

N-ARYL BENZAMIDE DERIVATIVES AS P2X7 RECEPTOR ANTAGONISTS

FIELD OF THE INVENTION

The present invention relates to N-Aryl benzamide derivatives of formula (I), or their isotopic forms, stereoisomers, or a pharmaceutical acceptable salt thereof, as P2X7 receptor antagonists. The present invention also describes the method of making such compounds, pharmaceutical compositions comprising such compounds, and their use in the treatment or prophylaxis of diseases or disorders related to P2X7 receptor activity.

BACKGROUND OF THE INVENTION

P2X7 receptor (P2X7R) belongs to the family of purinergic P2X ionotropic receptors. It is activated by extracellular nucleotides, notably adenosine triphosphate (ATP). P2X7R are ligand-gated ion channel receptors and are present on a variety of cell types, largely those known to be involved in the inflammatory and/or immune process, specifically, macrophages, mast cells and lymphocytes (T and B). Activation of the P2X7R by extracellular nucleotides, such as ATP leads to the release of interleukin-10 (IL-10) and giant cell formation (macrophages/microglial cells), degranulation (mast cells) and L-selectin shedding (lymphocytes). The P2X7R are also known to be a pain sensor in the nervous system. Experiments using P2X7R deficient mice demonstrated the role of P2X7R in the development of pain as these mice were protected from the development of both adjuvant-induced inflammatory pain and partial nerve ligation induced neuropathic pain. There is also growing evidence that P2X7R or their downstream effectors, such as IL-10, are involved in the pathophysiology of several neurological disorders, such as Alzheimer's disease (J.I. Diaz-Hernandez et al., *Neurobiology of Aging*. 2012 Aug; 33(8):1816-1828). P2X7R are thought to have an important function in neurotransmission within the central nervous system (CNS) through its activation on postsynaptic and/or presynaptic neurons and glia. In rat, areas of high P2X7R mRNA expression were found in the anterior olfactory nucleus, cerebral cortex, piriform cortex, lateral septal nucleus, and hippocampal pyramidal cell layers of CA1, CA3, CA4, pontine nuclei, external cuneate nucleus, and medial vestibular nucleus. P2X7R hybridization signals were also observed in the motor neurons of the trigeminal motor nucleus, facial nucleus, hypoglossal nucleus, and the anterior horn of the spinal cord.

Hence, there is a therapeutic rationale for the use of P2X7R antagonists in the treatment of a variety of disease states. These states include but are not limited to diseases or disorders related to CNS such as Alzheimer's disease, Parkinson's disease, Huntington's

disease, spinal cord injury, cerebral ischemia, head trauma, meningitis, post-traumatic stress disorder, epilepsy, traumatic brain injury, sleep disorder associated with neuropathic pain, psychiatric disorders, amyotrophic lateral sclerosis, multiple sclerosis and HIV-induced neuroinflammation. Furthermore, peripheral inflammatory disorders and autoimmune diseases including but not limited to rheumatoid arthritis, osteoarthritis, psoriasis, allergic dermatitis, asthma, chronic obstructive pulmonary disease, airways hyper-responsiveness, septic shock, bronchitis, glomerulonephritis, irritable bowel syndrome, fatty liver disease, liver fibrosis, skin injury, lung emphysema, muscular dystrophy, fibrosis, atherosclerosis, bum injury, Crohn's disease, ulcerative colitis, osteoporosis, acute kidney disease, endocrine or hormonal disorder are all examples where the involvement of P2X7R have been implicated. In view of the clinical importance of P2X7R, the identification of compounds that modulate P2X7 receptor function represents an attractive avenue for the development of new therapeutic agents.

WO2004/099146 discloses benzamide inhibitors of the P2X7R and their use in the treatment of inflammatory diseases.

WO2008/138876 discloses pyrazole derivatives as P2X7R modulators and their use in the treatment or prevention of disorders or diseases mediated by the P2X7R, for example pain, inflammation or a neurodegenerative disease.

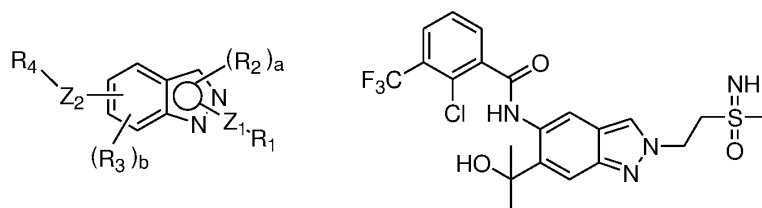
WO2009/108551 discloses heteroaryl amide analogs and their use in P2X7R mediated conditions.

