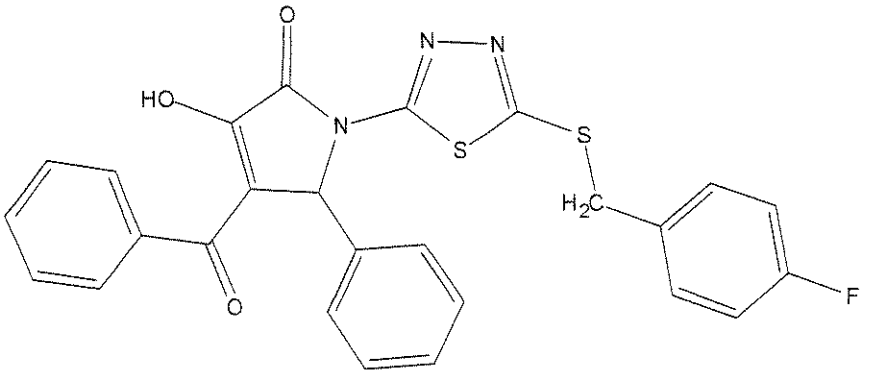
N
N
N
S
S
H2C
F
O
SCH3

;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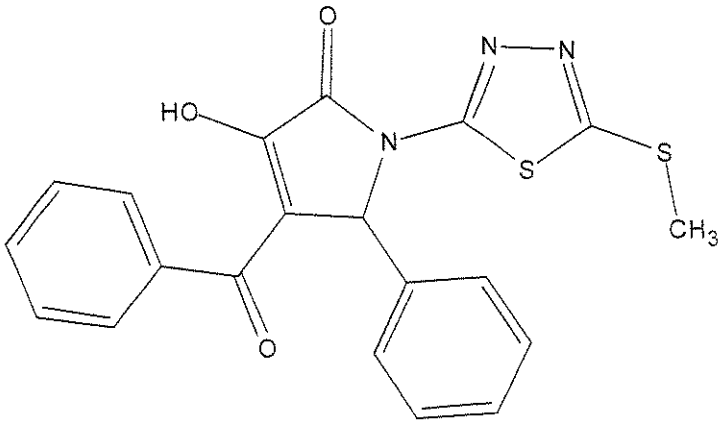
N
N
HO
N
S
S
H2C
O
F
;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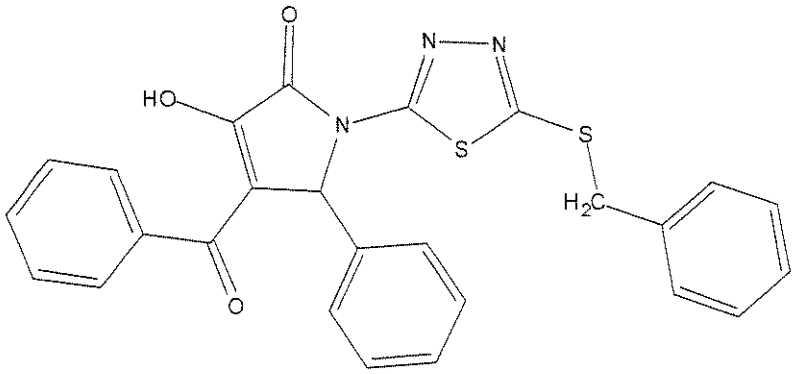
S
S
CH3
O
;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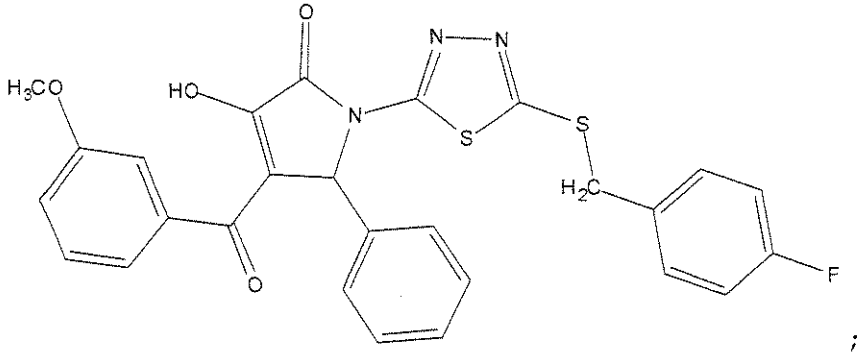
N
N
HO
N
S
S
H2C
O
;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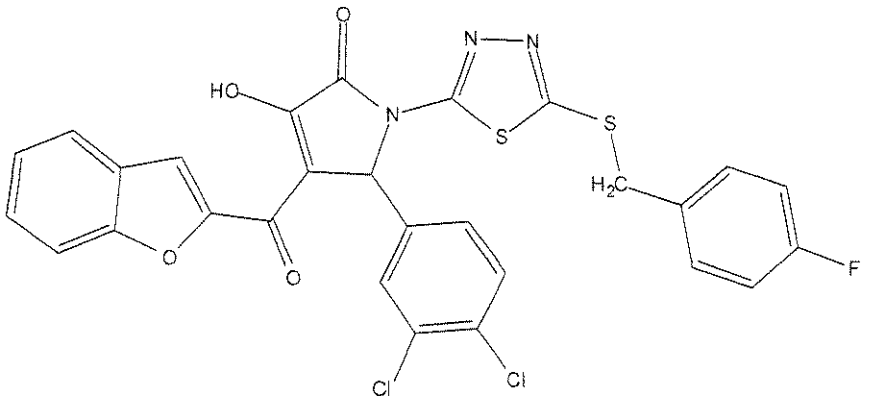
N
N
H3CO
HO
N
S
S
H2C
O
F
;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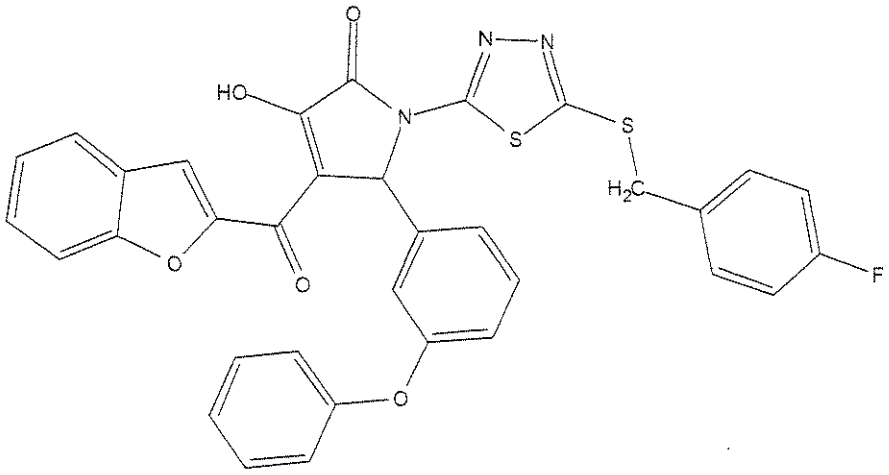
HO
N
N
N
S
S
H2C
F
O
O
Cl
Cl

;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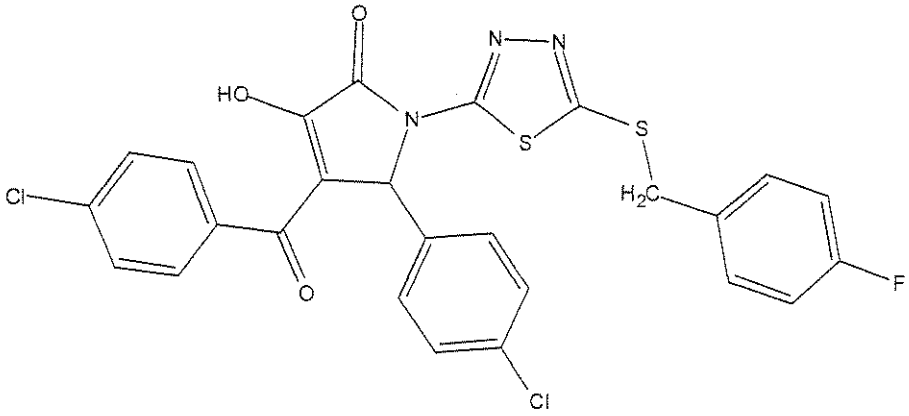
HO
N
N
N
S
S
H2C
F
O
O
O

;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S
S
H2C
F
Cl
O
Cl

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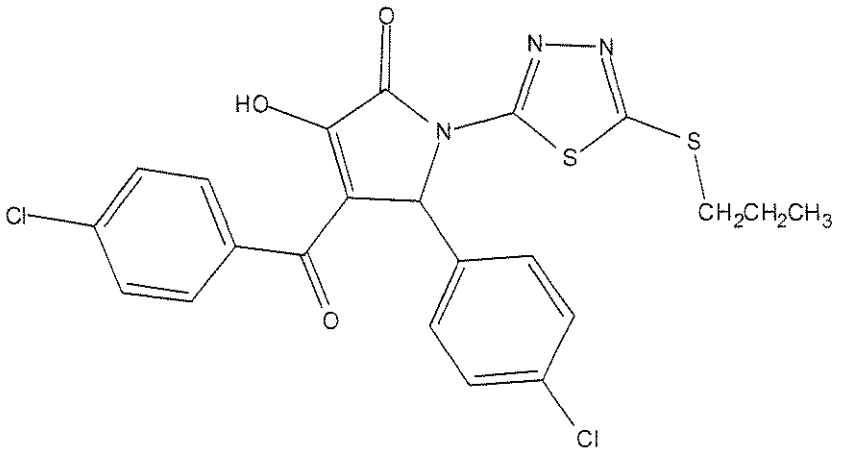
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HO
N
N
N
S
S
CH2CH2CH3
Cl
O
Cl

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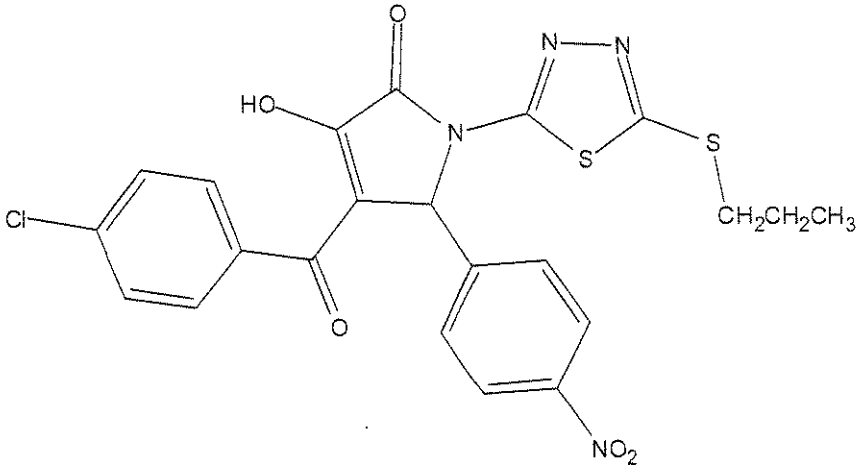
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Cl
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NO2

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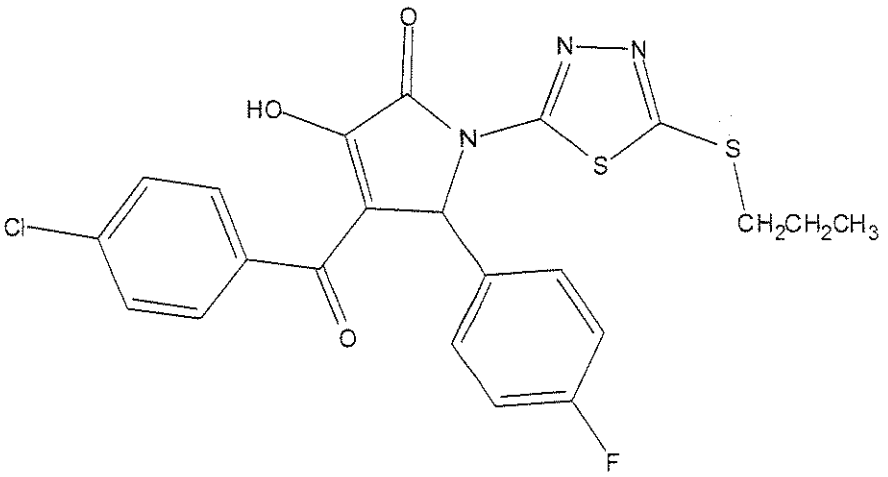
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S
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O
F

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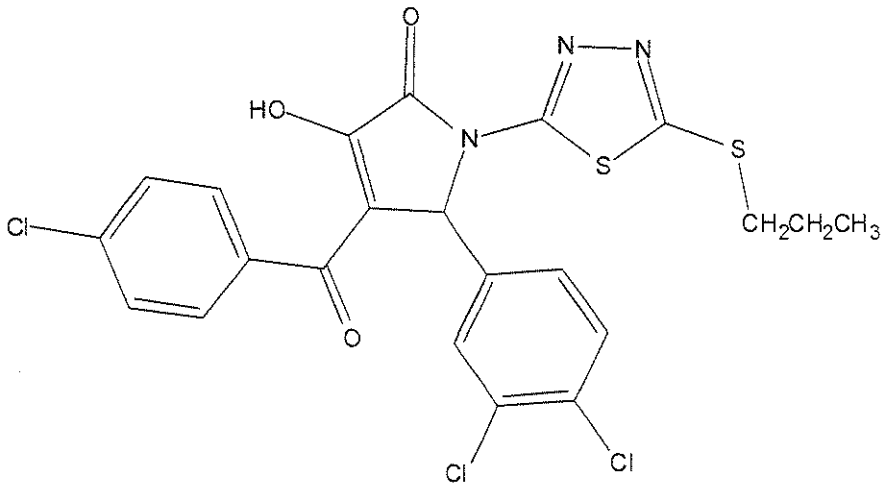
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N
HO
N
S
S
CH2CH2CH3
Cl
O
Cl
Cl

