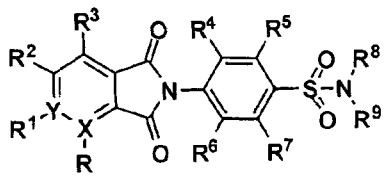
ABSTRACT

The present invention relates to novel compounds of 4-(1,3-dioxoisoindolin-2-yl)benzenesulfonamide derivatives of the general formula I, their pharmaceutically acceptable derivatives, tautomeric forms, stereoisomers, polymorphs, prodrugs, metabolites, salts or solvates thereof. The present invention also provides pharmaceutical compositions comprising compounds of general formula I and their use of such compounds and compositions in medicines and process for preparing the same for antiviral activity.

We Claim,

1. A compound of formula (I), their synthesis and methods thereof,



I

or a racemic or enantiomerically enriched compounds, or mixtures thereof.

2. The compounds as claimed in claim 1, wherein R represents Phenyl, (o,m,p) Toluene, (o,m,p) Alkoxy phenyl, (o,m,p) benzoic acid, (o,m,p) Nitro phenyl, (o,m,p) Ethyl phenyl, (o,m,p) Halo phenyl, (o,m,p) cyano phenyl, (o,m,p) Hydroxy phenyl, alkyl, thio phenyl. R1 represents H, alkyl and their pharmaceutically acceptable derivatives, tautomeric forms, stereoisomers including R and S isomers, polymorphs, prodrugs, metabolites, salts or solvates thereof.

In which:

X = C, N

Y = C, N

R = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^1 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^2 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

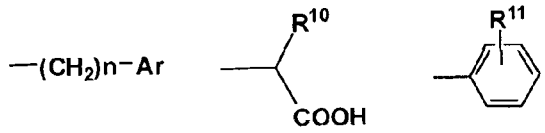
R^3 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^4 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

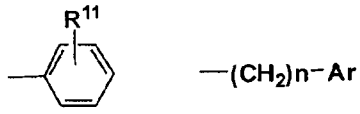
R^5 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^6 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^7 = -H, Halo, $-NH_2$, -OH, -CN, $-NO_2$, -COOH, alkyl(C_1-C_6), $-CF_3$, $-OCF_3$, $-OCH_3$, $-OC_2H_5$

R^8 = -H, Ph, alkyl(C_1-C_6), aryl, 

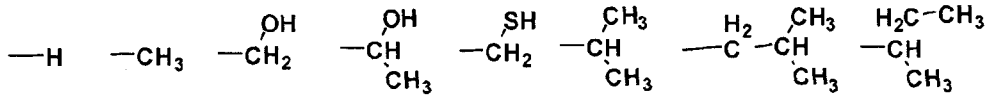
n = 1-2.

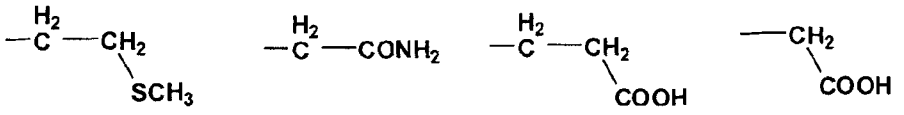


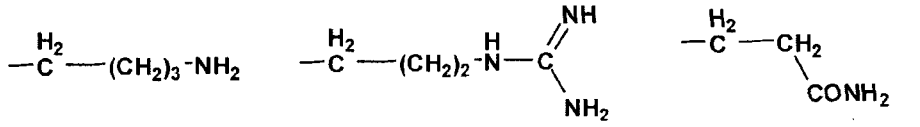
R^9 = -H, aryl, alkyl(C_1-C_6), napth-1-yl, napth-2-yl,

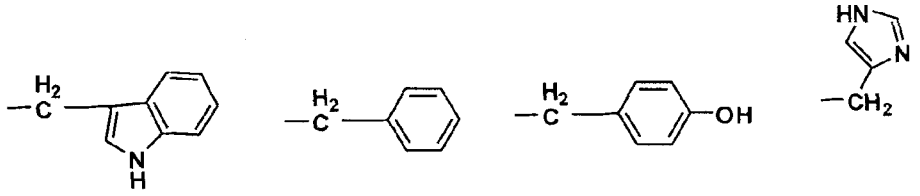
n = 1-2.

aryl = saturated or an unsaturated 3 to 8-membered heterocycles optionally comprising one or more further heteroatom selected from N, O, S, or P; the heterocycles being optionally substituted with one or more substituents.

R^{10} = 

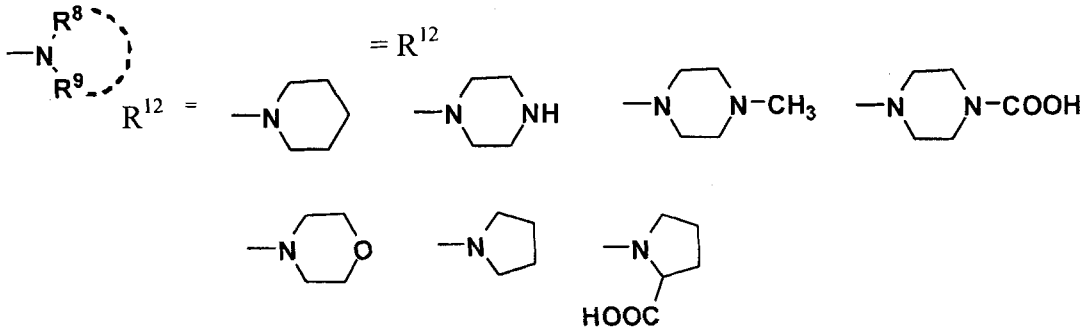






R^{11} = -H, halo, $-NH_2$, -OH, -CN, $-NO_2$, -COOH, alkyl(C_1-C_6), $-CF_3$, SO_2NH_2, $-OCH_3$, $-OC_2H_5$ at ortho or meta or para positions.

Halo = F, Cl, Br, I.



3. A method as claimed in claim 1, wherein is synthesized using organic bases as catalyst in the presence of chlorinated solvents, heterocyclic or aromatic hydrocarbons or aliphatic hydroxylic solvents at a temperature of 20 to 1500 °C.
4. A method as claimed in claim 1, wherein the organic bases are selected from pyridine or substituted pyridines, tertiary amines like Triethyl amine, Trimethyl amine etc, secondary amines such as dimethyl amine, diethyl amine, imidazole or substituted imidazole preferable bases from secondary amines.
5. A method as claimed in claim 1, wherein chlorinated solvents are selected from dichloromethane, chloroform, carbon tetrachloride. Heterocyclic solvents include tetrahydrofuran, dioxane and its isomers Aromatic hydrocarbons are selected from toluene, xylene isomers and aliphatic hydroxylic solvents selected from methyl alcohol, ethyl alcohol and isopropyl alcohol.
6. The compounds as claimed in claim 1, wherein exhibits antiviral activity against viruses including Dengue virus, Chikungunya virus, Coxsackie virus for instance Coxsackie virus B3, Yellow fever virus, Hepatitis C virus, Human Rhino virus, Entero virus, Polio virus.
7. The compounds as claimed in claim 1, wherein shows potential viral protease activity.

Dated at Chennai this Feb 01 , 2014

Signature:

(Barij Nayan Sinha)
Professor
Department of Pharmaceutical Sciences,
Birla Institute of Technology,
Mesra, Ranchi-835215 (JH), India

COMPLETE SPECIFICATION

TITLE OF THE INVENTION

4-(1, 3-DIOXOISOINDOLIN-2-YL)-BENZENESULFONAMIDE DERIVATIVES, THEIR SYNTHESIS, ANTIVIRAL ACTIVITY AND METHODS THEREOF

FIELD OF THE INVENTION

The present invention relates to novel compounds of the general formula I and to a process for preparing such compounds, to pharmaceutical compositions containing compounds and to use of such compounds and composition in medicines.