WO2009/132000 discloses quinoline and isoquinoline substituted P2X7R antagonists and their use in P2X7R mediated conditions.

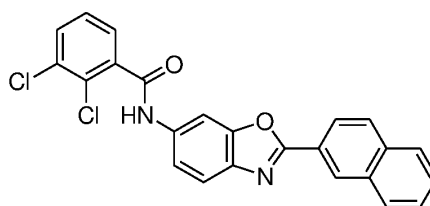
WO2018/202694 discloses oxadiazolinone compounds as P2X7R antagonists and their use in P2X7R mediated conditions.

The following patents disclose compounds that exhibit structural similarities with the compounds of the present invention however for different target/indication.

CN111362920 discloses heterocyclic compound intermediate, preparation methods thereof and application thereof as IRAK inhibitors. Following are the Markush structure and example reported therein.

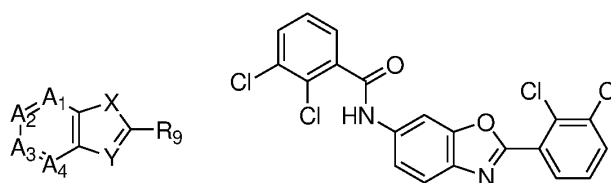


WO2019/200310 discloses compounds reported therein as E3 ubiquitin ligase agonists, pharmaceutical compositions including the E3 ubiquitin ligase agonists, related methods of use. Following structure is reported therein.



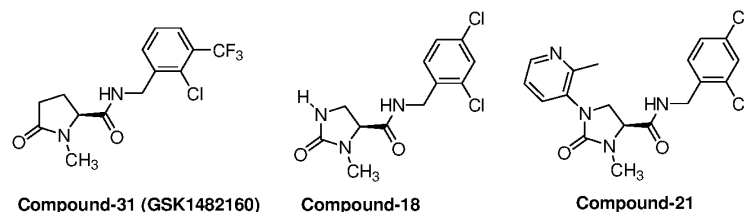
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WO2007/091106 discloses compounds reported therein for the treatment of Duchenne muscular dystrophy. Following structure is reported therein.



There is still an unmet need for compounds which are able to efficiently antagonize P2X7R and that can be delivered to the different target organs which are sites of P2X7R mediated pathology, including the brain. Many P2X7R compounds either lack sufficient CNS penetration or sufficient rodent P2X7R potency for testing in preclinical models, a common issue with P2X7R antagonists due to poor brain penetration and wide differences in receptor homology across species (*Front Cell Neurosci.* 2021 Apr 6;15:617036). The P2X7R antagonist compound 31 (GSK1482160), with a human pIC_{50} of 8.5 and a rat pIC_{50} of 6.5 were reported recently. While compound 31 is 100-fold less active in rat, efficacy was still observed in a rat model of Freund's complete adjuvant (FCA)-induced centralized inflammatory knee joint pain and in a chronic constriction injury model of neuropathic pain. Exposure at the minimal effective doses provided free drug fractions in excess of the rat pIC_{50} in both the periphery and the CNS (*Bioorg. Med. Chem. Lett.* 2010, 20, 5080–5084). The importance of CNS activity in the FCA induced hypersensitivity in the knee joint model of chronic inflammatory pain was supported by a comparative study of 2-oxo-4-

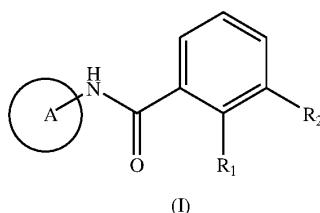
imidazolidinecarboxamide analogs 18 and 21 differing in their brain penetration capabilities. Compound 21 which was inactive in the FCA model had superior free plasma exposure and P2X7R potency, but poor brain penetration. On the other hand, the less potent, brain penetrant P2X7R antagonist, compound 18 showed efficacy in this knee joint pain model (*Bioorg. Med. Chem. Lett.* 2010, 20, 6370–6374).



Compounds of the present invention are highly brain penetrant and showed efficacy in diabetic and chronic constriction injury model of neuropathic pain.