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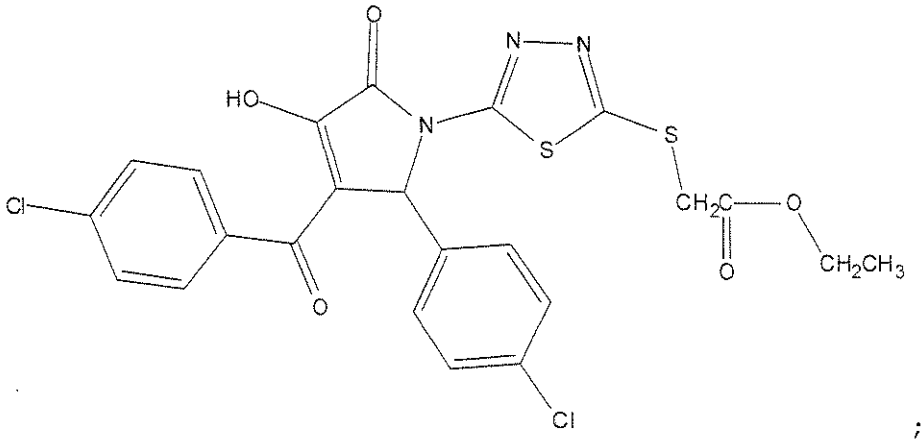
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Cl
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Cl

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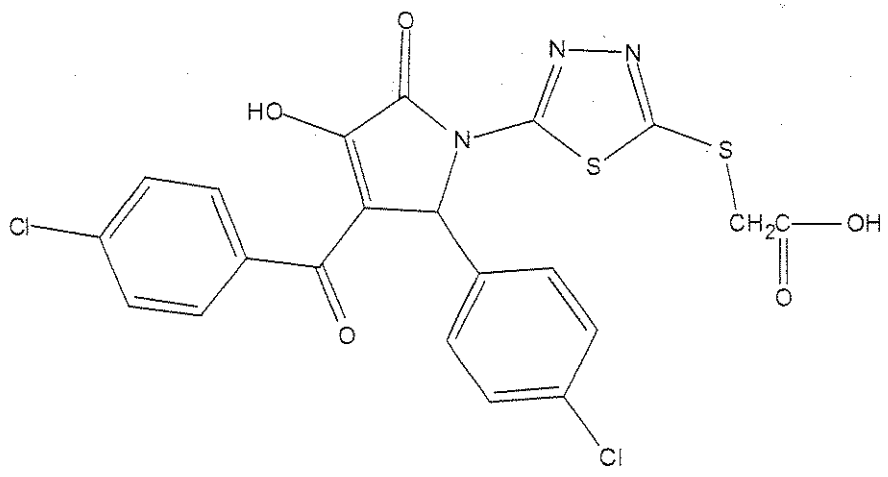
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HO
N
S
S
CH2C
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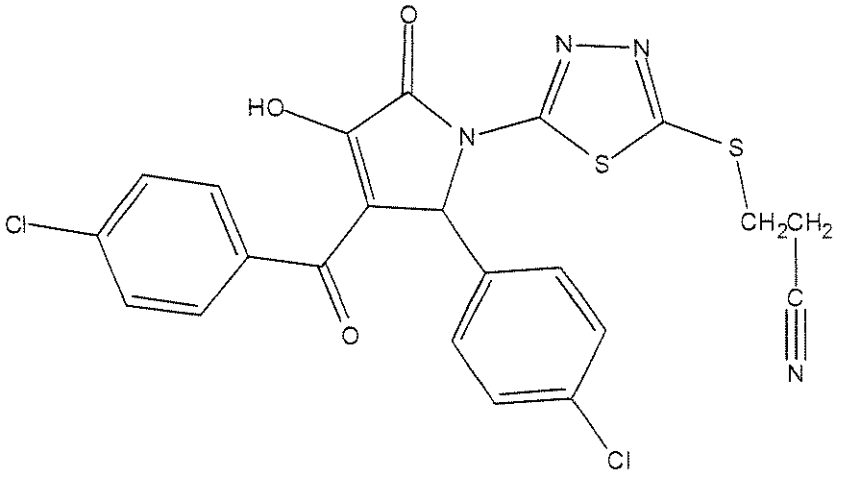
Cl
O
Cl

;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HO
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S
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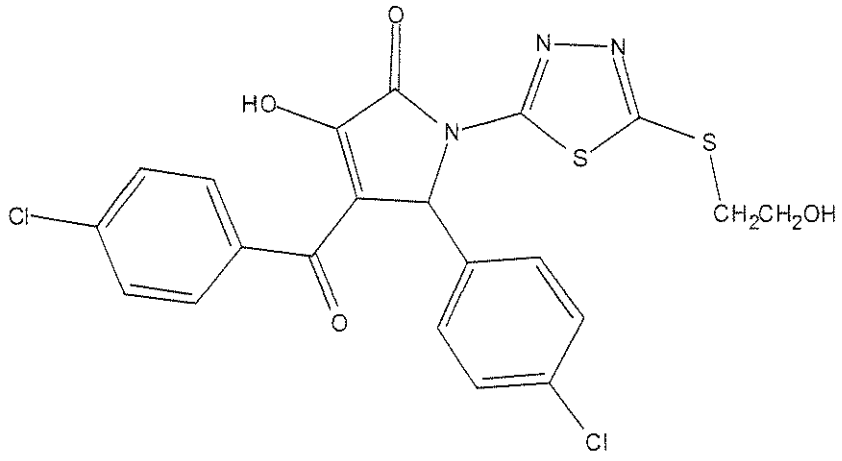
C
N
Cl
O
Cl

;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Cl

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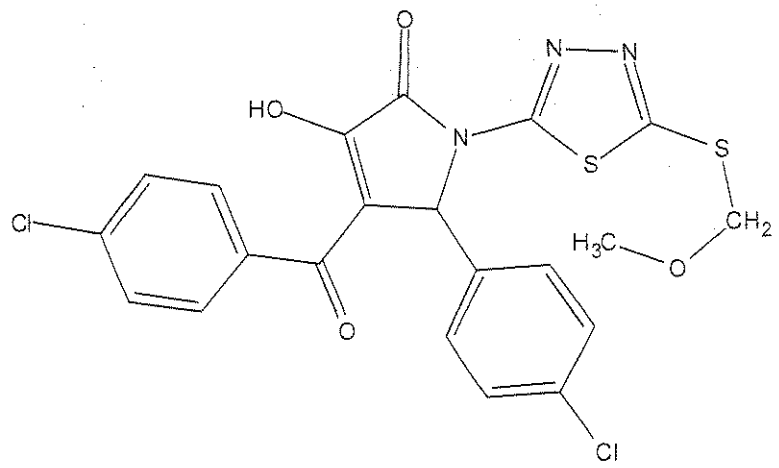
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Cl
O
Cl

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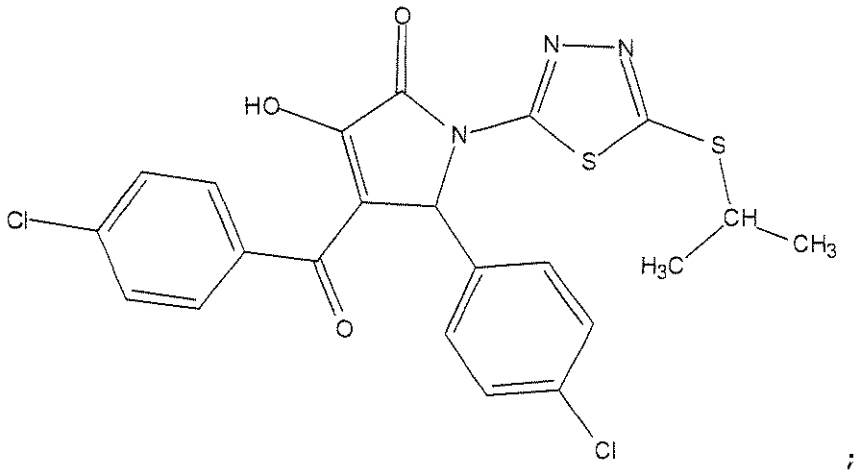
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HO
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S
CH
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H3C
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O
Cl

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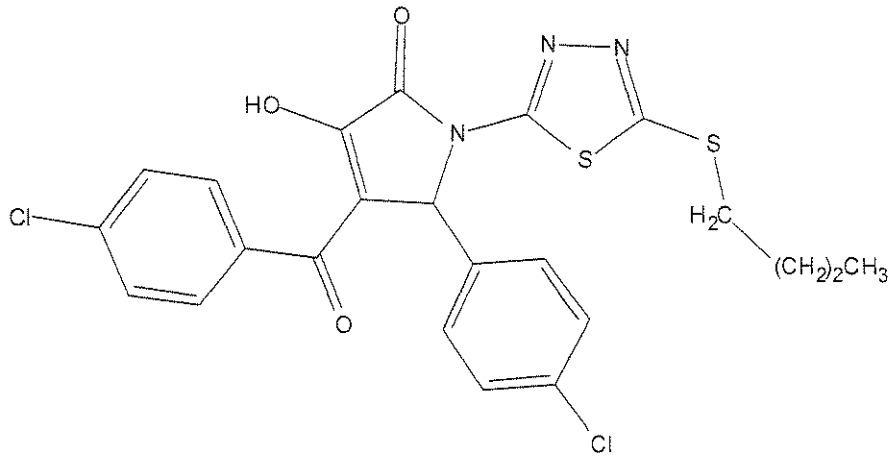
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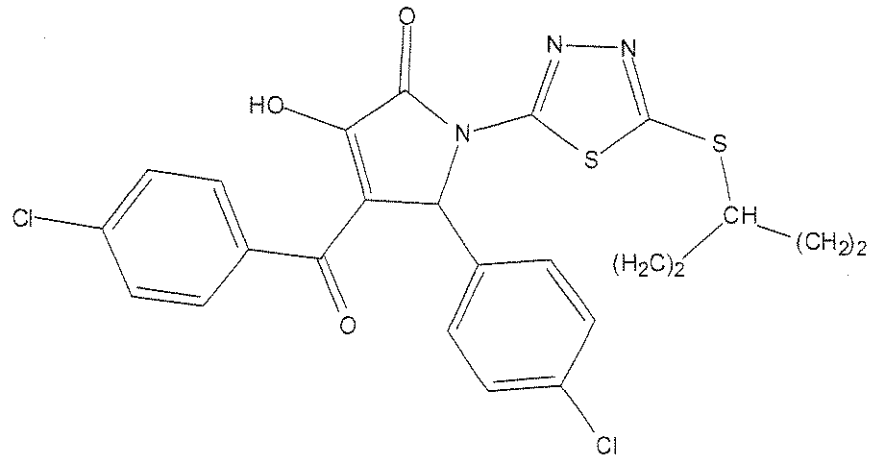
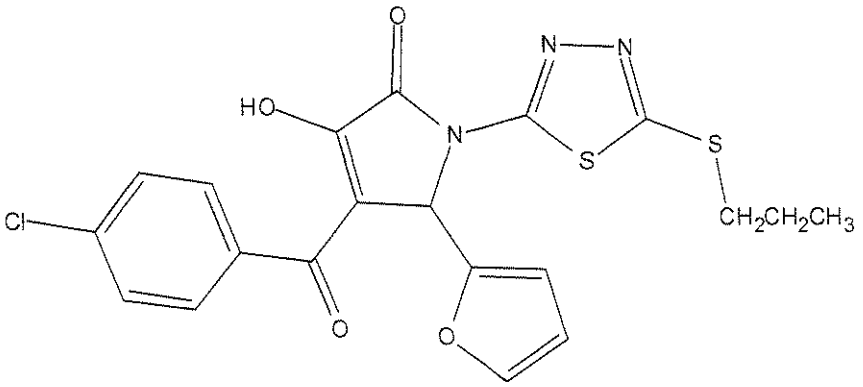
N
N
HO
N
S
S
H2C
(CH2)2CH3
Cl
O
Cl

;


O
N
N
HO
N
S
S
CH
(CH2)2
(H2C)2
Cl
O
Cl

; and

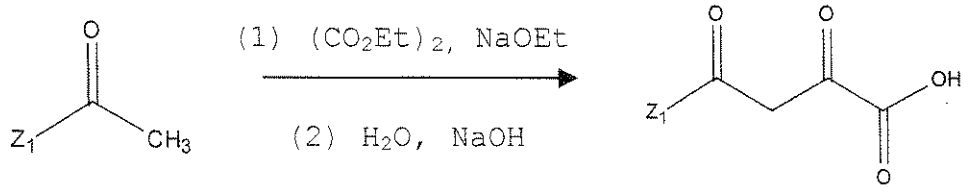


Since the disclosed active compounds may have asymmetric carbon centers, they can be present in the form of racemate, diastereomers or mixtures thereof. Therefore, the present invention also includes all these isomers and their mixtures.

Synthesis of Active Compounds

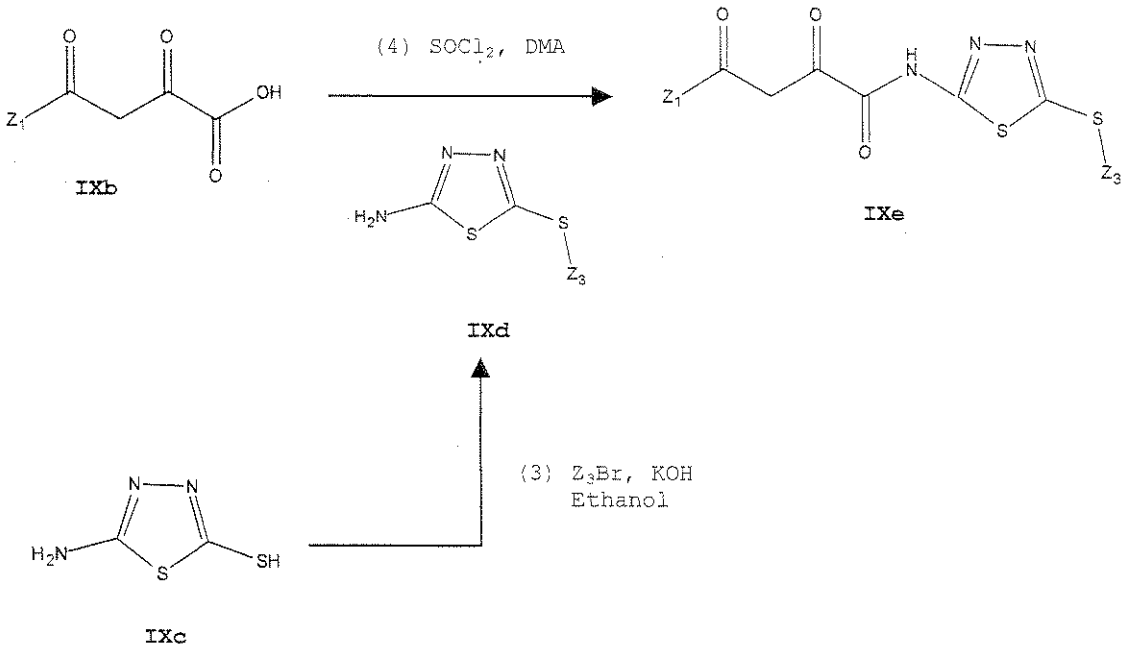
The disclosed active compounds having the general formula (I), wherein X_1 is N, Y_1 is S, X_2 is O when ------ is a double bond and OH when ------ is a single bond and Y_2 is S, may be prepared by a process described in the following schema.