BACKGROUND OF INVENTION

In the quest for designing inhibitors against dengue virus protease, a high throughput virtual screening was performed against dengue protease, which resulted in 4-(5,7-dioxo-5H-pyrrolo[3,4-b]pyridin-6(7H)-yl)- benzenesulfonamide derivative as one of the HIT molecule. In a trial to synthesize analogues of the same, 4-(1, 3-dioxoisoindolin-2-yl)-benzenesulfonamide, Ia-t were selected for synthesis and they have been synthesized.

The present invention discloses as for "novel methodology for the synthesis of 4-(1, 3-dioxoisoindolin-2-yl)-benzenesulfonamide derivatives (Ia-t)"

A series of 4-(1, 3-dioxoisoindolin-2-yl)-N-benzene sulfonamide derivatives were reported for its anticonvulsant activity, anti-inflammatory, anti tubercular activity, anti-CCR8 activity and anti-carbonic anhydrase activity.

a) Lima, L. d. M.; Castro, P.; Machado, A. L.; Fraga, C. A. M.; Lugnier, C.; de Moraes, V. L. G.; Barreiro, E. J. Bioorganic & medicinal chemistry 2002, 10, 3067

b) Santos, J. L.; Yamasaki, P. R.; Chin, C. M.; Takashi, C. H.; Pavan, F. R.; Leite, C. Q. Bioorganic & medicinal chemistry 2009, 17, 3795.

c) Arti; Sandeep, K.; Devendre, P. International Journal of PharmTech Research 2011, 3 (4), 2014.

d) Jenkins, T. J.; Guan, B.; Dai, M.; Li, G.; Lightburn, T. E.; Huang, S.; Freeze, B. S.; Burdi, D. F.; Jacutin-Porte, S.; Bennett, R. Journal of medicinal chemistry 2007, 50, 566.

e) Scozzafava, A.; Mincione, F.; Menabuoni, L.; Supuran, C. T. Drug design and discovery 2000, 17, 337.

DEFINITIONS AND ABBREVIATIONS

1. As used herein, "carrier" or "pharmaceutical carrier" refers to a diluents, adjuvant, excipient, or vehicle with which a compound of the invention is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soyabean oil, mineral oil, sesame oil, and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectible solutions. The pharmaceutical carriers can be gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition auxillary, stabilizing, thickening, lubricating, and coloring agents may be used. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, polyethylene glycol 300, water, ethanol, polysorbate 20, wetting or emulsifying agents, or pH buffering agents.

2. As used herein, "isolated" means that the compounds of the invention are separated from other components of either (a) a natural source, such as a plant or cell, preferably bacterial culture, or (b) a synthetic organic chemical reaction mixture. An isolated compound can be, for example, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% pure.

3. By "isomer" is meant any stereoisomer, enantiomer, or diastereomers of any compound of the invention. Representative stereoisomers include isomers such as double bond isomers. Exemplary double bond isomers that are encompassed by the invention are the compounds of Formula.

4. It is recognized that the compounds of the invention can have one or chiral centers and or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric isomers), enantiomers, or diastereomers. According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all of the corresponding enantiomers and stereoisomers, that is, both the stereomerically pure form (e.g.,

geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates.

5. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as column chromatography, chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compounds as chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

6. In one embodiment, when administered to a patient, e.g., a mammal for veterinary use or a human for clinical use, the compounds are administered in isolated form. In another embodiment, via conventional techniques, the compounds are purified.

7. It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure controls. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

8. As used herein, "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

9. As used herein, "pharmaceutically acceptable salt(s)," includes but are not limited to salts of acidic basic groups that may be present in compounds used in present compositions. The compounds included in the present compositions that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such compounds are those that form non-toxic acid addition salts. i.e., salts containing pharmacologically acceptable anions, including but not limited to sulfuric, acetic, nitrate, hydrochloride, hydrobromide, acetate, phosphate, citric, oxalic, hydroiodide, maleic, sulfate, bisulfate, acid phosphate, isonicotinate, lactate, salicylate, citrate, acid citrate, tartrate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methasulfonate, ethanesulfonte, benzensulfonate, p-toluenesulfonate, mesylate,

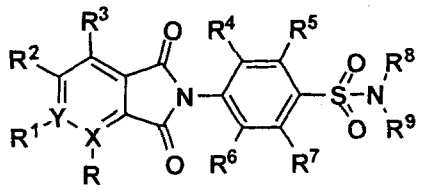
hydroxymethylsulfonate, and pamoate salts. Similar, compounds of the invention that include ionizable hydrogens can be combined with various inorganic and organic bases to form salts.

10. As used herein, a "prodrug" is a compound that is rapidly transformed in vivo to the parent compound of the compounds of the invention may be esters, carbonates, phosphorus (III) esters, or phosphorus (V) esters. Some common esters that have been utilized as prodrugs are phenylesters, aliphaticesters, cholesterol esters, acycloxymethyl esters, and amino acid esters. Compounds of the invention can be converted to their corresponding prodrugs according to methods known in the art. For example, the thiouronium group of compounds of invention can be treated with an electrophile (e.g., an acid chloride, an anhydride, a carboxylic ester, a carbonate, a carbomyl chloride, or a phosphorus (III) or (IV) electrophile) to prepare the corresponding prodrug. Exemplary methods for the preparation of prodrugs are described herein. A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S Symposium series, Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, and Judkins et al., Synthetic Communications 26(23):4351-4367, 1996, each of which is incorporated herein by reference. As used herein, "purified" means that when isolated, the isolate contains at least 95% preferably at least 98%, of a single compound by weight of isolate.

11. As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of stereoisomer of the compound and less than20% by weight of other stereoisomers the compound, more preferably greater than about 90% by weight of one stereoisomers of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of the other stereoisomers of the compound and less than about 5% by weight of other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

SUMMARY OF THE INVENTION

The present invention relates to a compound of formula I



I

or a racemic or enantiomerically enriched compounds, or mixtures thereof

In which:

X = C, N

Y = C, N

R = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^1 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^2 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

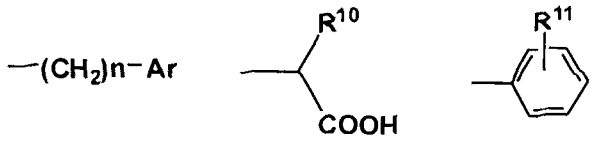
R^3 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^4 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^5 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

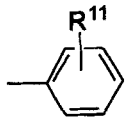
R^6 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^7 = -H, Halo, $-NH_2$, -OH, -CN, $-NO_2$, -COOH, alkyl(C_1-C_6), $-CF_3$, $-OCF_3$, $-OCH_3$, $-OC_2H_5$



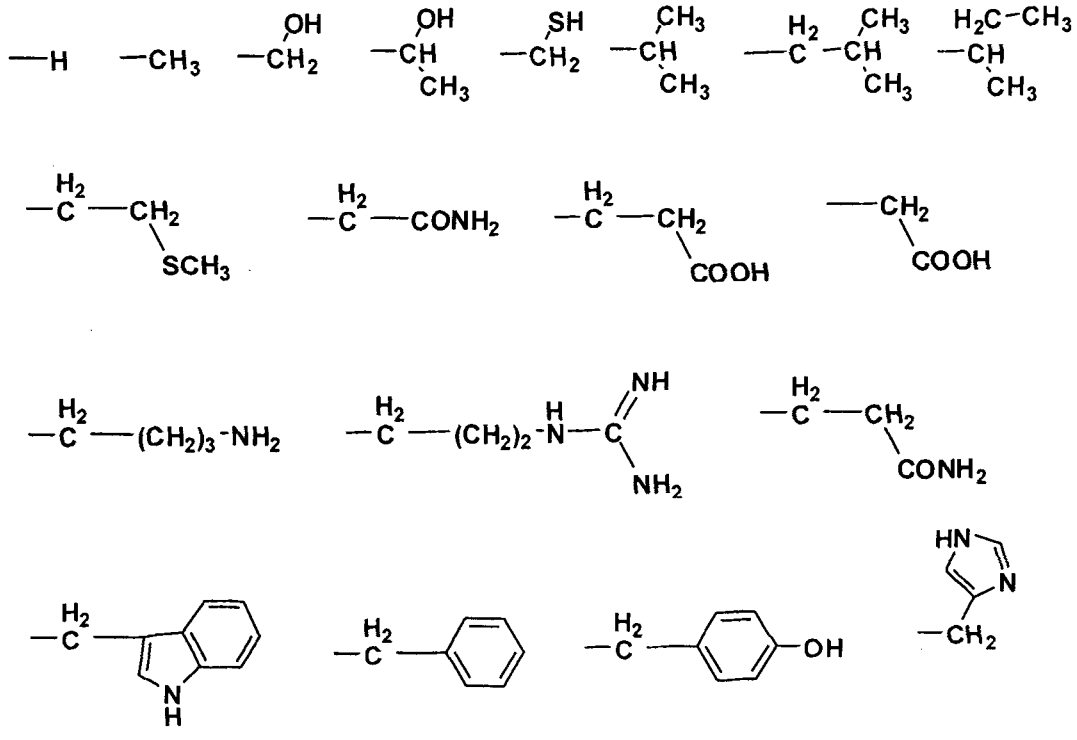
R^8 = -H, Ph, alkyl(C_1-C_6), aryl,

n = 1-2.