SUMMARY OF THE INVENTION

In first aspect, the present invention relates to a compound of formula (I), or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof,

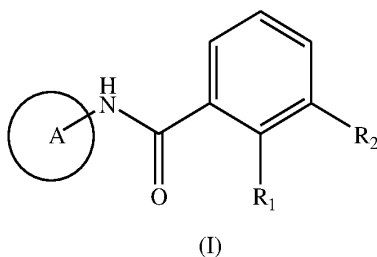


wherein,

Ring A is selected from:

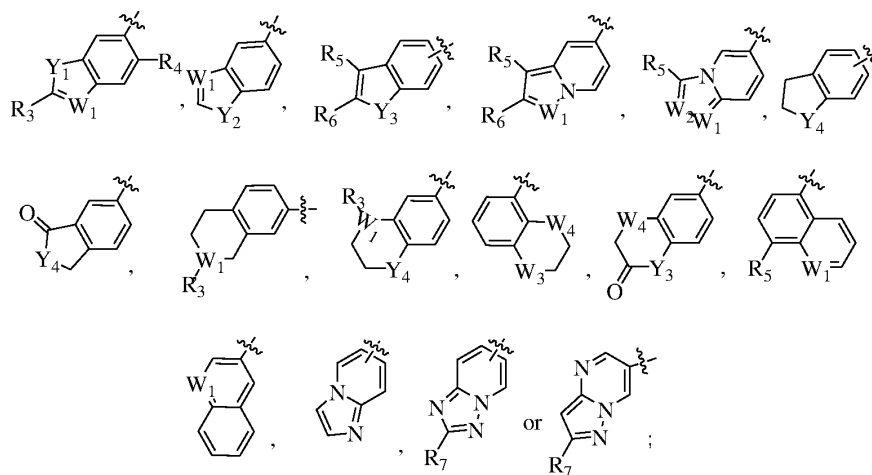
- (i) 9 to 10 membered heterocyclyl comprising one to three heteroatoms selected from N, O and S,
 - (ii) 9 to 10 membered aryl, or
 - (iii) 9 to 10 membered non-aromatic carbocyclyl; wherein the said 9 to 10 membered heterocyclyl, 9 to 10 membered aryl and 9 to 10 membered non-aromatic carbocyclyl is unsubstituted or substituted with one or two groups selected from halogen, $-(C_{1-6})$ -alkyl, $-O-(C_{1-6})$ -alkyl, carbonyl, cyano, $-(C_{1-6})$ -alkyl- $O-(C_{1-6})$ -alkyl, and $-C(O)O-(C_{1-6})$ -alkyl;
- R_1 is selected from CF_3 or halogen;
- R_2 is selected from CF_3 or halogen;

In another aspect, the present invention relates to a compound of formula (I), or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof,



wherein,

- 5 Ring A is selected from,



wherein,

- 10 “” represents the point of attachment;
 R₁ is selected from halogen;
 R₂ is selected from CF₃ or halogen;
 R₃ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl, -(C₁₋₆)-alkyl-O-(C₁₋₆)-alkyl, -C(O)O-(C₁₋₆)-alkyl or cyano;
 15 R₄ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;
 R₅ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;
 R₆ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;
 R₇ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;
 W₁ is N or CH;
 20 W₂ is N or CH;

W₃ is CH₂ or O;

W₄ is CH₂, N or O;

Y₁ is CH₂, NH, O or S;

Y₂ is CH₂, NH, O or S;

5 Y₃ is NH or CH₂; and

Y₄ is CH₂, NH, O or S.

In another aspect, the present invention provides a process for preparing the compound of formula (I), or an isotopic form, a stereoisomer or a pharmaceutically acceptable salt thereof.

10 In another aspect, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of the compound of formula (I), or an isotopic form, a stereoisomer or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable excipients.

In another aspect, the present invention provides a compound of formula (I), or an isotopic form, a stereoisomer or a pharmaceutically acceptable salt thereof, for use as P2X7R antagonist.

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are P2X7R antagonists, methods of making such compounds, pharmaceutical compositions comprising such compounds, and method of treating diseases or disorder related to P2X7R activity.

Definitions

Unless otherwise stated, the following terms used in the specification and claims have the meanings given below:

The term, “-(C₁₋₆)-alkyl” as used herein refers to a branched or straight-chain aliphatic hydrocarbon containing one to six carbon atoms. Non-limiting examples of -(C₁₋₆)-alkyl include methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl and *tert*-butyl.