A solution of a compound of formula IXa, wherein Z_1 is as described above, in anhydrous ethanol is added to diethyl oxalate and sodium ethoxide in ethanol in the reaction represented by (1). Sodium hydroxide and water are added to the mixture in the reaction represented by (2) to yield the compound of formula IXb:



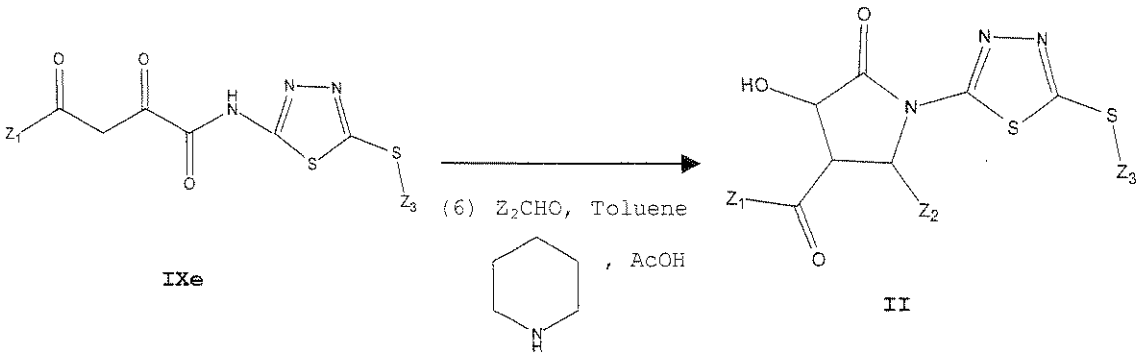
IXa **IXb**

In the reation represented by (3) below, 5-amino-1,3,4-thiadiazole-2-thiol having the formula IXc below is mixed with an alkyl halide (compound Z_3Br below, wherein Z_3 is as described above) and potassium hydroxide in ethanol to yield a compound of formula IXd. The compound of formula IXb from reaction (2) in dimethylacetamide (anhydrous) is reacted with thionyl chloride followed by the compound of formula IXd in the reaction represented by (4) below to form a compound having the formula IXe:



The compound of formula IXe is converted to the compound of formula II, wherein Z_1, Z_2 and Z_3 are as

described above, by dissolving the compound of formula IXe and an aldehyde having the formula Z_2CHO, wherein Z_2 is as described above, in toluene containing acetic acid and piperidine as catalyst as shown in the reaction represented by (5) below:



IXe

II

Active Compound Salts

In some forms, it may be desirable to formulate the active compounds in pharmaceutically acceptable salt form, generally to improve the solubility and bioavailability and to provide an active drug that may be capable of being assimilated readily. Preferably, the pharmaceutically acceptable salts are suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio.

The active compounds may form pharmaceutically acceptable salts with both organic and inorganic acids. Suitable physiologically tolerated acids for salt formation may be organic and inorganic acids, such as hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic, lactic,

gluconic, glucuronic, sulfamic, benzoic, tartaric, pamoic, and the like.

The salts may be prepared by contacting a free base form with an equivalent amount of the desired acid in the conventional manner. The free base forms may be regenerated by treating the salt form with a base. For example, dilute aqueous base solutions may be utilized. Dilute aqueous sodium hydroxide, potassium carbonate, ammonia, and sodium bicarbonate solutions may be suitable for this purpose.

The free base forms may differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvent. Otherwise, the salts may be equivalent to their respective free base forms for purposes of the invention.

The active compounds may exist in unsolvated as well as solvated forms, including hydrated forms. Such salt forms of the active compound may be provided or mixed prior to use with a physiologically acceptable solvent such as water or ethanol.

Pharmaceutically Acceptable Carriers

The active compounds disclosed herein may include a conventional pharmaceutical carrier or excipient, and in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc. Examples of suitable pharmaceutical carriers include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Other suitable pharmaceutical carriers are described in Remington's Pharmaceutical

Sciences (Mack Publishing Company), a standard reference text in this field, or in U.S. Pharmacopeia National Formulary, 1857-1853, (1990). Compositions comprising such carriers may be formulated by conventional methods.

Mode of Administration

Administration of the active compounds disclosed herein, in pure form or in an appropriate pharmaceutical composition, may be carried out via any of the acceptable modes of administration or pharmaceutically acceptable means of delivery. The modes of administration and pharmaceutically acceptable means of delivery may include oral administration or delivery in the form of solid, semi-solid, lyophilized powder, or liquid dosage forms. The dosage forms may include tablets, suppositories, pills, soft elastic and hard gelatin capsules, powders, solutions, suspensions, or aerosols, or the like, preferably in unit dosage forms suitable for simple administration of precise dosages.

Oral administration of the disclosed active compounds may be effected by preparing a mixture of the disclosed active compounds with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsules, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the disclosed active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations may contain the active

compounds in an amount by weight percent selected from the group consisting of about 0.1% to about 70%, about 0.5% to about 65%, about 1% to about 60%, about 2% to about 55% and about 3% to about 50%.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, such as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compounds sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavorings, such as cherry or orange flavor. Apparently, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

The active compounds disclosed herein may be administered parenterally or intraperitoneally. Solutions of the disclosed active compounds as free base or pharmacologically acceptable salts may be prepared in

water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use may include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms may be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Prolonged absorption of the injectable compositions can also be brought about by including in the composition an agent which delays absorption, for example, aluminium monostearate and gelatin.

Preferably, the pharmaceutical form may further include a suitable buffer to minimise acid hydrolysis. Suitable buffer agent agents are well known to those skilled in the art and include, but are not limited to, phosphates, citrates, carbonates and mixtures thereof.

The active compounds may also be administered in the form of a "prodrug". A prodrug is an inactive form of a compound which is transformed *in vivo* to the active form. Suitable prodrugs include esters, phosphonate esters etc, of the active form of the compound.

Administration Dosage and Regime

The active compounds in pharmaceutically acceptable form may be administered in a therapeutically effective amount which will vary depending upon a variety of factors including the activity of the specific compounds employed; the metabolic stability and length of action of the compounds; the age, body weight, general health, sex and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disease states; and the patient undergoing treatment.

Single or multiple administrations of the pharmaceutical compositions according to the invention may be carried out. One skilled in the art would be able, by routine experimentation, to determine effective, non-toxic dosage levels of the compound and/or composition of the invention and an administration regime which would be suitable for treating the diseases and/or infections to which the compounds and compositions are applicable.

It will be apparent to one of ordinary skill in the art that the optimal course of treatment, such as the number of doses of the compound or composition of the invention given per day for a defined number of days, can be ascertained using conventional course of treatment determination tests.

Generally, an effective dosage per 24 hours may be in the range of about 0.0001 mg to about 1000 mg per kg body weight; suitably, about 0.001 mg to about 750 mg per kg body weight; about 0.01 mg to about 500 mg per kg body weight; about 0.1 mg to about 500 mg per kg body weight; about 0.1 mg to about 250 mg per kg body weight; or about 1.0 mg to about 250 mg per kg body weight. More suitably, an effective dosage per 24 hours may be in the range of about 1.0 mg to about 200 mg per kg body weight; about 1.0 mg to about 100 mg per kg body weight; about 1.0 mg to about 50 mg per kg body weight; about 1.0 mg to about 25 mg per kg body weight; about 5.0 mg to about 50 mg per kg body weight; about 5.0 mg to about 20 mg per kg body weight; or about 5.0 mg to about 15 mg per kg body weight.

Alternatively, an effective dosage may be up to about 500 mg per kg body weight. For example, generally, an effective dosage is expected to be in the range of about 25 to about 500 mg per kg body weight, about 25 to about 350 mg per kg body weight, about 25 to about 300 mg per kg body weight, about 25 to about 250 mg per kg body weight, about 50 to about 250 mg per kg body weight, and about 75 to about 150 mg per kg body weight.

The active compounds of the invention may be used in combination with other known treatments or anti-viral

agents. Combinations of active agents, including the active compounds of the invention, may be synergistic.

Active Compounds as a Therapeutic Agent

It has been surprisingly found that the active compounds of general formula (I) may be capable of inhibiting the WNV and dengue virus NS3 protease. Hence, the disclosed active compounds may be capable of inhibiting the viral replication of the WNV and dengue virus. The active compounds may be envisaged as being useful in the treatment, prevention, inhibition or amelioration of one or more diseases associated with West Nile Virus and dengue virus replication in patients suffering from diseases caused by WNV and dengue virus infection. Diseases associated with West Nile Virus replication include, but are not limited to, fever, headache, fatigue, variable malaise, anorexia, nausea, myalgia, lymphadenopathy, a non-pruritic generalized maculopapular rash, hepatitis, pancreatitis, myocarditis, rhabdomyolysis, orchitis, ocular manifestations, meningitis, encephalitis, acute flacid paralysis (AFP)/poliomyelitis, Parkinsonism, pharyngitis, conjunctivitis, coma, and seizure/status epilepticus. Diseases associated with dengue virus replication include, but are not limited to non-specific febrile illness with rash, abrupt onset of high fever, severe headache, pain behind the eyes, muscle and joint pains, and dengue haemorrhagic fever (DHF).

Brief Description of Drawings

The accompanying drawings illustrate non-limiting embodiments of the disclosed active compounds and serve to explain the principles of the disclosed embodiments. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Figure 1 shows the proton and carbon NMR spectra for the compound of formula II' synthesized according to the protocol described in Example 1. Figure 1(a) represents the proton NMR spectrum and Figure 1(b) represents the carbon NMR spectrum.

Figure 2 shows the fluorescence readings plotted against the enzymatic reaction time for experiments on SPNUS 20 inhibition of the WNV NS3 protease at different concentrations of SPNUS 20. The fluorescence readings are represented as Relative Fluorescence Units (RFU). **D1** represents the RFU versus time profile for the positive control (in the absence of SPNUS 20). D2-D7 represent the RFU versus time profiles for SPNUS 20 inhibition of the WNV protease at various concentrations of SPNUS 20: at $1x10^{-3}$ mg/ml (1.63μM) (**D2**), at $1x10^{-2}$ mg/ml (16.3μM) (**D3**), at $3x10^{-2}$ mg/ml (48.9μM) (**D4**), at $6x10^{-2}$ mg/ml (97.8μM) (**D5**), at $1x10^{-1}$mg/ml (163.0μM) (**D6**), and at $2x10^{-1}$ mg/ml (326.0μM) (**D7**). **E2** represents the negative control.

Figure 3 shows a sigmoidal dose-response curve that illustrates the percentage inhibition of the WNV NS3 protease at various concentrations of SPNUS 20.

Figure 4 shows the fluorescence readings plotted against the enzymatic reaction time for experiments on SPNUS 21 inhibition of the WNV NS3 protease at different concentrations of SPNUS 21. The fluorescence readings are represented as Relative Fluorescence Units (RFU). **D1** represents the RFU versus time profile for the positive control (in the absence of SPNUS 21). D8-D12 represent the RFU versus time profiles for SPNUS 21 inhibition of the WNV protease at various concentrations of SPNUS 21: at $1x10^{-3}$ mg/ml (1.57μM) (**D8**), at $1x10^{-2}$ mg/ml (15.7μM) (**D9**), at $3x10^{-2}$ mg/ml (41.7μM) (**D10**), at $6x10^{-2}$ mg/ml (94.2μM) (**D11**), at $1x10^{-1}$ mg/ml (157.0μM) (**D12**), and at $2x10^{-1}$ mg/ml (314.0μM) (**E1**). **E2** represents the negative control.

Figure 5 shows a sigmoidal dose-response curve that illustrates the percentage inhibition of the WNV NS3 protease at various concentrations of SPNUS 21.

Examples

Non-limiting embodiments of the disclosed active compounds will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Preparation of Active Compounds

Example 1

***Reactions (1) and (2)*: Synthesis of 4-substituted-2,4-dioxo-butanoic acid (compound IXb)**

A solution of acetophenone (5 mmol) in 15 ml anhydrous ethanol was added to diethyl oxalate (10 mmol) and sodium ethoxide (21% solution in ethanol) (15 mmol), and the mixture was stirred at room temperature for 24 hours. 40 ml water and NaOH (20 mmol) were added to the mixture, which was further stirred for 4 hours. The mixture was then extracted three times with ethyl acetate (60 mL). The organic layer was discarded, the aqueous layer collected, and the pH adjusted to pH 1-2 with 4 M hydrochloric acid solution. The mixture was again extracted three times with ethyl acetate (60 mL). The aqueous layer was discarded, and the organic layer was collected and dried over sodium sulfate anhydrous, followed by evaporation under vacuum to provide a compound having the formula IXb. The yields ranged from 78% to 85%.

***Reaction (3)*: Synthesis of compound IXd**

A mixture of 5-amino-1,3,4-thiadiazole-2-thiol (compound IXc) (10 mmol), an alkyl halide (10 mmol) and potassium hydroxide (10 mmol) was dissovled in ethanol. The resulting solution was either stirred at room temperature or refluxed. The completion of the reaction was monitored by thin layer chromatography (TLC). The compound IXd was purified by column chromatography, using

an ethyl acetate and hexance elution system. The yields ranged from 88% to 93%.

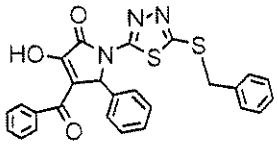
Reaction (4): **Synthesis of compound IXe**

The solution of compound IXb (3 mmol) from *Reactions (1)* and *(2)* in 5 ml dimethylacetamide (anhydrous) was stirred at 0-5°C for 10 mins. Thionyl chloride ($SOCl_2$, 3 mmol) was then added dropwise. Following stirring at 0-5°C for 15 mins, compound IXd (3 mmol) was added and stirred at room temperature for 2-4 hours. 40 ml cool water was added to the mixture to yield a precipitate which was then isolated by suction filtration. The isolated precipitate was further washed with water, followed by diethyl ether to yield pure compound IXe. The yields ranged from 80% to 87%.