R^9 = -H, aryl, alkyl(C_1-C_6), napth-1-yl, napth-2-yl,  —$(CH_2)n$–Ar

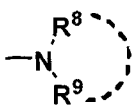
n = 1-2.

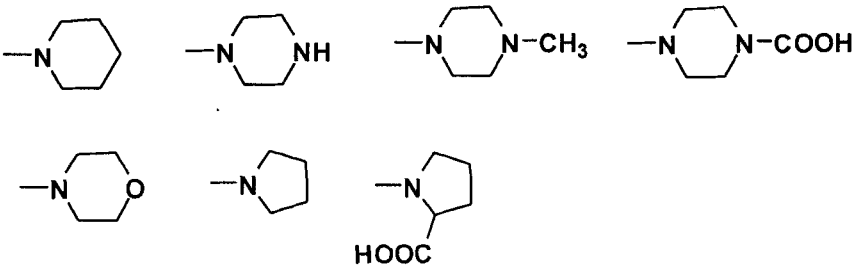
aryl = saturated or an unsaturated 3 to 8-membered heterocycles optionally comprising one or more further heteroatom selected from N, O, S, or P; the heterocycles being optionally substituted with one or more substituents.

R^{10} = 

R^{11} = -H, halo, $-NH_2$, -OH, -CN, $-NO_2$, -COOH, alkyl(C_1-C_6), $-CF_3$, SO_2NH_2, $-OCH_3$, $-OC_2H_5$ at ortho or meta or para positions.

Halo = F, Cl, Br, I.

 = R^{12}

R^{12} = 

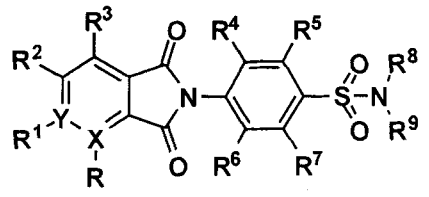
Their salts or pharmaceutical solvents or isomers or enantiomers are novel compounds and their pharmaceutical compositions containing such compounds and the use of such compounds in medicines and process for preparing the same.

Wherein, the said compounds of formula I are of 4-(1,3-dioxoisoindolin-2-yl)benzenesulfonamide derivatives and mixtures thereof. The compounds of formula I exhibits excellent antiviral activity.

In another aspect of the invention, the compounds of formula I are used along with pharmaceutically acceptable excipeints.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a compound of formula I



I

or a racemic or enantiomerically enriched compounds, or mixtures thereof

In which:

X = C, N

Y = C, N

R = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^1 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^2 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

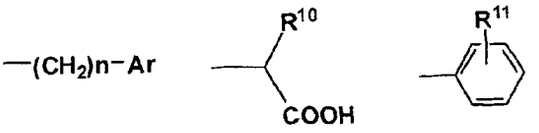
R^3 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^4 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

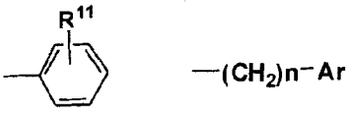
R^5 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^6 = -H, Halo, -NH2, -OH, -CN, -NO2, -COOH, alkyl(C1-C6), -CF3, -OCF3, -OCH3, -OC2H5

R^7 = -H, Halo, $-NH_2$, -OH, -CN, $-NO_2$, -COOH, alkyl(C_1-C_6), $-CF_3$, $-OCF_3$, $-OCH_3$, -OC_2H_5

R^8 = -H, Ph, alkyl(C_1-C_6), aryl, 

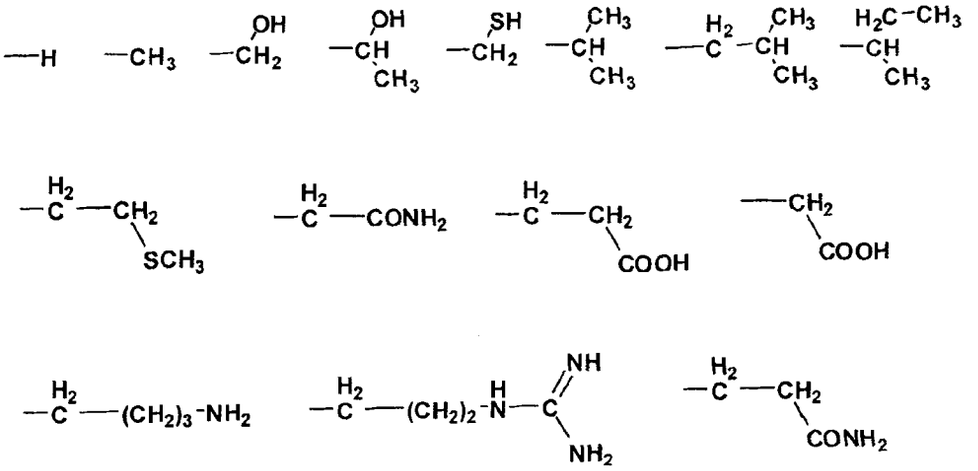
n = 1-2.

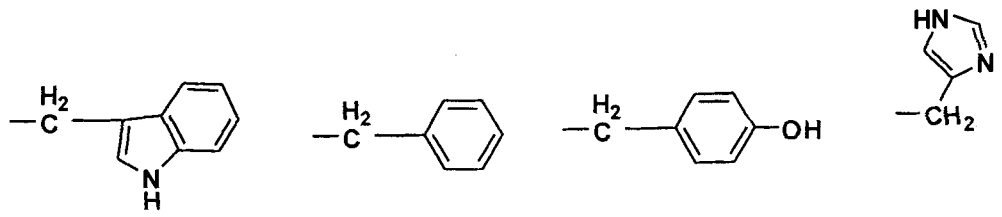


R^9 = -H, aryl, alkyl(C_1-C_6), napth-1-yl, napth-2-yl,

n = 1-2.

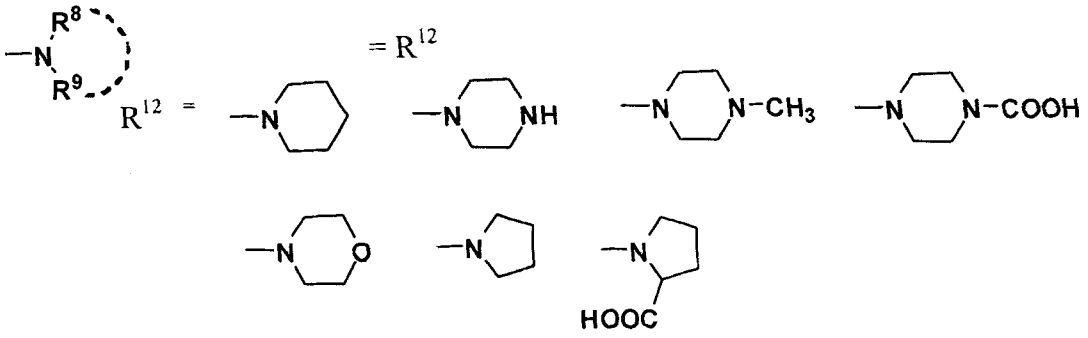
aryl = saturated or an unsaturated 3 to 8-membered heterocycles optionally comprising one or more further heteroatom selected from N, O, S, or P; the heterocycles being optionally substituted with one or more substituents.