The term “-O-(C₁₋₆)-alkyl” as used herein refers to an -(C₁₋₆)-alkyl group as defined above that is bonded to the oxygen atom. Non-limiting examples of the -O-(C₁₋₆)-alkyl include methoxy, ethoxy, propyloxy, and isopropyloxy.

30 The term “-(C₁₋₆)-alkyl-O-(C₁₋₆)-alkyl” as used herein refers to two -(C₁₋₆)-alkyl groups as defined above are bonded by oxygen atom. Non-limiting examples of -(C₁₋₆)-alkyl-O-(C₁₋₆)-alkyl include methoxyethane, ethoxypropane, butoxymethane, hexoxyethane, and propoxybutane.

The term “-C(O)O-(C₁₋₆)-alkyl” as used herein refers to -(C₁₋₆)-alkyl groups as defined above bonded to the -C(O)O- group. Non-limiting examples of -C(O)O-(C₁₋₆)-alkyl include methyl ester, ethyl ester, isopropyl ester, *sec*-butyl ester and *tert*-butyl ester.

The term “9 to 10 membered heterocyclyl” as used herein refers to 9 or 10 membered bicyclic aromatic or non-aromatic heterocyclic rings containing one to three hetero atoms selected from N, O, and S. Non-limiting examples of 9 to 10 membered heterocyclyl include imidazopyridine, benzoimidazole, benzthiazole, benzoxazole, indole, pyrazolopyridine, triazolopyridine, benzofuran, dihydro benzofuran, dihydro isobenzofuran, quinolone, isoquinoline, dihydroisoquinoline, oxotetrahydroquinoline, tetrahydroquinoline, tetrahydroisoquinoline, dihydrobenzoxazine, dihydrobenzodioxine, and benzodihdropyran.

The term “9 to 10 membered aryl” as used herein refers to bicyclic aromatic ring comprising 9 to 10 carbon atoms for example indane, and naphthalene.

The term “9 to 10 membered non-aromatic carbocyclyl” as used herein refers to bicyclic non-aromatic ring comprising 9 to 10 carbon atoms for example indene and tetrahydro naphthalene.

The term, “halogen” as used herein refers to fluorine, chlorine, bromine or iodine. Preferably, halogen is fluorine, chlorine or bromine.

The term, “isotopic form” as used herein refers to the compound of formula (I) wherein one or more atoms of the compound of formula (I) are substituted by their respective isotopes. For example, isotopes of hydrogen include ²H (deuterium) and ³H (tritium).

The term, “stereoisomer” as used herein refers to isomers of the compound of formula (I) that differ in the arrangement of their atoms in space. Compounds disclosed herein may exist as a single stereoisomer, racemates and/or mixtures of enantiomers and/or diastereomers. All such single stereoisomer, racemates and mixtures thereof are intended to be within the scope of the present invention.

The term, “pharmaceutically acceptable salt” as used herein refers to salts of the active compound *i.e.* the compound of formula (I), and are prepared by reaction with the appropriate acid or acid derivative, depending on the particular substituents found on the compounds described herein.

The phrase, “therapeutically effective amount” is defined as an amount of a compound of the present invention that (i) treats the particular disease, condition or disorder, (ii) eliminates one or more symptoms of the particular disease, condition or disorder, and/or (iii)

delays the onset of one or more symptoms of the particular disease, condition or disorder described herein.

The term, “patient” as used herein refers to an animal. Preferably the term “patient” refers to a mammal. The term mammal includes animals such as mice, rats, dogs, rabbits, pigs,
5 monkeys, horses, pigeons, xenopus laevis, zebrafish, guinea pigs, elephant, camel, chimpanzee and humans. More preferably the patient is human.

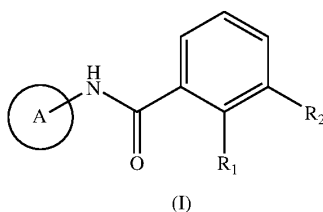
The term “treating” and “treatment”, as used herein, refers to curative, palliative and prophylactic treatment, including reversing, alleviating, inhibiting the progress of, or preventing the disease, disorder or condition to which such term applies, or one or more
10 symptoms of such disease, disorder or condition.

The term “preventive” and “preventing” as used herein, refers to intended to stop or prevent disease, disorder or condition from happening.