Reaction (5): **Synthesis of compound II**

The compound IXe (1 mmol) and an aldehyde (1 mmol) were dissolved in toluene (6 ml) containing acetic acid (0.1 mmol) and piperidine (0.1 mmol) as catalyst. The solution was heated at 70°C overnight then cooled to room temperature. The precipitate was isolated by suction filtration and washed with diethyl ether to obtain compound II with yields ranging from 35% to 60%. Compound II with a purity of >95% was isolated by semi-preparative RP-HPLC for use in the bioassays of Examples 2 and 3.

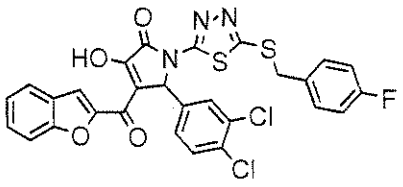
A representative compound having the formula II is as shown below as compound II':



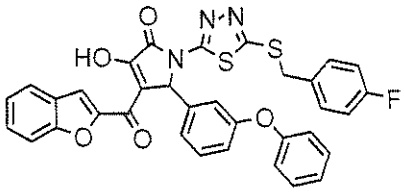
II'

The structure of compound II' above was confirmed using proton and carbon nuclear magnetic resonance (NMR) and mass spectrometry (MS). The spectra are shown in Figure 1.

Two derivative compounds prepared using the protocol above and tested for inhibitory activity on the WNV NS3 protease are:



SPNUS 20 (MW: 612.49)



SPNUS 21 (MW: 635.69)

Other derivative compounds having Z_1, Z_2 and Z_3 groups as shown in Table 1 below were also prepared using the above protocol and their respective molecular weights (MW) determined using NMR and MS.

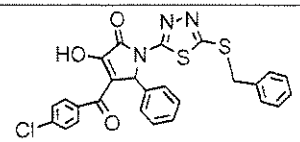
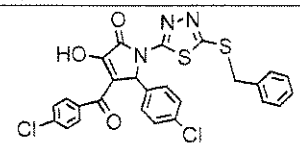
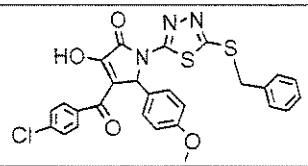
Table 1. Derivatives of compounds of formula II and their molecular weights (MW)

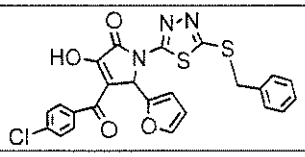
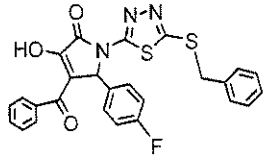
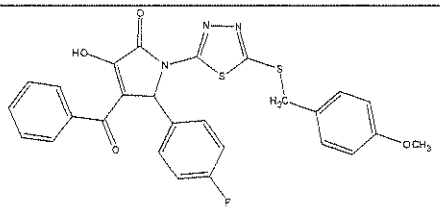
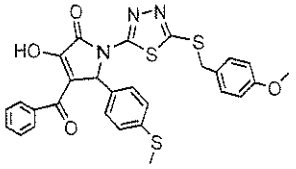
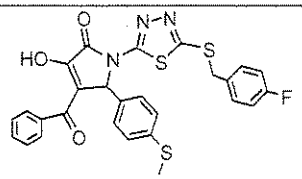
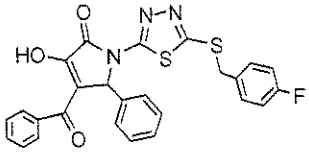
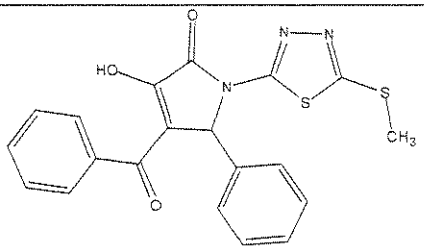
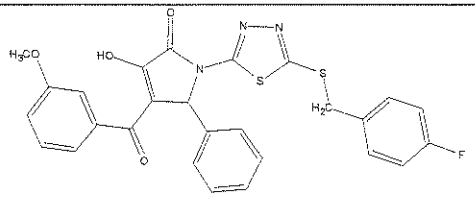
No.	Z_1	Z_2	Z_3	MW
1	4-Cl-Ph	Ph	$PhCH_2$	520.0
2	4-Cl-Ph	4-Cl-Ph	$PhCH_2$	553.5
3	4-Cl-Ph	4-MeO-Ph	$PhCH_2$	550.1
4	4-Cl-Ph		$PhCH_2$	509.9
5	Ph	4-F-Ph	$PhCH_2$	503.6
6	Ph	4-F-Ph	4-MeO-Ph CH_2	533.6
7	Ph	4-MeS-Ph	4-MeO-Ph CH_2	561.7

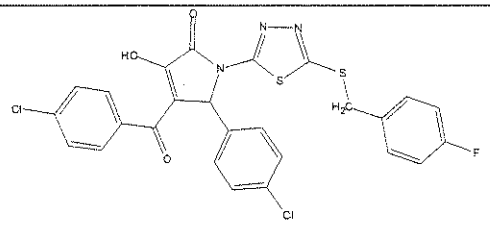
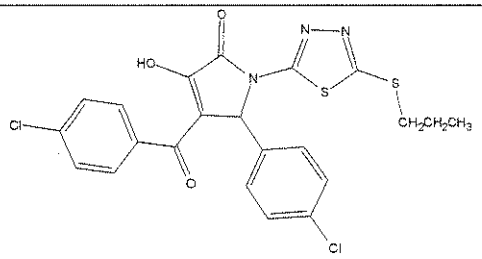
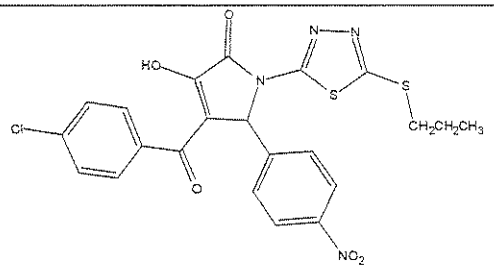
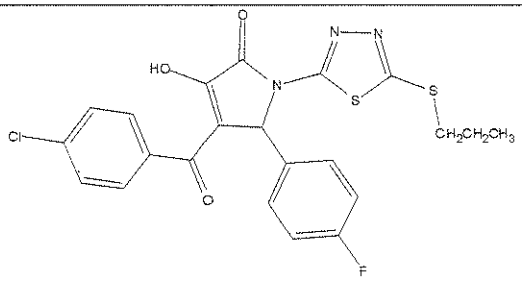
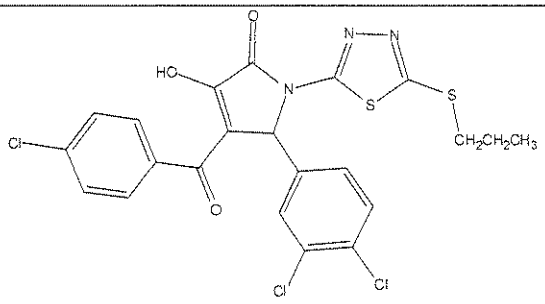
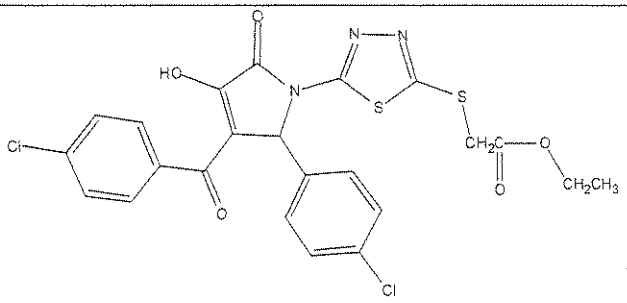
8	Ph	4-MeS-Ph	4-F-$PhCH_2$	549.7
9	Ph	Ph	4-F-$PhCH_2$	503.6
10	Ph	Ph	CH_3	409.5
11	3-MeO-Ph	Ph	4-F-$PhCH_2$	533.6
12	4-Cl-Ph	4-Cl-Ph	4-F-$PhCH_2$	606.9
13	4-Cl-Ph	4-Cl-Ph	$CH_2CH_2CH_3$	506.4
14	4-Cl-Ph	4-NO_2- Ph	$CH_2CH_2CH_3$	516.9
15	4-Cl-Ph	4-F-Ph	$CH_2CH_2CH_3$	489.7
16	4-Cl-Ph		$CH_2CH_2CH_3$	540.9
17	4-Cl-Ph	4-Cl-Ph	CH_2COOEt	550.4
18	4-Cl-Ph	4-Cl-Ph	CH_2COOH	522.4
19	4-Cl-Ph	4-Cl-Ph	CH_2CH_2CN	517.4
20	4-Cl-Ph	4-Cl-Ph	CH_2CH_2OH	508.4
21	4-Cl-Ph	4-Cl-Ph	CH_2OCH_3	508.4
22	4-Cl-Ph	4-Cl-Ph	$CH(CH_3)_2$	506.4
23	4-Cl-Ph	4-Cl-Ph	$CH_2(CH_2)_2CH_3$	534.5
24	4-Cl-Ph	4-Cl-Ph	$CH(CH_2)_4$	532.5
25	4-Cl-Ph		$CH_2CH_2CH_3$	461.9

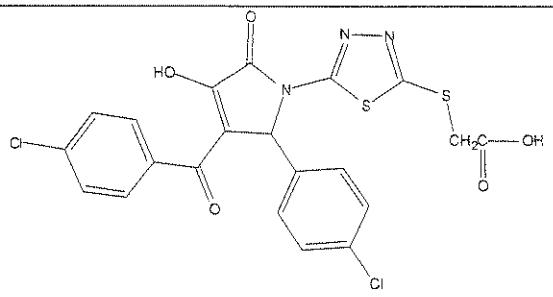
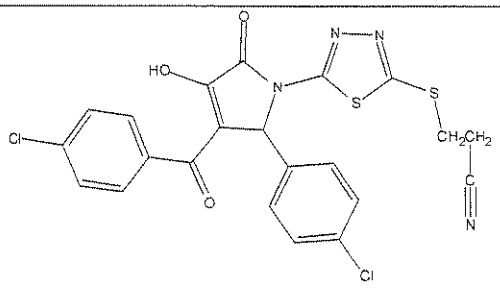
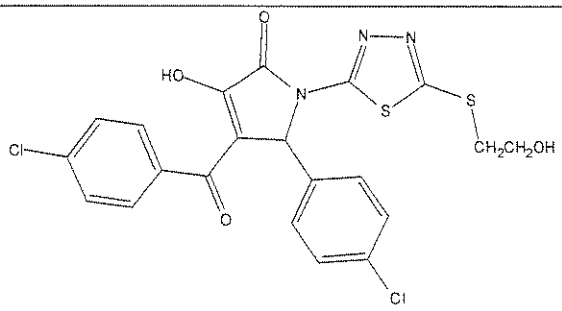
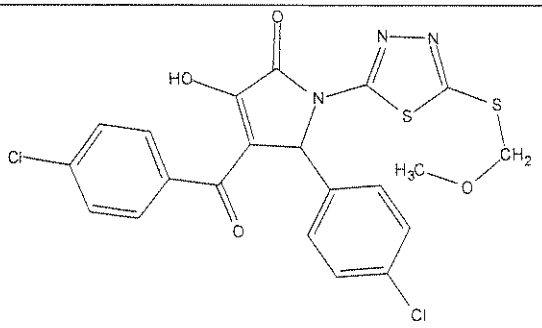
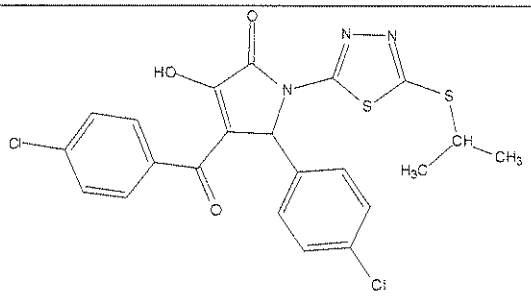
The structures of some of the above derivatives are shown in Table 2 below.

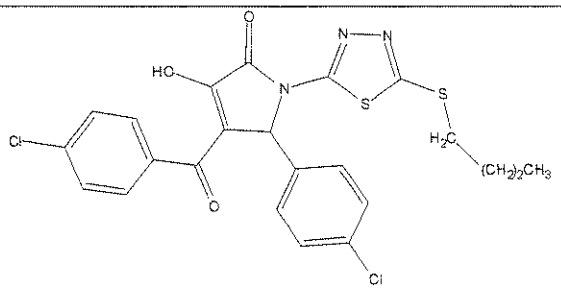
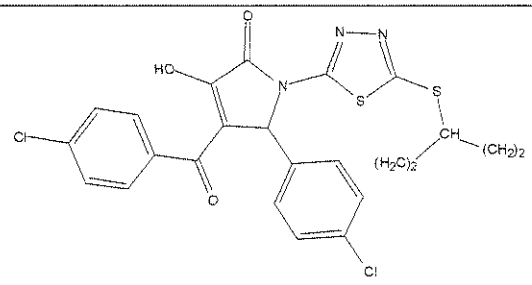
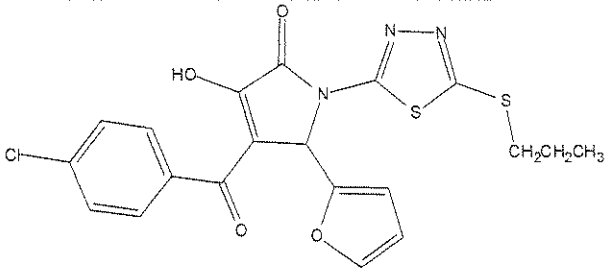
Table 2. Structures of derivatives of compounds of formula II

NO.	Structure	MW.
1		520.02
2		553.47
3		550.05

4		509.98
5		503.57
6		533.6
7		561.69
8		549.66
9		503.57
10		409.5
11		533.59

12		606.9
13		506.4
14		516.9
15		489.7
16		540.9
17		550.4

18		522.4
19		517.4
20		508.4
21		508.4
22		506.4

23		534.5
24		532.5
25		461.9

Protease Inhibition Studies

Example 2
Protocol for WNV Protease Inhibitory Assay

The inhibitory property of the disclosed active compounds, SPNUS 20 and SPNUS 21, was measured using a commercially available kit, SensoLyte™ 440 West Nile Virus Protease Assay Kit (AnaSpec, Inc., USA). The kit provides a homogenous assay for high throughput screening for compounds having inhibition activity on the WNV NS3 protease. The kit enables continuous quantification of protease activity using a fluorogenic peptide. This peptide, Pyr-RTKR-AMC, generates the 7-amino-4-methylcoumarin (AMC) fluorophore upon NS3 protease

cleavage. AMC has a bright blue fluorescence that can then be detected with excitation at a wavelength of 354 nm and emission at a wavelength of 442 nm.