R^{10} = 



R^{11} = -H, halo, $-NH_2$, -OH, -CN, $-NO_2$, -COOH, alkyl(C_1-C_6), $-CF_3$, SO_2NH_2, $-OCH_3$, $-OC_2H_5$ at ortho or meta or para positions.

Halo = F, Cl, Br, I.



Their salts or pharmaceutical solvents or isomers or enantiomers are novel compounds and their pharmaceutical compositions containing such compounds and the use of such compounds in medicines and process for preparing the same.

Wherein, the said compounds of formula I are of 4-(1,3-dioxoisoindolin-2-yl)benzenesulfonamide derivatives and mixtures thereof. The compounds of formula I exhibits excellent antiviral activity.

In another aspect of the invention, the compounds of formula I are used along with pharmaceutically acceptable excipeints.

Pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. When administered to a patient, the

compounds of the invention and pharmaceutically acceptable carriers can be sterile. In one embodiment, water is a carrier when the compound is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectible solutions. Suitable pharmaceutical carriers also include excipeints such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, polyethylene glycol 300, water, ethanol, polysorbate 20, and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, and capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, sprays, suspensions, or any other suitable for use. In one embodiment, the pharmaceutically acceptable carriers is capsule (see e.g., U.S. Patent No. 5698155). Other examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

Compounds of the invention included in the present compositions that include an amino moiety may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Compounds, included in the present compositions, which are acidic in nature, are capable of forming base salts with various pharmacologically or cosmetically acceptable cations. Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium, lithium, zinc, potassium, and iron salts.

In another embodiment, the compounds of the invention are formulated in accordance with routine procedures as a pharmaceutical compositions adopted for intravenous administration to human beings. Typically, compounds for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the compositions may also include a local anesthetic such as lignociane to ease pain at the site of infection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the active agent. Where the compound of the invention is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the compound of the invention is

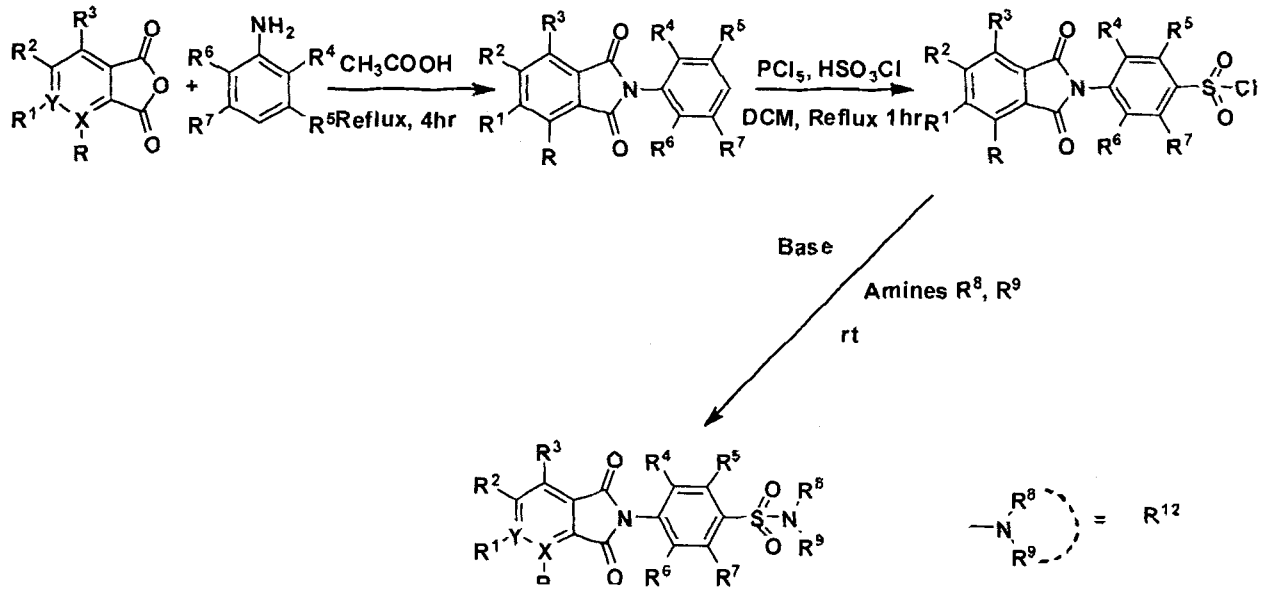
administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration

Compounds for oral delivery may be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixers, for example, sweetening agents such as fructose, aspartame or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions may be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered compounds. In these later platforms, fluid from the environment surrounded the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time-delay material such as glycerol monostearate or glycerol stearate may also be used. Oral compositions can include standard carriers such as mannitol, lactose, starch, magnesium stearate, sodium stearate, sodium saccharine, cellulose, or magnesium carbonate. Such carriers can be of pharmaceutical grade.

The following scheme shows the preparation of respective compounds of the invention. There is no particular restriction on the nature of the solvent to be employed, provided that there is no adverse effect on the reaction or on the reagents involved.

1. CHEMISTRY

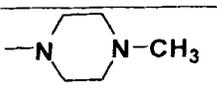
1. a. GENERAL REACTION SCHEME



Base used are: Pyridine, Triethylamine, Diethyl amine, Diisopropylamine
X = Y = C
$R = R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = R^7 = H$
Whereas the corresponding R^8, R^9 and R^{12} are provided in table below

Table 1

Compound Code	R^8	R^9	R^{12}
Ia	3-Methoxy Phenyl	H	—
Ib	4-Methoxy Phenyl	H	—
Ic	3-Chloro Phenyl	H	—
Id	4-Chloro Phenyl	H	—
Ie	2-Methyl phenyl	H	—
If	3-Methyl phenyl	H	—
Ig	4-Methyl phenyl	H	—
Ih	2-Hydroxy Phenyl	H	—
Ii	Phenyl-4-carboxylic acid	H	—
Ij	4-Ethyl phenyl	H	—
Ik	4-Nitro phenyl	H	—
I l	Phenyl	H	—
Im	1-Napthyl	H	—
In	Phenyl ethyl	H	—
Io	H	H	—
Ip	Isopropyl	H	—

Iq	Ethyl	Ethyl	——
Ir	Methyl	Methyl	——
Is	——	——	
It	——	——	

The invention compounds of formula I may be prepared by considering intermediates to result in compounds of formula I.

The invention compounds of formula were synthesized following the reactions outlined in the REACTION SCHEME. Pthalic anhydride was used as initial starting material for the synthesis of final intermediate 4-(1,3-dioxoisoindolin-2-yl)benzene-1-sulfonyl chloride. Nucleophillic substitution of Pthalic anhydride was done with aniline to form 2-phenylisoindoline-1,3-dione. Electrophillic substitution by Chlorosulfonyl group to 2-phenylisoindoline-1,3-dione resulted in 4-(1,3-dioxoisoindolin-2-yl)benzene-1-sulfonyl chloride. Sulfuryldichloride was insitu formed by the reaction of chlorosulfonic acid with phosphorus pentachloride at cold temperature. Electrophillic substitution of sulfuryl dichloride to 2-phenylisoindoline-1,3-dione was done at fourth position as second and third positions are sterically hindered. The resulted final intermediate 4-(1,3-dioxoisoindolin-2-yl)benzene-1-sulfonyl chloride was reacted with different anilines and amines to give compounds Ia-t.

Substitution is referred to substitution with suitable substituting group which can be present in the starting materials, added to any one of the intermediate or added after formation of the final products by known methods of substitution or conversion reactions.