Embodiments

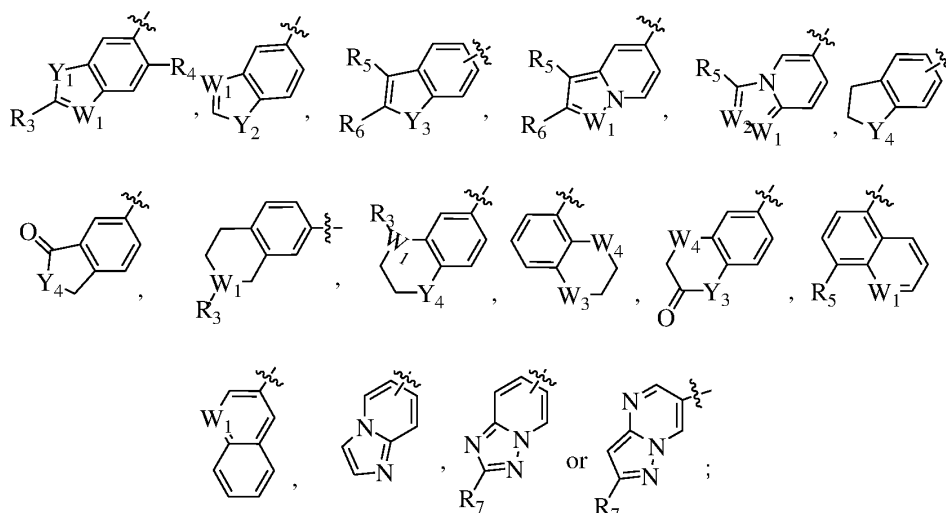
The present invention encompasses all the compounds described by the compound of
15 formula (I) without any limitation, however, preferred aspects and elements of the invention are discussed herein below in the following embodiments.

In one embodiment, the present invention relates to the compound of formula (I) or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof,



20 wherein,

Ring A is selected from,



wherein,

“” represents the point of attachment;

R₁ is selected from halogen;

5 R₂ is selected from CF₃ or halogen;

R₃ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl, -(C₁₋₆)-alkyl-O-(C₁₋₆)-alkyl, -C(O)O-(C₁₋₆)-alkyl or cyano;

R₄ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

R₅ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

10 R₆ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

R₇ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

W₁ is N or CH;

W₂ is N or CH;

W₃ is CH₂ or O;

15 W₄ is CH₂, N or O;

Y₁ is CH₂, NH, O or S;

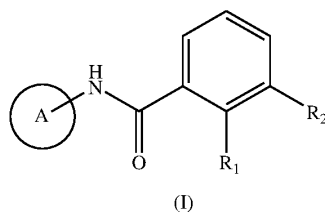
Y₂ is CH₂, NH, O or S;

Y₃ is NH or CH₂; and

Y₄ is CH₂, NH, O or S.

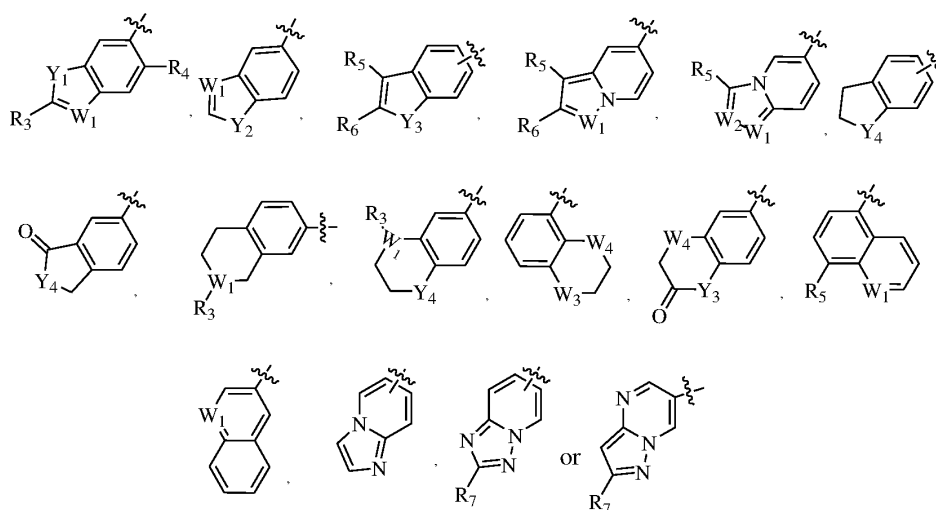
20 In another embodiment, the present invention relates to method of treating or preventing diseases or disorders mediated by P2X7 receptor activity, comprising administering to a patient a therapeutically effective amount of a compound having the

structure of formula (I), or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof,



wherein,

- 5 Ring A is selected from,



wherein,

“” represents the point of attachment;