The kit has the following components: WNV protease substrate (Component A), AMC fluorescence reference standard (Component B), assay buffer (Component C) and WNV protease inhibitor (Component D). Working solutions of the WNV protease substrate, diluent and inhibitor were prepared following the manufacturer's instructions. Briefly, a 1:100 dilution of the protease substrate (Component A) was prepared using the assay buffer (Component C). The working solution for the WNV protease inhibitor was prepared by diluting 1 mM of the WNV protease inhibitor (Component D) to 10 μM in the assay buffer (Component C).

Appropriate concentrations of a WNV protease were also prepared using the assay buffer (Component C). The WNV protease used was a recombinant WNV NS3 protease obtained from AnaSpec, Inc. (USA), Catalog #72081. A protease solution was prepared by diluting the WNV protease to 0.3 ng/μL in the assay buffer prior to use.

Different concentrations of a stock solution for the active compounds were prepared. For SPNUS 20 and SPNUS 21, the concentration ranged from $1x10^{-3}$ mg/mL to $2x10^{-1}$ mg/mL.

15 μL of the 10 μM WNV protease inhibitor working solution was added to each inhibitor control well of a 96-well microplate. Care was taken to ensure that the concentration of dimethylsulfoxide (DMSO), used as a vehicle for delivering the test compound, did not exceed 1%. To set up the enzymatic reactions, the test

compounds (SPNUS 20 and SPNUS 21) and the protease were added to wells on a 96-well microplate. For each well of the microplate, 85 μL of the protease solution and 15 μL of the test compound were added. The following controls were also set up:

- Positive Control: which contained the protease without the test compound
- Inhibitor Control: which contained the protease and protease inhibitor
- Vehicle Control: which contained the protease and vehicle used in delivering the test compound (the vehicle used was DMSO, with its concentration not exceeding 1%)
- Test Compound Control: which contained the assay buffer (Component C) and test compound
- Substrate Control: which contained the assay buffer (Component C)

The total volume of all the controls was made up to 100 μL using the assay buffer (Component C). Plates with or without test compounds were incubated for 10 minutes at 37°C. To initiate the enzymatic reaction, 50 μL of the WNV protease substrate solution at 37°C was added into each well except the test compound control wells. The reagents were mixed completely by shaking the plate gently for no more than 30 seconds.

To obtain kinetic readings, fluorescence intensity was immediately measured at Ex/Em = 354nm /442 nm continuously and data were recorded every 5 minutes for 30 to 60 minutes at 37°C.

The fluorescence reading from the substrate control well was the background fluorescence, and was subtracted from the other wells containing the substrate. The fluorescence readings are expressed in relative fluorescence units (RFU).

The data were plotted as RFU against enzymatic reaction time for both SPNUS 20 (Figure 2) and SPNUS 21 (Figure 4).

To determine percentage inhibition and IC_{50}, the initial reaction velocity (V_0) in RFU/min was determined. Initial reaction velocity was determined by calculating the slope value of the linear portion of the data plot (Figure 2 for SPNUS 20 and Figure 4 for SPNUS 21). The linear portion is typically the first 10-15% of the reaction. The slope value was calculated using the GraphPad prism software (GraphPad Software, Inc., La Jolla, USA).

Percentage inhibition was calculated with the following formula (Equation 1):

$$\frac{(V_o \text{ positive control} - V_{oA})}{V_o \text{ positive control}} \times 100\%$$

where V_{oA} is the slope value for the different concentrations of the test compound.

The percentage inhibition for each concentration of the test compound was then plotted against the normalized (Log) value of each concentration of the test compound to obtain a dose-response curve. The IC_{50} value for each test compound was determined from the dose-response curve using the variable slope fitting function of the GraphPad

prism software (GraphPad Software, Inc., La Jolla, USA). The IC_{50} value indicates the concentration of the test compound which inhibits 50% of the enzymatic activity. The formula (Equation 2) used for the slope fitting was:

$$Y = Bottom + (Top-Bottom)/(1+10^{((Log\ IC_{50}-X)*HillSlope)})$$

where the Bottom value is the baseline response, the Top value is the maximum response, X is the logarithm of concentration, and the HillSlope is the slope factor.

Example 3

SPNUS 20 Inhibitory Activity on the WNV NS3 Protease

Fluorescence readings from the enzymatic reactions with SPNUS 20 were plotted as RFU against enzymatic reaction time as shown in Figure 2. As discussed above, the slope of each plot represents the initial reaction velocity (V_o) of the WNV protease inhibition for each concentration of the compound SPNUS 20. The V_o value for each concentration of SPNUS 20 was calculated using the GraphPad prism software and is tabulated in Table 3 below.

Table 3. Initial reaction velocity (V_o) of WNV protease inhibition at various concentrations of the compound SPNUS 20.

Concentration of SPNUS 20	**V_o (RFU/min)**
Positive control	93.68

$1x10^{-3}$ mg/ml (1.63 μM)	91.5
$1x10^{-2}$ mg/ml (16.3 μM)	90.3
$3x10^{-2}$ mg/ml (48.9 μM)	13.27
$6x10^{-2}$ mg/ml (97.8 μM)	2.471
$1x10^{-1}$ mg/ml (163.0 μM)	0.3852
$2x10^{-1}$ mg/ml (326.0 μM)	3.083
Negative control	0.9001

From the V_o values in Table 3, percentage inhibition by SPNUS 20 was determined using Equation 1 and tabulated in Table 4 below. These inhibition percentages were then used to plot a dose-response curve of SPNUS 20 inhibition of WNV protease as shown in Figure 3. The dose-response curve is sigmoidal. From this curve, the IC_{50} value of SPNUS 20 was determined to be 34.7 μM, which indicates strong inhibitory activity.

Table 4. Percentage inhibition of WNV protease by SPNUS 20 as compared with positive control

SPNUS 20(MW: 612.49)				
mg/ml	**nM**	**Log (nM)**	**V_o**	**Inhibition(%)**
$1x10^{-3}$	1630	3.212188	91.5	2.32707088
$1x10^{-2}$	16300	4.212188	90.3	3.608027327
$3x10^{-2}$	48900	4.689309	13.27	85.83475662
$6x10^{-2}$	97800	4.990339	2.471	97.36229718
$1x10^{-1}$	163000	5.212188	0.3852	99.58881298
$2x10^{-1}$	326000	5.513218	3.083	96.70900939

Example 4

SPNUS 21 Inhibitory Activity on the WNV NS3 Protease

Fluorescence readings from the enzymatic reactions with SPNUS 21 were plotted as RFU against enzymatic reaction time as shown in Figure 4. As discussed above, the slope of each plot represents the initial reaction velocity (V_o) of the WNV protease inhibition for each concentration of the compound SPNUS 21. The V_o value for each concentration of SPNUS 21 was calculated using the GraphPad prism software and is tabulated in Table 5 below.

Table 5. Initial reaction velocity (V_o) of WNV protease inhibition by various concentrations of the compound SPNUS 21.

Concentration of SPNUS 21	**V_o (RFU/min)**
Positive control	93.68
$1x10^{-3}$mg/ml(1.57uM)	92.75
$1x10^{-2}$mg/ml(15.7uM)	88.04
$3x10^{-2}$mg/ml(41.7uM)	30.15
$6x10^{-2}$mg/ml(94.2uM)	10.84
$1x10^{-1}$mg/ml(157.0uM)	4.286
$2x10^{-1}$mg/ml(314.0uM)	0.7481
Negative control	0.9001

From the V_o values in Table 5, percentage inhibition by SPNUS 21 was determined using Equation 1 and tabulated in Table 6 below. These inhibition percentages were then

used to plot a dose-response curve of SPNUS 21 inhibition of WNV protease as shown in Figure 5. The dose-response curve is sigmoidal. From this curve, the IC_{50} value of SPNUS 20 was determined to be 34.7 μM, which indicates strong inhibitory activity.

Table 6. Percentage inhibition of WNV protease by SPNUS 21 as compared with the positive control

SPNUS 21(MW: 635.69)				
mg/ml	**nM**	**Log (nM)**	**V_o**	**Inhibition(%)**
$1x10^{-3}$	1570	3.1959	92.75	0.992741247
$1x10^{-2}$	15700	4.1959	88.04	6.020495303
$3x10^{-2}$	47100	4.673021	30.15	67.81596926
$6x10^{-2}$	94200	4.974051	10.84	88.42869342
$1x10^{-1}$	157000	5.1959	4.286	95.42485056
$2x10^{-1}$	314000	5.49693	0.7481	99.2014304

Both SPNUS 20 and SPNUS 21 showed strong inhibitory activity on the WNV NS3 protease, thus reflecting the potential of these compounds for use as an anti-viral agent for treating WNV infection and disease by inhibiting viral replication.

Applications

The active compounds disclosed herein have demonstrated strong inhibitory activity on the WNV NS3 protease. WNV NS3 protein is important in the replication of the WNV. Advantageously, by inhibiting WNV NS3 protease activity, the disclosed compounds are useful for inhibiting WNV replication and disease onset and/or progression. Accordingly, the disclosed active compounds are useful as medicines to treat a patient suffering from

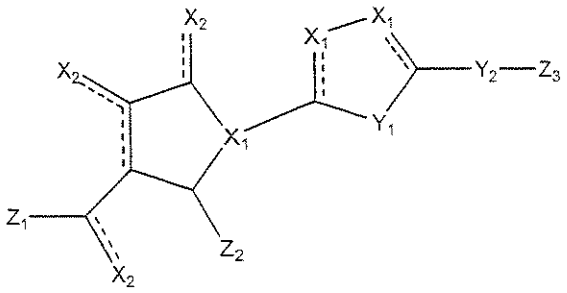
WNV infection and related diseases. The disclosed active compounds have very low toxicity to humans and therefore are ideal for use in medicine, particularly for use in medicines for treating patients suffering from WNV infections and related diseases.

Advantageously, the active compounds disclosed herein can also be used to inhibit the dengue virus NS3 protease because of the structural similarity between the dengue virus and WNV NS3 proteases. Hence, the active compounds disclosed herein are expected to be useful as medicines to treat a patient suffering from dengue virus infection and related diseases.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A compound having the general formula (I)



(I)

and the pharmaceutically acceptable salts thereof, wherein

$\overset{\text{------}}{\text{———}}$ is an optional double bond;

X_1 and Y_1 are heteroatoms;

Y_2 is optional and is a heteroatom;

X_2 is independently selected from oxygen (O), nitrogen (N) and sulfur (S) when $\overset{\text{------}}{\text{———}}$ is a double bond and is independently selected from a hydroxyl (-OH), thiol (-SH) when $\overset{\text{------}}{\text{———}}$ is a single bond; and

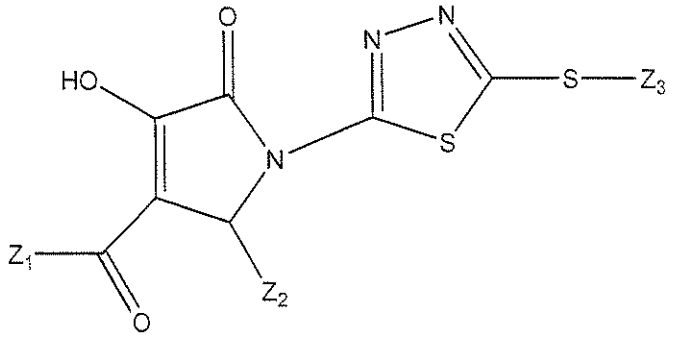
Z_1, Z_2 and Z_3 are independently selected from the group consisting of optionally substituted aromatic ring group, heterocyclic group, straight or branched chain alkyl group, alkenyl group, alkynyl group, hydroxyl group, alcohols, straight or branched chain alkoxy group, cyano group, carboxyl group and ester group.

2. A compound as claimed in claim 1, wherein the heteroatom X_1 and Y_1 are independently selected from nitrogen (N), sulfur (S) and phosphorous (P).

3. A compound as claimed in claim 2, wherein X_1 is nitrogen (N).

4. A compound as claimed in claim 2, wherein Y_1 is sulfur (S).

5. A compound as claimed in claim 1, wherein the compound has the general formula (II):



(II)

6. A compound as claimed in claim 1, wherein the optionally substituted aromatic group of Z_1, Z_2 and Z_3 comprises an aryl group.

7. A compound as claimed in claim 6, wherein Z_1, Z_2 and Z_3 are independently a 5 to 7-membered ring aryl group.

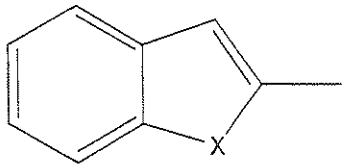
8. A compound as claimed in claim 7, wherein the aryl group of Z_1, Z_2 and Z_3 comprise a phenyl ring or naphthalene ring.

9. A compound as claimed in claim 1, wherein the heterocyclic group of Z_1, Z_2 and Z_3 comprises a 4 to 6-membered ring.

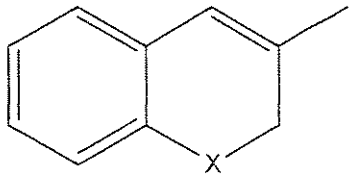
10. A compound as claimed in claim 9, wherein the heterocyclic group of Z_1, Z_2 and Z_3 is a 5-membered ring comprising an oxygen atom.

11. A compound as claimed in claim 1, wherein Z_1, Z_2 and Z_3 comprise a heterocyclic group fused to an aryl group.

12. A compound as claimed in claim 11, wherein Z_1 comprises the heterocyclic group fused to the aryl group having one of the following structures:



; and



;

wherein X is O, S or NH.

13. A compound as claimed in claim 12, wherein X is oxygen (O).

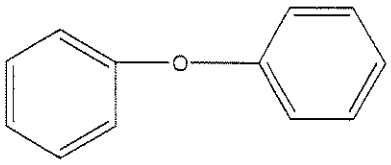
14. A compound as claimed in claim 1, wherein Z_1, Z_2 and Z_3 comprise an aryl group substituted with at least one of an oxygen (O) atom, a sulfur (S) atom and a halide atom.