It may be used with salts organic and inorganic acids salt such as acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, mandelic, malic, phthalic, hydrochloric, phosphoric, nitric, sulfuric, methanesulfonic, benzenesulfonic, camphorsulfonic acid salt. Acceptable inorganic and organic acid salts which are pharmaceutically acceptable are prepared, when compound of this invention contains basic moiety.

Thus the present invention may provide a pharmaceutical composition, containing the compounds of the general formula I as defined above in combination with the usual pharmaceutically employed carriers, diluents and the like, useful for the any treatment of Human and animal diseases.

The pharmaceutical composition may be in the forms normally employed, such as tablets, capsules, powders, syrups, solutions, suspensions and the like, may contain flavourant, sweetners etc. in suitable solid or liquid carriers or diluents, or in suitable sterile media to form injectible solutions or suspensions.

Certain modifications and equivalents will be apparent to those skilled in the art and are intended to be included within the scope of the present invention.

1.b. GENERAL SYNTHETIC PROCEDURE

General procedure for 2-Phenyl-1,3-isoindolinedione

A mixture of Aniline (4,0 mmol), phthalic anhydride (0.5 g, 3.4 mmol), and 20 mL of glacial acetic acid was stirred under nitrogen at 130 0°C for 2 h. After that the mixture was cooled and 30 mL of cold water was added. The product was filtered and washed with cold water. The product was recrystallized in ethanol without further purification to lead to compound with yield 90%.

General procedure for 4- (1,3-Dioxo- 2,3-dihydro- 1H- 2- isoindolyl) - 1-benzenesulfonyl chloride

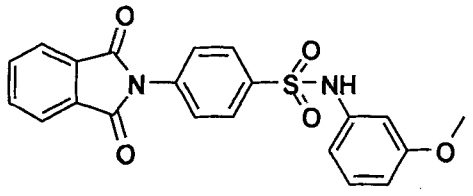
To a solution of 0.16 g (0.09 mL, 1.35 mmol) of chlorosulfonic acid and 0.14 g (0.67 mmol) of phosphorus pentachloride that had been stirred for 10 min, was added 0.15 g (0.67 mmol) of N-phenyl-phthalimide in small portion. The resulting mixture was stirred and heated at 500°C for 30 min. The reaction mixture was poured onto ice and extracted with chloroform. The organic phase was separated, washed with brine, dried over anhydride Na2SO4 and evaporated at reduced pressure to furnish compound in 70% yield, as a white solid mp 180–182 0°C.

General procedure for sulphonamides (Ia-It)

To a solution of sulfonyl chloride derivative (0.5 g; 1.56 mmol) in 50 mL of methylene chloride in R.B.flask kept under icebath added triethylamine or Pyridine (1.872mmol) and stirred

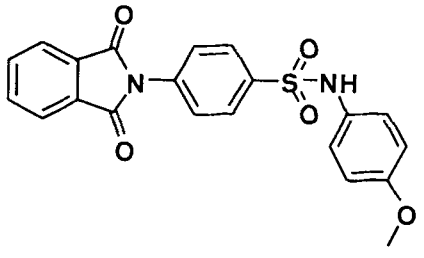
for half an hour and to this mixture added the functionalized amine derivatives (3.12 mmol). The reaction mixture was stirred for about 0.5-4 hr, at room temperature, when the end of reaction was observed by TLC. Next, the phthalimide derivatives **I** were isolated by addition of 50 mL of methylene chloride and extraction with 10% aq HCl and brine. The organic layer was dried over anhydrous sodium sulphate and evaporated at reduced pressure to give the sulfonamide derivatives in good yields.

Example 1: 4-(1,3-dioxoisoindolin-2-yl)-N-(3-methoxyphenyl)benzenesulfonamide (Ia): **Ia** was prepared following the procedure described in REACTION SCHEME using 3-Methoxy aniline as funtionalised amine. The product is pale white amorphous powder having a yield of 85%. 1H NMR (DMSO-D6) δ 3.67(s, 3H, OCH3), 6.71(m, 2H, Ar), 7.15(t, 1H, Ar, J= 8.4Hz), 7.6(d, 2H, Ar, J= 8.4 Hz), 7.92(m, 4H, Ar), 7.98(m, 2H, Ar), 10.42(s, 1H, NH). ESI-MS (m/z) 409



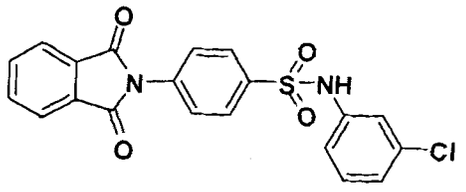
Ia

Example 2: 2-(4-(4-methoxybenzylsulfonyl)phenyl)isoindoline-1,3-dione (Ib): Ib was prepared following the procedure described in REACTION SCHEME using 4-Methoxy aniline as funtionalised amine. The product is pale white amorphous powder having a yield of 79%. 1H NMR (DMSO-D6) δ 3.67(s, 3H, OCH3), 6.83(d, 2H, Ar, J= 9.2 Hz), 7.03(d, 2H, Ar, J= 8.8 Hz), 7.16(m, 1H, Ar), 7.25(m, 1H, Ar), 7.65(d, 2H, Ar, J= 8.8 Hz), 7.84(d, 2H, Ar, J= 8.8Hz), 7.92(m, 2H, Ar), 7.98(m, 2H, Ar), 10.05(s, 1H, NH). ESI-MS (m/z) 409



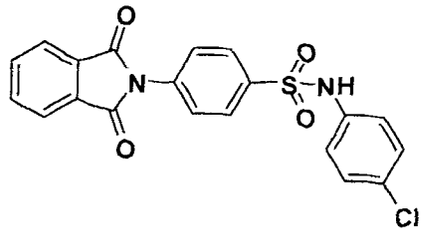
Ib

Example 3: N-(3-chlorophenyl)-4-(1,3-dioxoisoindolin-2-yl)benzenesulfonamide (Ic): **Ic** was prepared following the procedure described in REACTION SCHEME using 3-Chloro aniline as funtionalised amine. The product is buff white amorphous powder having a yield of 81%. 1H NMR (DMSO-D6) δ 7.140(t, 2H, Ar, J= 8Hz), 7.172(s, 1H, Ar), 7.30(t, 1H, Ar, J= 7.6Hz), 7.70(d, 2H, Ar, J= 8.4 Hz), 7.91(m, 2H, Ar), 7.93(m, 2H, Ar), 7.98(m, 2H, Ar), 10.7(s, 1H, NH). ESI-MS (m/z) 412



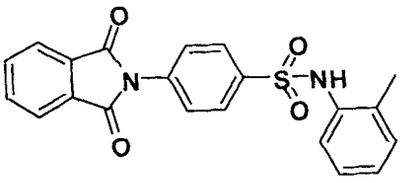
Ic

Example 4: N-(4-chlorophenyl)-4-(1,3-dioxoisoindolin-2-yl)benzenesulfonamide [Id]: **Id** was prepared following the procedure described in REACTION SCHEME using 4-Chloro aniline as funtionalised amine. Yield = %; pale white amorphous powder. 1H NMR (DMSO-D6) δ 7.16(d, 2H, Ar, J= 9.2 Hz), 7.32(d, 2H, Ar, J= 8.8 Hz), 7.68(d, 2H, Ar, J= 8 Hz), 7.92(m, 4H, Ar), 7.99(m, 2H, Ar), 10.57(s, 1H, NH). ESI-MS (m/z) 412



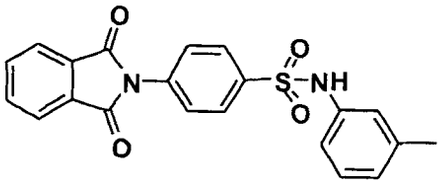
Id

Example 5: 2-(4-(2-methylbenzylsulfonyl)phenyl)isoindoline-1,3-dione (Ie): **Ie** was prepared following the procedure described in REACTION SCHEME using 2-Methyl aniline as funtionalised amine. The product is off white amorphous powder having a yield of 89%. 1H NMR (DMSO-D6) δ 2.03(s, 3H, CH3), 7.01(m, 1H, Ar), 7.14(m, 3H, Ar), 7.67(d, 2H, Ar, J= 8.4 Hz), 7.82(d, 2H, Ar, J= 8.8 Hz), 7.92(m, 2H, Ar), 7.99(m, 2H, Ar), 9.72(s, 1H, NH). ESI-MS (m/z) 393



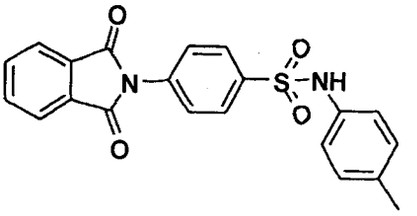
Ie

Example 6: 2-(4-(3-methylbenzylsulfonyl)phenyl)isoindoline-1,3-dione (If): If was prepared following the procedure described in REACTION SCHEME using 3-Methyl aniline as funtionalised amine. The product is pale pinkish white amorphous powder having a yield of 90%. 1H NMR (DMSO-D6) δ 2.21(s, 3H, CH3), 6.86(d, 1H, Ar, J= 7.2Hz), 6.95(d, 2H, Ar, J= 6.8 Hz), 7.13(t, 1H, Ar, J= 8Hz), 7.66(d, 2H, Ar, J= 8.8 Hz), 7.91 (m, 4H, Ar), 7.98(m, 2H, Ar), 10.33(s, 1H, NH). ESI-MS (m/z) 393



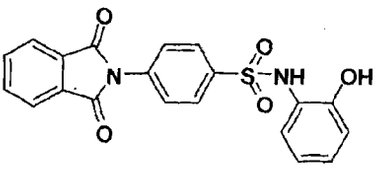
If

Example 7: 2-(4-(4-methylbenzylsulfonyl)phenyl)isoindoline-1,3-dione (Ig): Ig was prepared following the procedure described in REACTION SCHEME using 4-Methyl aniline as funtionalised amine. The product is off white amorphous powder having a yield of 84%. 1H NMR (DMSO-D6) δ 2.19(s, 3H, CH3), 7.04(dd, 4H, Ar, J= 13.2 Hz, 8.8Hz), 7.65 (d, 2H, Ar, J= 8Hz), 7.91(m, 4H, Ar), 7.98(m, 2H, Ar), 10.24 (s, 1H, NH). ESI-MS (m/z) 393



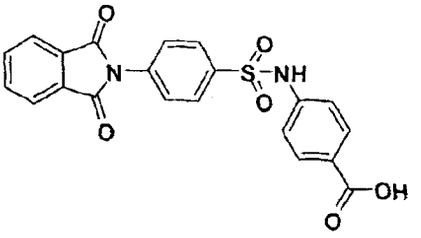
Ig

Example 8: 4-(1,3-dioxoisoindolin-2-yl)-N-(2-hydroxyphenyl)benzenesulfonamide (Ih): Ih was prepared following the procedure described in REACTION SCHEME using 2-Hydroxy aniline as funtionalised amine. The product is pale brownish white amorphous powder having a yield of 86%. 1H NMR (DMSO-D6) δ 6.73(m, 2H, Ar), 6.95(m, 1H, Ar), 7.18(d, 1H, Ar, J= 8.8 Hz), 7.68(m, 2H, Ar), 7.94(m, 6H, Ar), 9.45(s, 1H, NH), 9.7(s, 1H, OH). ESI-MS (m/z) 394.



Ih

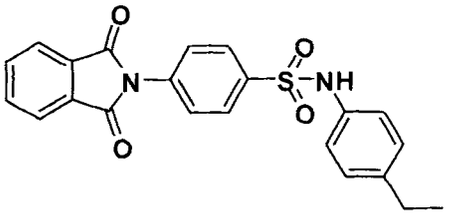
Example 9: 4-(4-(1,3-dioxoisoindolin-2-yl)phenylsulfonamido)benzoic acid (Ii): Ii was prepared following the procedure described in REACTION SCHEME using Paraamino benzoic acid as funtionalised amine. The product is pale pinkish white amorphous powder having yield of 76%. 1H NMR (DMSO-D6) δ 7.26(d, 2H, Ar, J= 8.4 Hz), 7.70(d, 2H, Ar, J= 9.2 Hz), 7.83(d, 2H, Ar, J= 8.8 Hz), 7.91(m, 2H, Ar), 8.02 (m, 4H, Ar), 10.94 (s, 1H, NH).



Ii

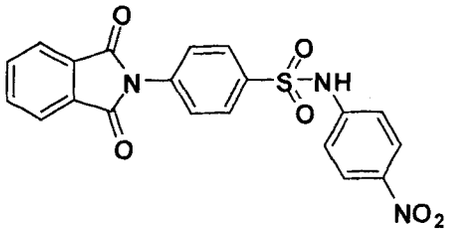
Example 10: 4-(1,3-dioxoisoindolin-2-yl)-N-(4-ethylphenyl)benzenesulfonamide (Ij): Ij was prepared following the procedure described in REACTION SCHEME using 4-Ethyl aniline as funtionalised amine. The product is pale pinkish white amorphous powder having yield as 88%. 1H NMR (DMSO-D6) δ 1.19(t, 3H, -CH3, J= 7.2 Hz), 2.59(q, 2H, -CH2-, J= 7.4 Hz),

7.01(d, 2H, Ar, J= 8.4 Hz), 7.09(d, 2H, Ar, J= 8.0 Hz), 7.63(d, 2H, Ar, J= 8.4 Hz), 7.84(m, 4H, Ar), 7.96 (m, 4H, Ar)



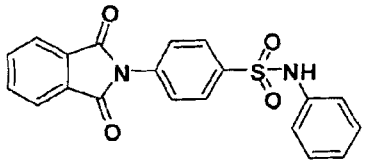
Ij

Example 11: 4-(1,3-dioxoisoindolin-2-yl)-N-(4-nitrophenyl)benzenesulfonamide (Ik): **Ik** was prepared following the procedure described in REACTION SCHEME using 4-Nitro aniline as funtionalised amine. The product is pale yellow color amorphous powder having yield as Yield = 75%;. 1H NMR (DMSO-D6) δ 7.37(d, 2H, Ar, J= 9.2 Hz), 7.72(d, 2H, Ar, J= 8.8 Hz), 7.91(d, 2H, Ar, J=3.2 Hz), 7.98(d, 2H, Ar, J=3.2 Hz), 8.05(d, 2H, Ar, J=8.4 Hz), 8.17(d, 2H, Ar, J=9.2 Hz), 11.43(s, 1H, NH). ESI-MS (m/z) 423.



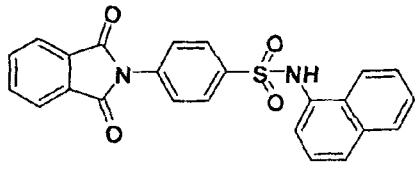
Ik

Example 12: 4-(1,3-dioxoisoindolin-2-yl)-N-phenylbenzenesulfonamide (Il): **Il** was prepared following the procedure described in REACTION SCHEME using Aniline as funtionalised amine. Yield = 68%; off white amorphous powder (purified by column chromatography using hexane/ethyl acetate 1:1 as eluent); 1H NMR (DMSO-D6) δ 7.045(t, 1H, Ar, J= 7.6 Hz), 7.15(d, 2H, Ar, J= 4 Hz), 7.259(t, 2H, Ar, J= 7.6 Hz), 7.67(d, 2H, 8.4 Hz), 7.93(m, 4H, Ar), 7.98(m, 2H, Ar), 10.42(s, 1H, NH). Anal. C20H14N2O4S (C, H, N, S). ESI-MS (m/z) 379.



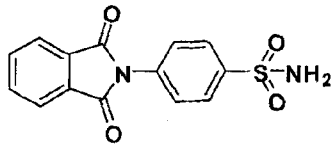
Il

Example 13: 4-(1,3-dioxoisoindolin-2-yl)-N-(naphthalen-1-yl)benzenesulfonamide (Im): **Im** was prepared following the procedure described in REACTION SCHEME using 1-Napthyl amine as funtionalised amine. The product is pale purple color amorphous powder having a yield of 88%;. 1H NMR (DMSO-D6) δ 7.21(d, 1H, Ar, J= 8 Hz), 7.40-7.50(m, 3H, Ar), 7.62(s,1H, Ar), 7.64 (s,1H, Ar), 7.86(d, 1H, Ar, J= 8Hz), 7.89-7.99 (m, 7H, Ar), 8.04(d,1H, Ar, J= 8Hz), 10.37(s, 1H, NH). ESI-MS (m/z) 429.