15. A compound as claimed in claim 14, wherein Z_2 is substituted with at least one of a fluoride (F) and a chloride (Cl).

16. A compound as claimed in claim 14, wherein Z_2 is substituted with two or more halides selected from the group of fluoride (F) and chloride (Cl).

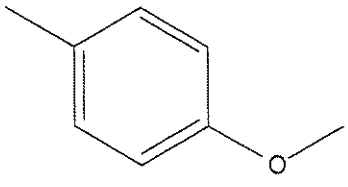
17. A compound as claimed in claim 1, wherein Z_1, Z_2 and Z_3 comprise two aryl groups linked by a heteroatom.

18. A compound as claimed in claim 17, wherein Z_2 has the structure (III):



(III)

19. A compound as claimed in claim 1, wherein Z_2 has one of the following structures:



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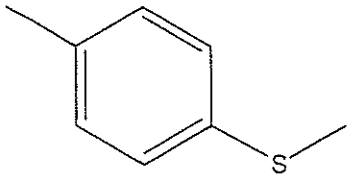

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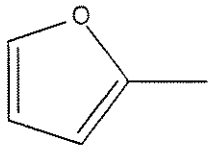

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20. A compound as claimed in claim 1, wherein Z_3 is a halide substituted aryl.

21. A compound as claimed in claim 1, wherein Z_3 is an aryl substituted with an oxygen atom.

22. A compound as claimed in claim 1, wherein Z_3 is an aryl substituted with an epoxide group.

23. A compound as claimed in claim 1, wherein Z_3 is a phenyl substituted with a fluoride (F) atom.

24. A compound as claimed in claim 1, wherein Z_3 is selected from the group consisting of lower alkyl, lower alkenyl, lower alkynyl, and lower alkoxy.

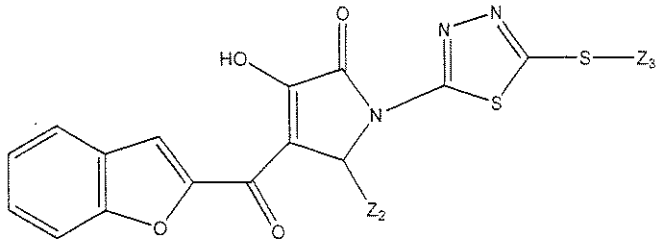
25. A compound as claimed in claim 1, wherein Z_3 is a hydroxyl group substituted with a methyl, ethyl or propyl group.

26. A compound as claimed in claim 1, wherein Z_3 is a cyano group substituted with a methyl, ethyl or propyl group.

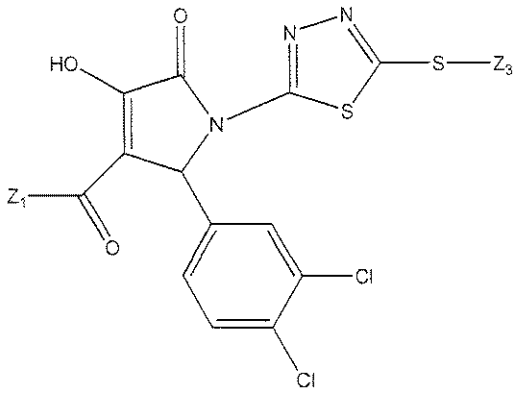
27. A compound as claimed in claim 1, wherein Z_3 is a carboxyl group substituted with a methyl, ethyl or propyl group.

28. A compound as claimed in claim 1, wherein Z_3 is an ester group substituted with a methyl, ethyl or propyl group.

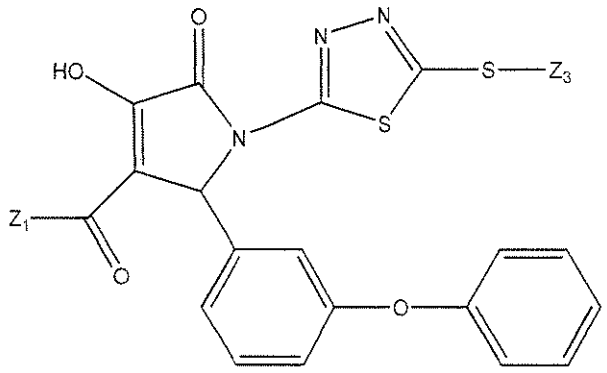
29. A compound as claimed in claim 1, having the structure selected from the following group:



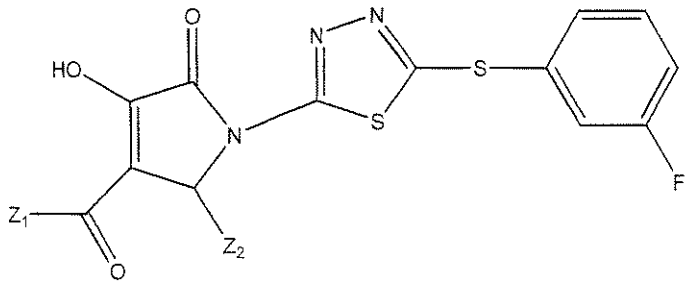
(IV) ;



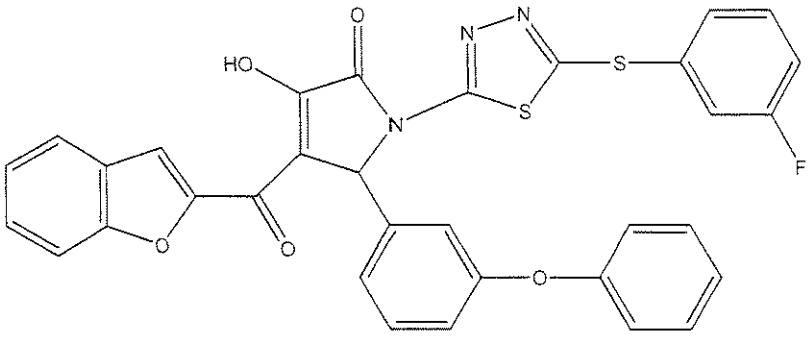
(V) ;



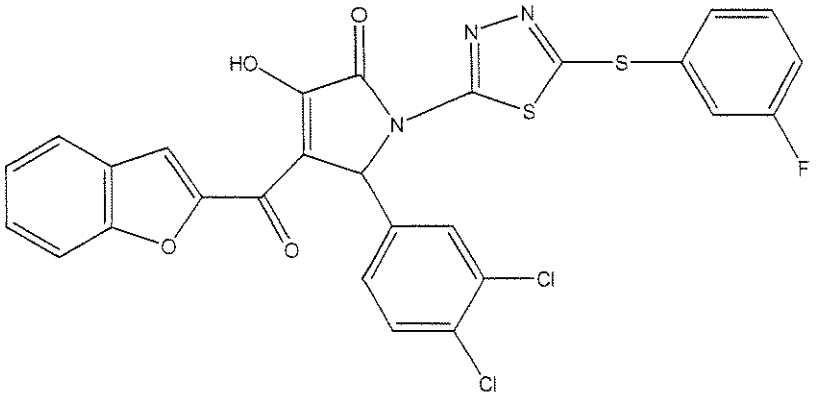
(VI) ;



(VII) ;

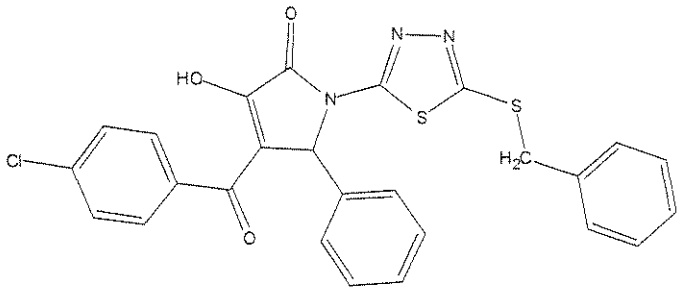


(VIII) ; and



(IX)

30. A compound as claimed in claim 1, having the structure selected from the following group:



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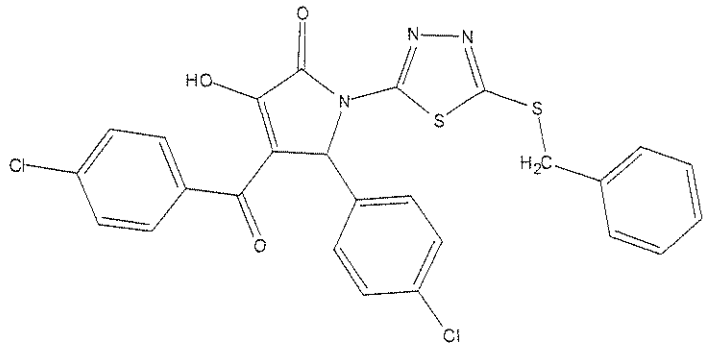

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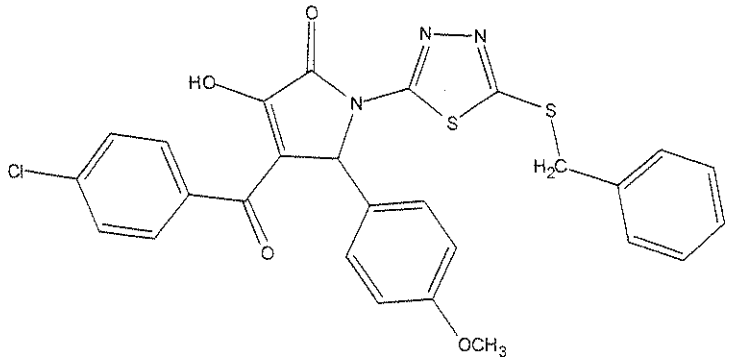
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S
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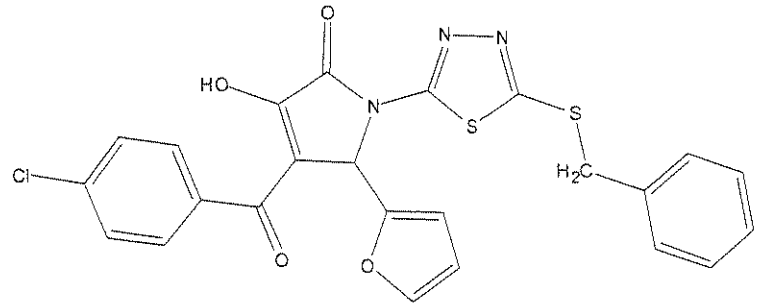
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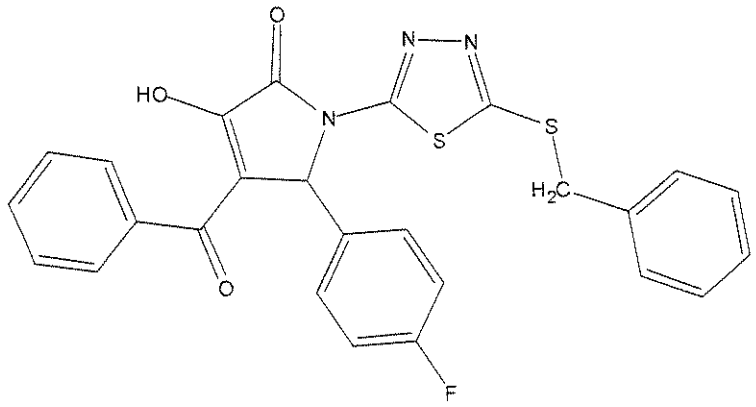
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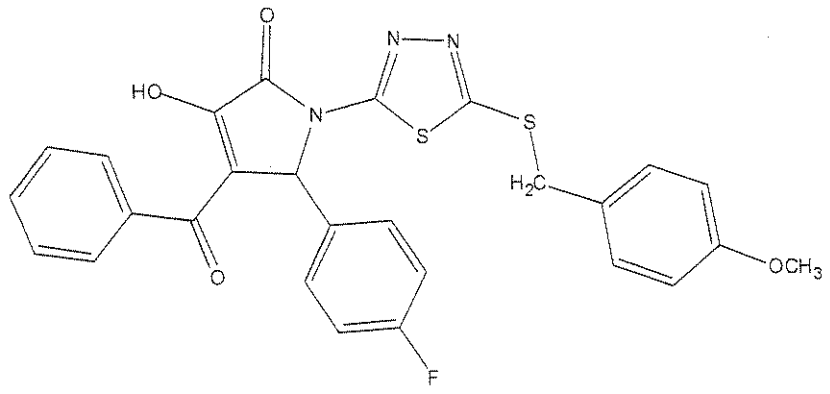
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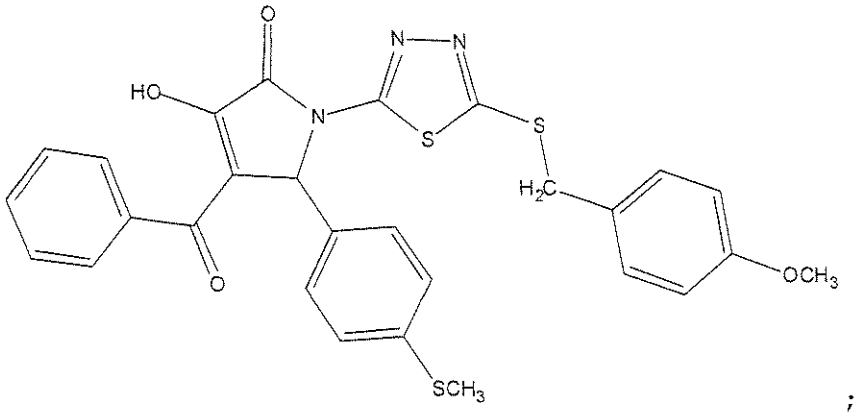
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SCH_3

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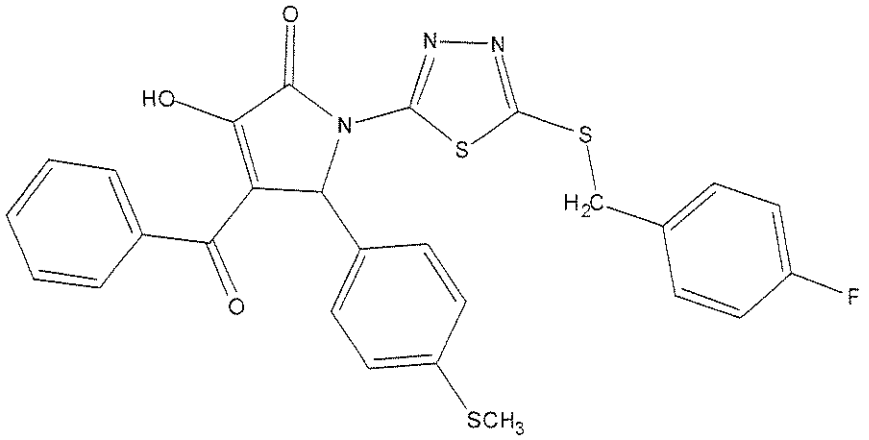
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SCH_3