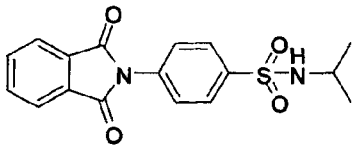
Im

Example 15: 4-(1,3-dioxoisoindolin-2-yl)benzenesulfonamide {Io}: **Io** was prepared following the procedure described in REACTION SCHEME using Ammonia solution as funtionalised amine. Yield = 81%; pale white amorphous powder. 1H NMR (DMSO-D6) δ 7.46(s, 1H, NH), 7.67(d, 2H, Ar, J= 8.4 Hz), 7.96 (m, 4H, Ar). ESI-MS (m/z) 303.



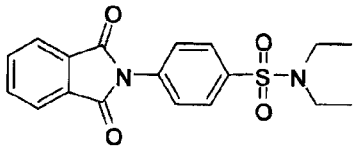
Io

Example 16: 4-(1,3-dioxoisoindolin-2-yl)-N-isopropylbenzenesulfonamide {Ip}: **Ip** was prepared following the procedure described in REACTION SCHEME using N-isopropyl amine as funtionalised amine. Yield = 95%; pale white amorphous powder. 1H NMR (DMSO-D6) δ 0.98(d, 6H, (CH3)2, J= 6.8 Hz), 1.09(m, 1H, -CH), 7.73 (m, 4H, Ar), 7.96 (m, 6H, Ar). ESI-MS (m/z) 345.



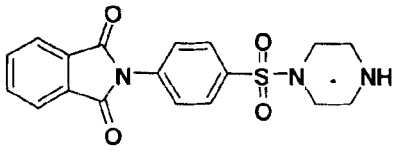
Ip

Example 17: 4-(1,3-dioxoisoindolin-2-yl)-N,N-diethylbenzenesulfonamide [Iq]: Iq was prepared following the procedure described in REACTION SCHEME using N-diethyl amine as funtionalised amine. Yield = 94%; pale white amorphous powder. 1H NMR (DMSO-D6) δ 1.08(t, 6H, -(CH3), J= 6.8 Hz), 3.21(q, 4H, -(CH2)-, J= 7.2 Hz), 7.71(d, 2H, Ar, J= 8.4 Hz), 7.96(m, 6H, Ar). ESI-MS (m/z) 359.



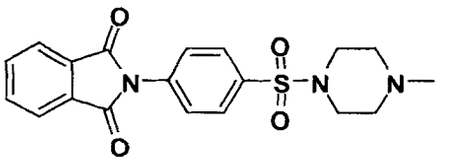
Iq

Example 18: 2-(4-(piperazin-1-ylsulfonyl)phenyl)isoindoline-1,3-dione [Ir]: Ir was prepared following the procedure described in REACTION SCHEME using Dimethyl amine as funtionalised amine. Yield = 80%; pale white amorphous powder. 1H NMR (DMSO-D6) δ 2.59(s, 3H, -CH3), 2.68(s, 3H, -CH3), 7.36(d, 1H, Ar, J= 6.4 Hz), 7.57(m, 1H, Ar), 7.76(m, 2H, Ar), 7.97(m, 4H, Ar). ESI-MS (m/z) 371.



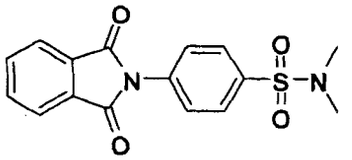
Ir

Example 19: 2-(4-(4-methylpiperazin-1-ylsulfonyl)phenyl)isoindoline-1,3-dione [Is]: **Is** was prepared following the procedure described in REACTION SCHEME using Piperazine as funtionalised amine. Yield = 80%; pale white amorphous powder. 1H NMR (DMSO-D6) δ 1.56(m, 4H,-(CH2)-NH), 2.95((m, 4H, -(CH2)-N), 7.76(m, 2H, Ar), 7.88(m, 4H, Ar), 8.00(m, 2H, Ar), 8.32(s, 1H, NH). ESI-MS (m/z) 386.



Is

Example 20: 4-(1,3-dioxoisoindolin-2-yl)-N,N-dimethylbenzenesulfonamide *[It]*: *It* was prepared following the procedure described in REACTION SCHEME using N-Methyl piperazine as funtionalised amine. Yield = 85%; pale white amorphous powder. 1H NMR (DMSO-D6) δ 2.15(s, 3H, -CH3), 2.38((m, 4H, -(CH2)-N), 2.94(m, 4H, Ar), 7.78(d, 2H, Ar, J= 8.4 Hz), 7.88(m, 4H, Ar), 8.00(m, 2H, Ar). ESI-MS (m/z) 330.



It

2. BIOLOGY

2.a. Cell-based antiviral assay(Chikungunya, Dengue, Yellow fever viruses):

Serial dilutions of extracts, fractions or compounds, as well as the reference compound chloroquine, were prepared in assay medium [MEM Rega3 (Cat. N°19993013; Invitrogen), 2% FCS (Integro), 5 mL 200 mM L-glutamine (25030024), and 5 mL 7.5% sodium bicarbonate (25080060)] that was added to empty wells of a 96-well microtiter plate (Falcon, BD). Subsequently, 50 μL of a 4× virus dilution in assay medium was added, followed by 50 μL of a cell suspension. This suspension, with a cell density of 25,000 cells/50 μL, was prepared from a Vero cell line subcultured in cell growth medium (MEM Rega3 supplemented with 10% FCS, 5mL L-glutamine, and 5 mL sodium bicarbonate) at a ratio of 1:4 and grown for 7 days in 150 cm 2 tissue culture flasks (Techno Plastic Products). The assay plates were returned to the incubator for 6–7days(37°C, 5% CO, 95–99% relative humidity), a time at which maximal virus-induced cell death or cytopathic effect (CPE) is observed in untreated, infected controls.

Subsequently, the assay medium was aspirated, replaced with 75 µL of a 5% MTS (Promega) solution in phenol redfree mediumand incubated for 1.5 hours. Absorbance was measured at a wavelength of 498 nm (Safire 2, Tecan); optical densities (OD values) reached 0.6–0.8 for the untreated, uninfected controls). Raw data were converted to percentage of controls and the EC (50% effective concentration or concentration which is calculated to inhibit virus induced cell death by 50%) and CC50(50% antimetabolic concentration or concentration which is calculated to inhibit the overall cell metabolism by 50%) were derived from the dose–response curves. All assay conditions producing an antiviral effect exceeding 50% were checked microscopically for minor signs of CPE or adverse effects on the host cell (i.e. altered cell morphology…). A compound is only considered to elicit a selective antiviral effect on virus replication when, following microscopic quality control, at least at one concentration of compound, no CPE nor any adverse effect is observed (image resembling untreated, uninfected cells). Multiple, independent experiments were performed.

CHIKUNGUNYA VIRUS:

Table 2

Sample code	CC50	EC50
Ia	----------	> 123
Ib	----------	> 123
Ic	----------	> 121
Id	----------	> 121
Ie	----------	> 128
If	----------	> 128
Ig	----------	> 128
Ih	----------	> 254
Ii	----------	> 118
Ij	----------	> 123
Il	----------	> 132

Im	----------	> 117
In	----------	> 246
Io	----------	> 166
Ip	----------	> 291
Iq	----------	> 279
Ir	----------	> 303
Is	----------	> 270
It	----------	> 260

DENGUE VIRUS:
Table 3

Sample code	CC50	EC50
Ia	= 95.5	----------
Ib	= 109	----------
Ic	= 187	----------
Id	= 83.5	----------
Ie	= 31.9	----------
If	= 101	----------
Ig	= 98.4	----------
Ih	= 82.8	= 35.4
Ii	-----	----------
Ij	= 131	----------
Il	= 149	----------
Im	= 84.5	----------
In	= 12	----------

Io	= 60.8	----------
Ip	= 73.6	----------
Iq	= 69.8	----------
Ir	= 145	----------
Is	= 113	----------
It	= 199	----------

YELLOW FEVER VIRUS:
Table 4

Sample code	CC50	EC50
Ia	----------	> 123
Ib	----------	> 123
Ic	----------	> 121
Id	----------	> 121
Ie	----------	> 128
If	----------	> 128
Ig	----------	> 128
Ih	----------	> 127
Ii	----------	> 118
Ij	----------	> 123
Il	----------	> 132
Im	----------	> 117
In	----------	> 123
Io	----------	> 166

Ip	----------	**> 145**
Iq	----------	**> 140**
Ir	----------	**> 152**
Is	----------	**> 135**
It	----------	**> 130**

2.b. Antiviral assay (Coxsackie, Rhino, Polio and Entero viruses)

The antiviral activity of the selected compounds was determined using an MTS-based CPE reduction assay and was expressed as the 50% effective concentration (EC), or the concentration of compound that inhibits virus-induced cytopathic effect formation by 50%. Cells, grown to confluency in 96-well plates, were infected with 100 CCID50 of virus, one CCID50 being the 50% cell culture infective dose. After an adsorption period of two hours at 370°C (350°C for rhinovirus), virus was removed and serial dilutions of the compounds were added. The cultures were further incubated at 370°C (350°C) for 3 days, until complete CPE was observed in the infected and untreated virus control (VC). After removal of the medium, 90 µl medium and 10 µl MTS/PMS (Promega, Leiden, The Netherlands) were added to each well. After an incubation period of 2 h at 370°C (350°C) the optical density of each well was read at 498 nm in a microplate reader. CPE values were calculated as follows: %CPE = [ODVC_ ODCVB3+Compound]/[ODCC_ ODVC]. In these formulae, ODCC corresponds to the optical density of the uninfected and untreated cell cultures, OD represents the infected and untreated cell cultures and OD CVB3+Compound VC are CVB3-infected cell cultures, treated with a given concentration of compound.

Cytotoxic assay. The cytotoxicity of the compounds was evaluated by the MTS-method and the 50% cytotoxic concentration (CC) was calculated. Briefly, the same experimental set-up was used as for the antiviral assay, but for cytotoxicity determination, uninfected cultures were incubated with serial dilution of compound for three days at 370°C. The cytotoxic activity was calculated using the following formula: %CPE = [ODCC_ OD Compound]/ODCC, where ODCC corresponds to the optical density of the uninfected and untreated cell cultures and OD Compound are uninfected cultures, treated with compound.

COXSACKIE VIRUS B3:

Table 5

Sample code	CC50	EC50
Ia	----------	> 123
Ib	----------	> 123
Ic	----------	> 121
Id	----------	> 121
Ie	----------	> 128
If	----------	> 128
Ig	----------	> 128
Ih	----------	> 127
Ii	> 118	= 9.54
Ij	----------	> 123
Il	----------	> 132
Im	----------	> 117
In	----------	> 123
Io	----------	> 166
Ip	----------	> 145
Iq	----------	> 140
Ir	----------	> 152
Is	----------	> 135
It	----------	> 130

RHINO VIRUS:

Table 6

Sample code	CC50	EC50
Ia	----------	> 123
Ib	----------	> 123
Ic	----------	> 121
Id	----------	> 121
Ie	----------	> 128
If	----------	> 128
Ig	----------	> 25.5
Ih	----------	> 127
Ii	----------	> 118
Ij	----------	> 123
Il	----------	> 132
Im	----------	> 117
In	----------	> 123
Io	----------	> 166
Ip	----------	> 145
Iq	----------	> 140
Ir	----------	> 152
Is	----------	> 135
It	----------	> 130

ENTEROVIRUS:

Table 7

Sample code	CC50	EC50
Ia	----------	> 123
Ib	----------	> 123
Ic	----------	> 121
Id	----------	> 121
Ie	----------	> 128
If	----------	> 128
Ig	----------	> 128
Ih	----------	> 127
Ii	----------	> 118
Ij	----------	> 123
Il	----------	> 132
Im	----------	> 117
In	----------	> 123
Io	----------	> 166
Ip	----------	> 145
Iq	----------	> 140
Ir	----------	> 152
Is	----------	> 135
It	----------	> 130

POLIO VIRUS:

Table 8

Sample code	CC50	EC50
Ia	----------	> 123
Ib	----------	> 123
Ic	----------	> 121
Id	----------	> 121
Ie	----------	> 128
If	----------	> 128
Ig	----------	> 128
Ih	----------	> 127
Ii	----------	> 118
Ij	----------	> 123
Il	----------	> 132
Im	----------	> 117
In	----------	> 123
Io	----------	> 166
Ip	----------	> 145
Iq	----------	> 140
Ir	----------	> 152
Is	----------	> 135
It	----------	> 130

2.c. Cell culture and dose-/response assay of HCV

Huh-7 human hepatoma cells carrying the HCV replicon were grown in Dulbecco's modified Eagle medium (DMEM; Life Technologies GmbH, Karlsruhe, Germany) supplemented with 2 mM L-glutamine, nonessential amino acids, 100 U of penicillin, 100 mg streptomycin, 10% fetal calf serum (complete DMEM) and 250 mg/ml G418 (Life Technologies). Cells were passaged twice per week at a dilution of 1:3_/1:5, depending on cell growth by using PBS containing 0.05% trypsin and 0.05% EDTA (Life Technologies). For the luciferase/RT-PCR assay, cells were seeded in 9.6 cm2 dishes at a density of 200,000 cells in complete DMEM supplemented with 250 mg/ml G418 (Life Technologies, Germany). After 24 h, medium was replaced by complete DMEM without G418 and containing 100, 50, 25, 10, 7.5, 5, 2.5, 1 or 0 U/ml of IFNa2a (Roche Applied Science, Mannheim, Germany) in triplicates. In all other cases, cells were seeded in 24-well plates (2 cm2 per well) at a density of 40,000 cells per well. After 24 h, medium was replaced by 1 ml complete DMEM per well containing 10% human serum and one of the following concentrations of interferon: 1000, 500, 250, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20 or 0 U/ml of IFN-a2a (kindly provided by K. Weyer and E.K. Weibel, Hoffmann-La Roche AG, Basel) or IFN a2b (Schering-Plough), or 180, 60, 30, 15, 7.5, 3.75, 1.88, 0.94, 0.47, 0.23, 0.12, 0.06, 0.02 or 0 ng/ml PEG_ /IFNa2a (Roche Applied Science). All experiments were carried out at least twice each in sextuple and on several plates, such that the positions of individual samples relative to the controls were varied in order to reduce positional effects. In case of the cell proliferation assay, the medium in triplicate wells was replaced by 1 ml complete DMEM containing increasing concentrations of ribavirin (Schering-Plough). Proliferation was determined by measuring the optical density after staining the cells with the tetrazolium salt WST-1 (Roche Applied Sciences). A positive (1000 U/ml IFN-a) and a negative control (no IFN-a) was included on each plate such that the position was different from plate to plate.

HEPATITIS C VIRUS:

Table 10

Sample code	CC50	EC50
Ia	> 123	> 123

Ib	> 123	> 123
Ic	> 121	> 121
Id	> 121	> 121
Ie	> 128	> 128
If	> 128	> 128
Ig	> 128	> 128
Ih	> 127	= 65.1
Ii	> 118	> 118
Ij	> 123	> 123
Il	> 132	> 132
Im	> 117	> 117
In	> 123	> 123
Io	> 166	> 166
Ip	> 145	> 145
Iq	> 140	> 140
Ir	> 152	> 152
Is	> 135	> 135
It	> 130	> 130

It may be appreciated by those skilled in the art that the drawings, examples and detailed description herein are to be regarded in an illustrative rather than a restrictive manner.