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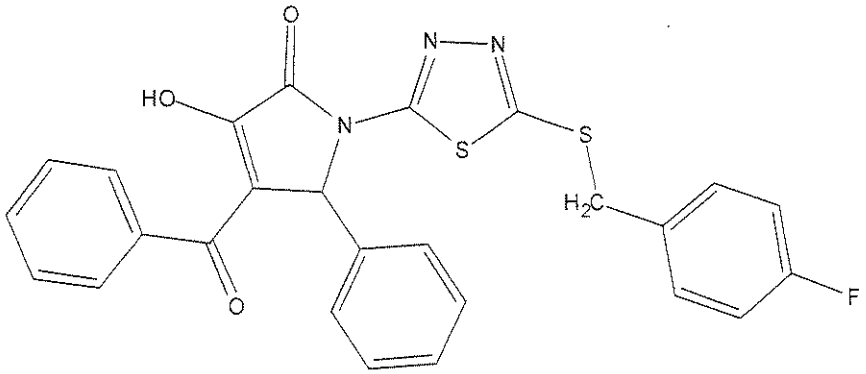
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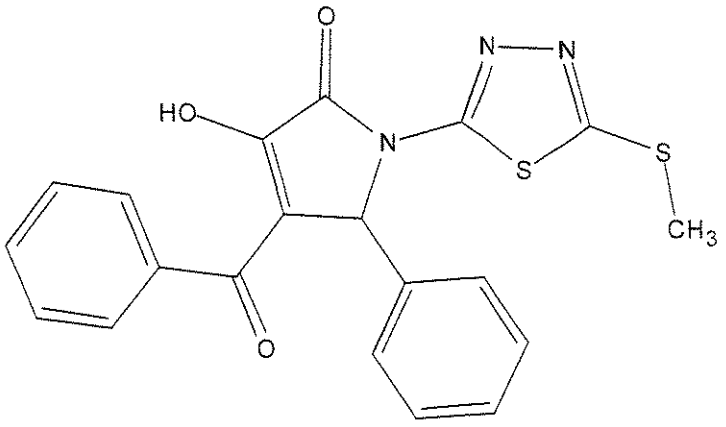
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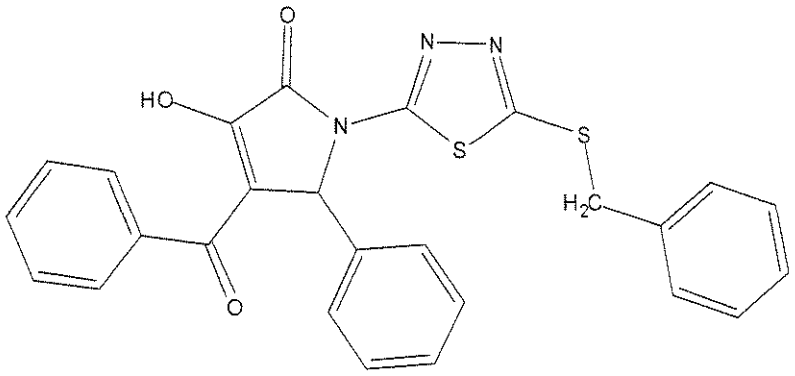
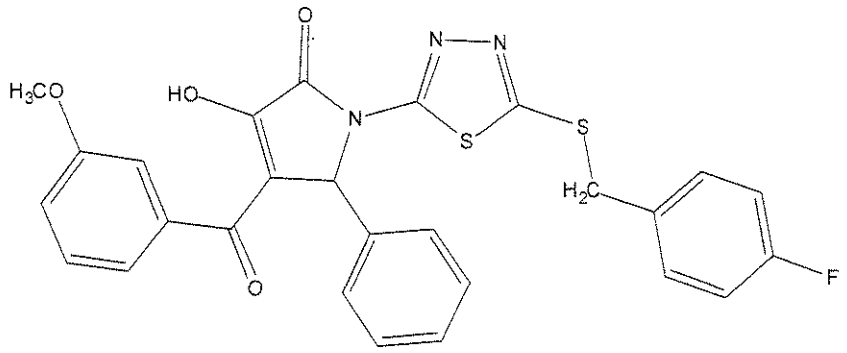
O

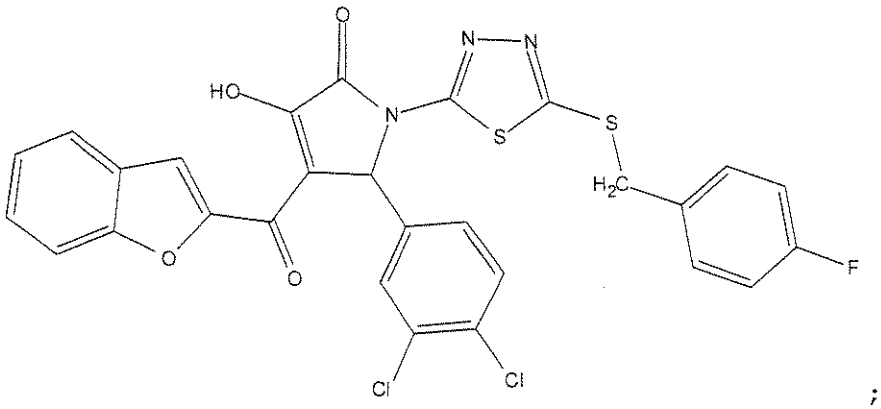
;


O
HO
N
N
N
S
S
H2C
O

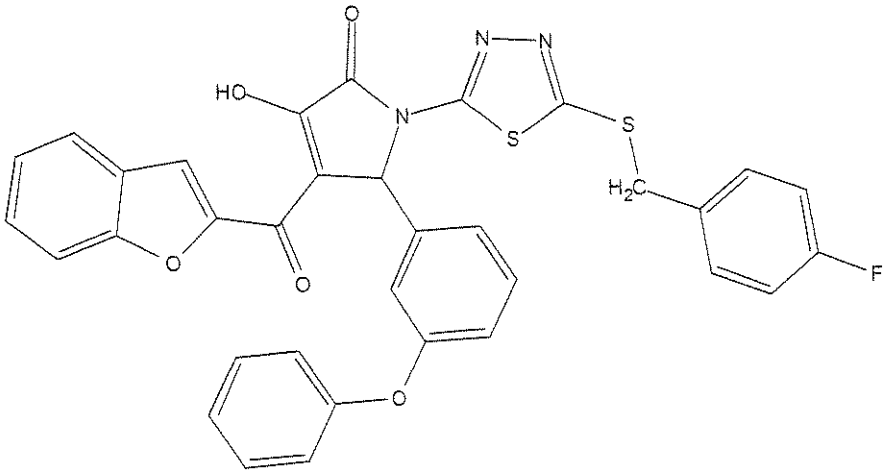
;


O
H3CO
HO
N
N
N
S
S
H2C
F
O

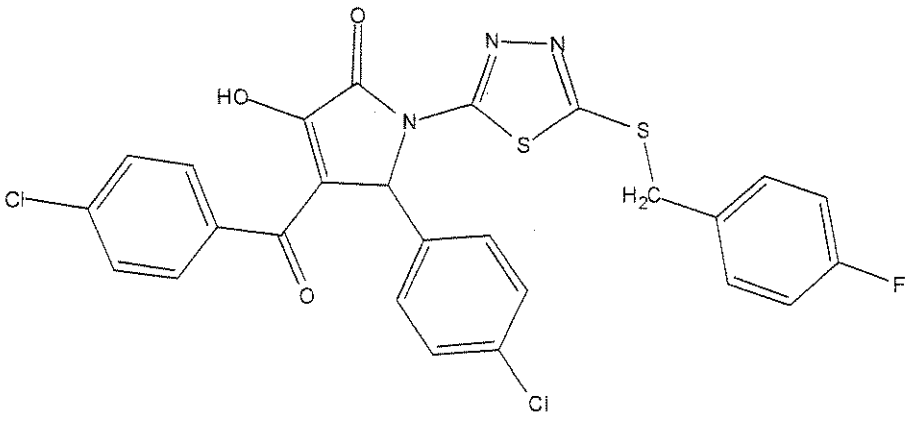
;


O
HO
N
N
N
S
S
H2C
F
O
O
Cl
Cl

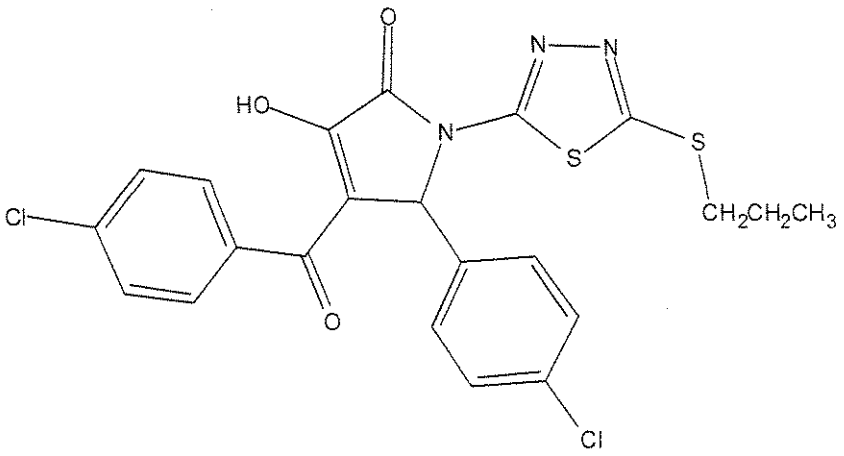
;


O
HO
N
N
N
S
S
H2C
F
O
O
O

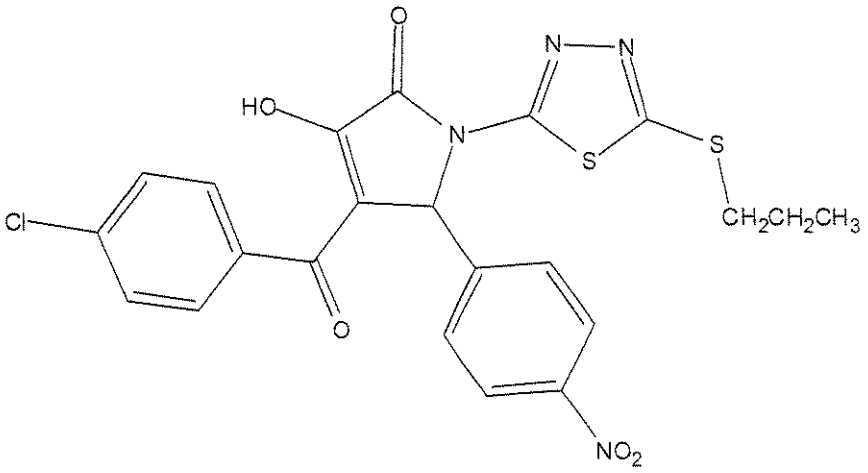
;


O
HO
N
N
N
S
S
H2C
F
Cl
O
Cl

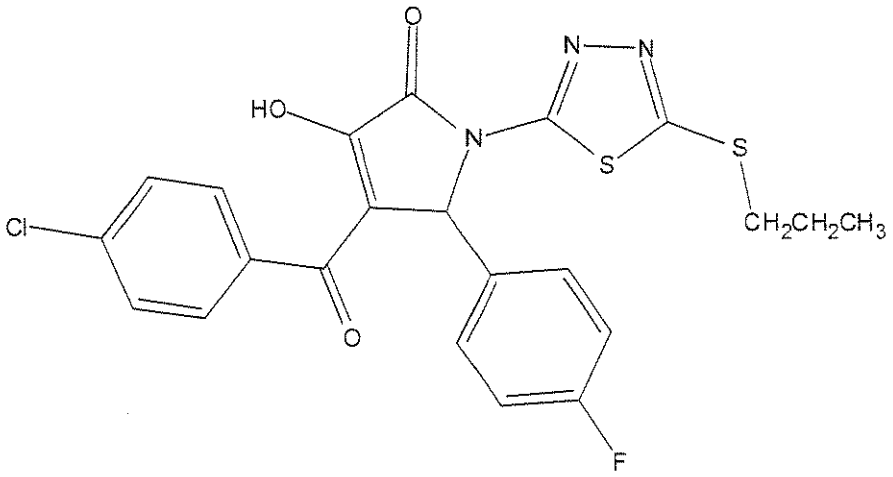
;


O
N
N
HO
N
S
S
CH2CH2CH3
Cl
O
Cl

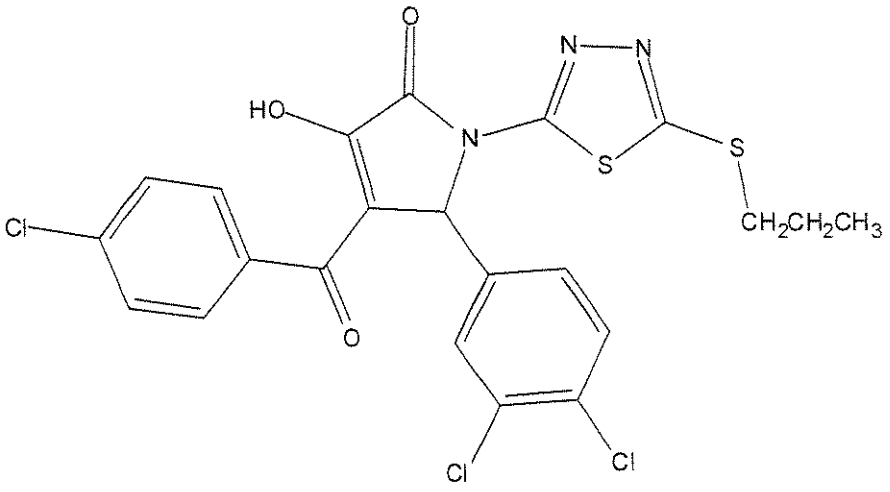
;


O
N
N
HO
N
S
S
CH2CH2CH3
Cl
O
NO2

;


O
N
N
HO
N
S
S
CH2CH2CH3
Cl
O
F

;



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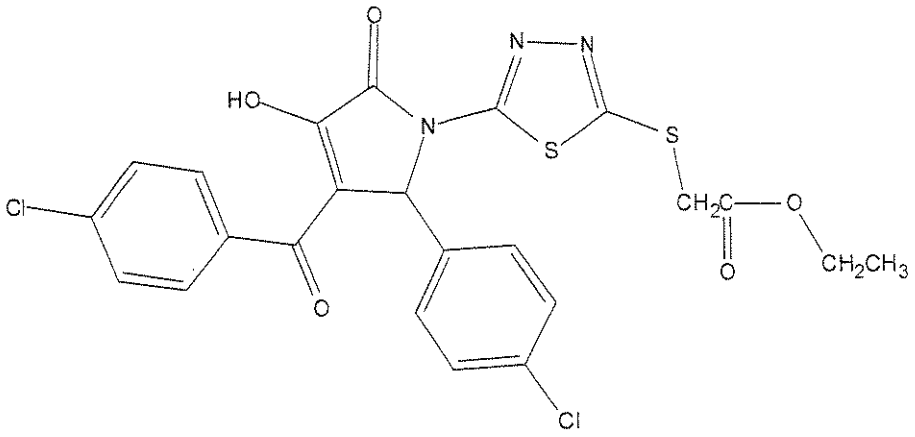

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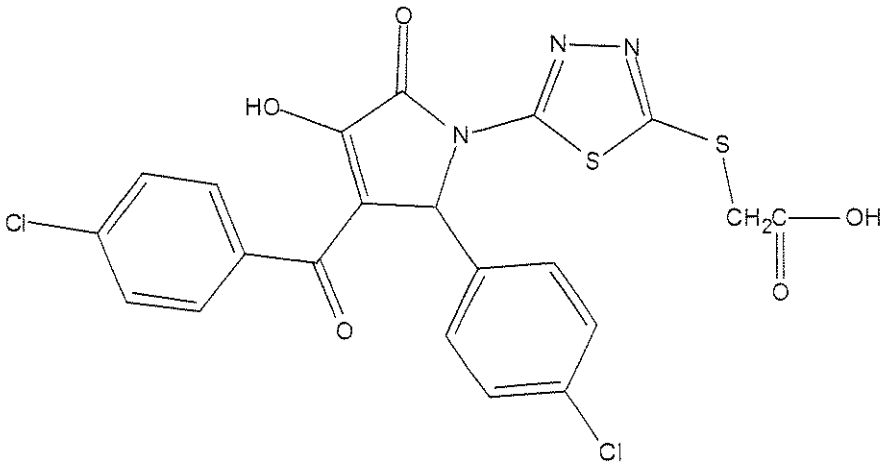
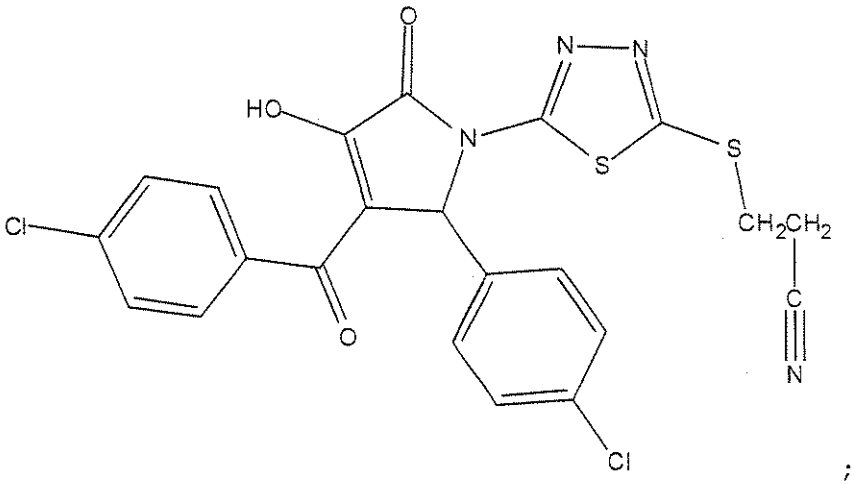
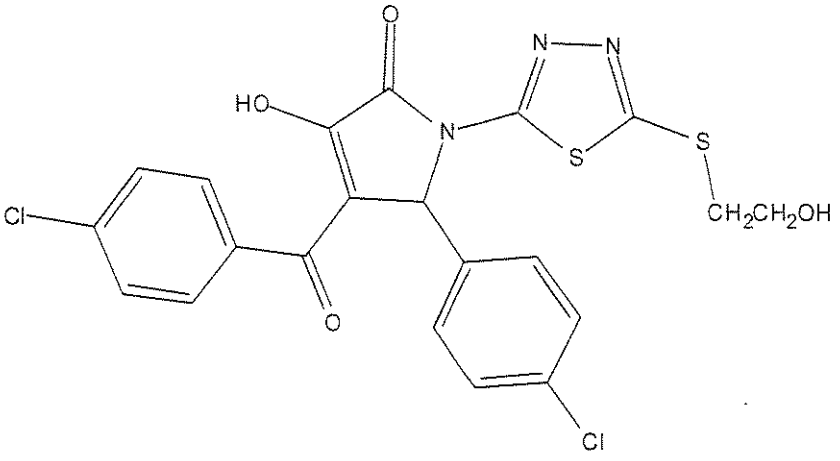


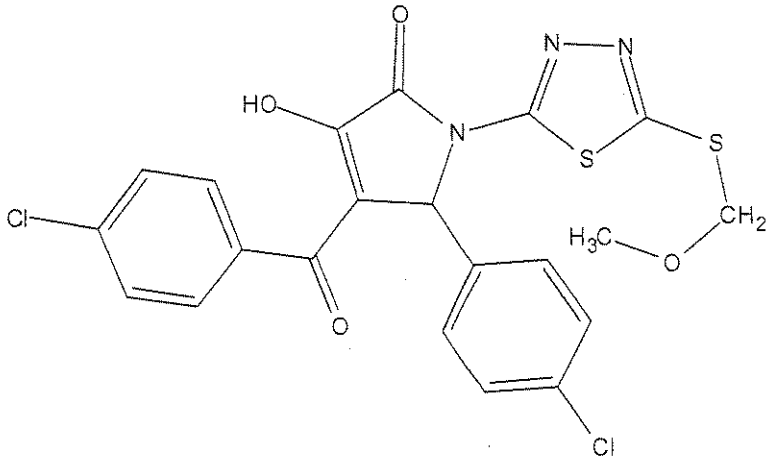
;


O
N
N
HO
N
S
S
CH2CH2
C
N
Cl
O
Cl

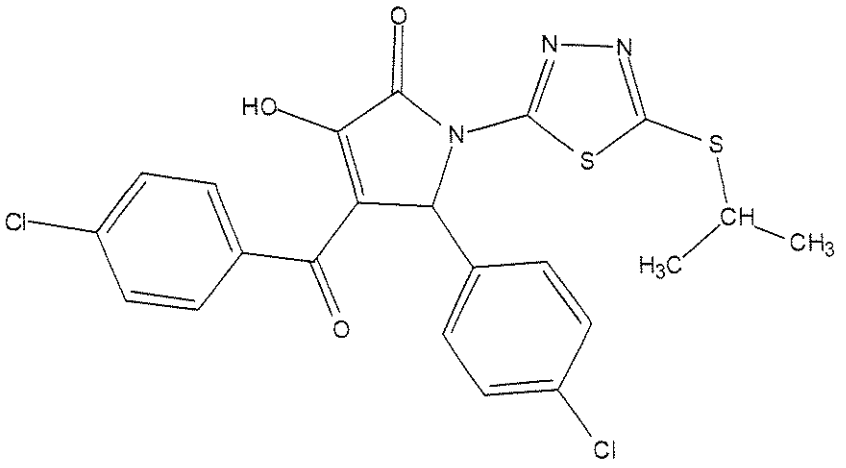
;


O
N
N
HO
N
S
S
CH2CH2OH
Cl
O
Cl

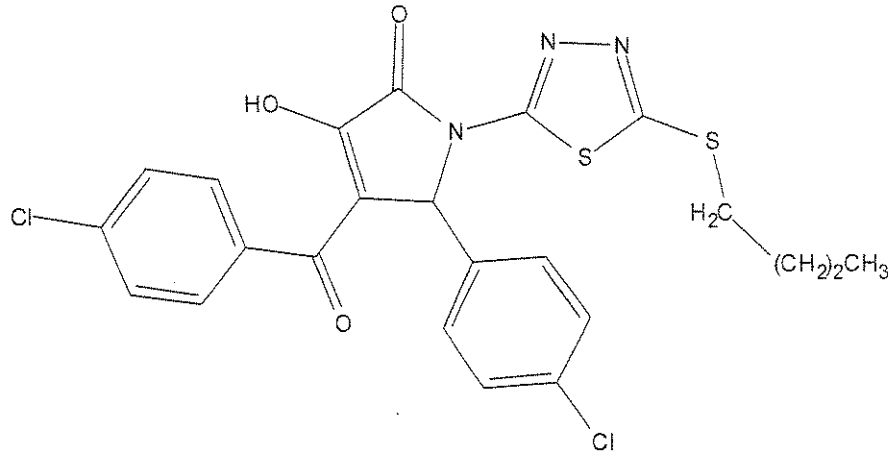
;


O
N
N
HO
N
S
S
CH2
H3C
O
Cl
O
Cl

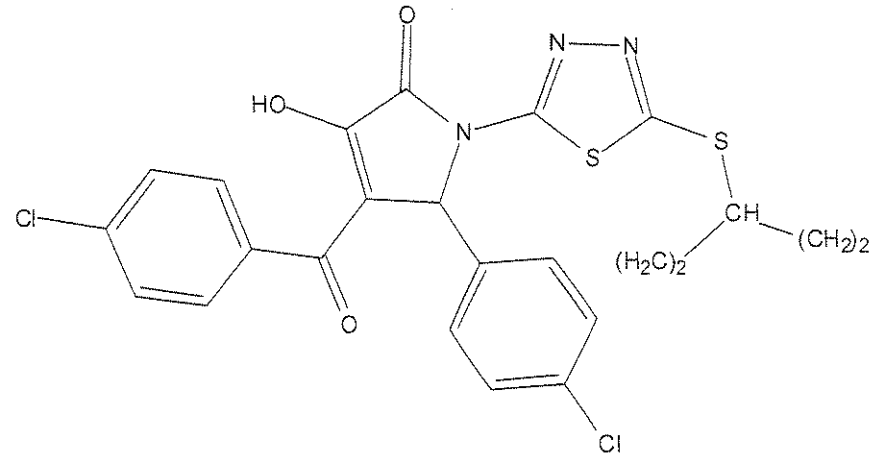
;


O
N
N
HO
N
S
S
CH
CH3
H3C
Cl
O
Cl

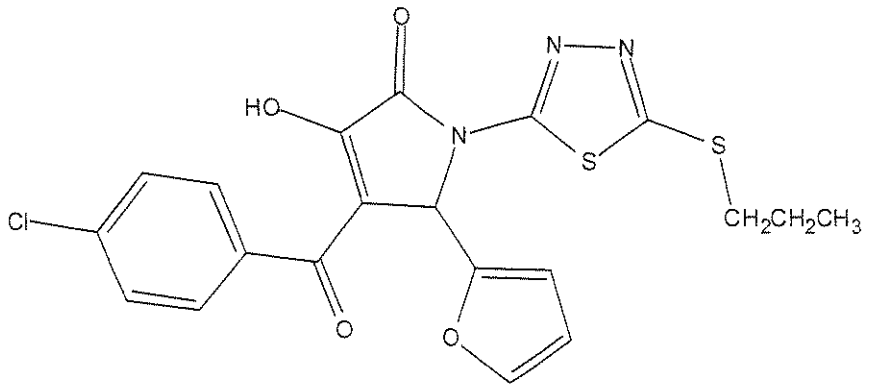
;


O
N
N
HO
N
S
S
H2C
(CH2)2CH3
Cl
O
Cl

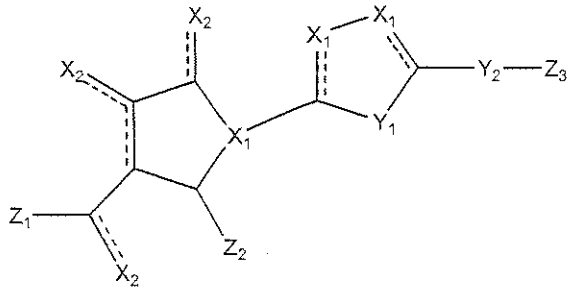
;


O
N
N
HO
N
S
S
CH
(CH2)2
(H2C)2
Cl
O
Cl

; and



31. A compound for use in medicine having the general formula (I)



(I)

and the pharmaceutically acceptable salts thereof, wherein

------ is an optional double bond;

X_1 and Y_1 are heteroatoms;

Y_2 is optional and is a heteroatom;

X_2 is independently selected from oxygen (O), nitrogen (N) and sulfur (S) when ------ is a double bond and is independently selected from a hydroxyl (-OH), thiol (-SH) when ------ is a single bond; and

Z_1, Z_2 and Z_3 are independently selected from the group consisting of optionally substituted aromatic ring group, heterocyclic group, straight or branched chain alkyl group, alkenyl group, alkynyl group, hydroxyl

group, alcohols, straight or branched chain alkoxy group, cyano group, carboxyl group and ester group.

32. Use of a compound of any one of claim 1 to 30 in the manufacture of a medicament for the treatment, prevention, inhibition or amelioration of one or more diseases associated with West Nile Virus replication in a subject.

33. Use as claimed in claim 32, wherein said one or more diseases associated with West Nile Virus replication is selected from the group consisting of fever, headache, fatigue, variable malaise, anorexia, nausea, myalgia, lymphadenopathy, a non-pruritic generalized maculopapular rash, hepatitis, pancreatitis, myocarditis, rhabdomyolysis, orchitis, ocular manifestations, meningitis, encephalitis, acute flacid paralysis (AFP)/poliomyelitis, Parkinsonism, pharyngitis, conjunctivitis, coma, and seizure/status epilepticus.

34. Use of a compound of any one of claim 1 to 30 in the manufacture of a medicament for the treatment, prevention, inhibition or amelioration of one or more diseases associated with dengue virus replication in a subject.