R₁ is selected from halogen;

- 10 R₂ is selected from CF₃ or halogen;

R₃ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl, -(C₁₋₆)-alkyl-O-(C₁₋₆)-alkyl, -C(O)O-(C₁₋₆)-alkyl or cyano;

R₄ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

R₅ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

- 15 R₆ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

R₇ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

W₁ is N or CH;

W₂ is N or CH;

W₃ is CH₂ or O;

W₄ is CH₂, N or O;

Y₁ is CH₂, NH, O or S;

Y₂ is CH₂, NH, O or S;

Y₃ is NH or CH₂; and

5 Y₄ is CH₂, NH, O or S.

In another embodiment, the present invention relates to the compound of formula (I) or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof, wherein the combination of W₁, W₂, W₃, W₄, Y₁, Y₂, Y₃, and Y₄ in ring A is selected from the group consisting of,

- 10 (i) W₁ = N and Y₁ = NH; W₁ = N and Y₁ = S; or W₁ = N and Y₁ = O;
- (ii) W₁ = N and Y₂ = S; or W₁ = N and Y₂ = O;
- (iii) W₁ = N and W₂ = C; or W₁ = N and W₂ = N;
- (iv) W₁ = N and Y₄ = O;
- (v) W₃ = CH₂ and W₄ = CH₂; W₃ = O and W₄ = O; or W₃ = O and W₄ = CH₂; and
- 15 (vi) W₄ = CH₂ and Y₃ = NH; or W₄ = O and Y₃ = NH.

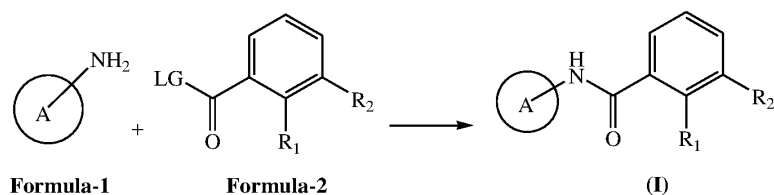
In another embodiment, the present invention relates to the compound of formula (I) or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof is selected from:

- N-imidazo[1,2-*a*]pyridin-6-yl-2-chloro-3-trifluoromethyl-benzamide;
- 20 N-(6-methoxy-3H-benzoimidazol-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
 - N-(6-methoxy-2-methyl-3H-benzoimidazol-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
 - N-Benzothiazol-6-yl-2-chloro-3-trifluoromethyl-benzamide;
 - N-(2-methyl-benzothiazol-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
 - N-Benzothiazol-6-yl-2,3-dichloro-benzamide;
 - 25 N-Benzothiazol-5-yl-2-chloro-3-trifluoromethyl-benzamide;
 - N-Benzothiazol-5-yl-2,3-dichloro-benzamide;
 - N-Benzoxazol-6-yl-2-chloro-3-trifluoromethyl-benzamide;
 - N-(5-chloro-2-methyl-benzoxazol-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
 - N-Benzoxazol-5-yl-2-chloro-3-trifluoromethyl-benzamide;
 - 30 N-Benzoxazol-5-yl-2,3-dichloro-benzamide;
 - N-(1H-indol-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
 - N-(1H-indol-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
 - N-pyrazolo[1,5-*a*]pyridin-5-yl-2-chloro-3-trifluoromethyl-benzamide;

- N-(2,3-dichloro-pyrazolo[1,5-a]pyridin-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-chloro-pyrazolo[1,5-a]pyridin-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-chloro-imidazo[1,2-a]pyridin-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-methyl-[1,2,4]triazolo[4,3-a]pyridin-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
5 N-(2,3-dihydro-benzofuran-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(2,3-dihydro-benzofuran-4-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-oxo-1,3-dihydro-isobenzofuran-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
2-Chloro-N-(1H-indol-4-yl)-3-trifluoromethyl-benzamide;
2-Chloro-N-(3-chloro-1H-indol-4-yl)-3-trifluoromethyl-benzamide;
10 7-(2-chloro-3-trifluoromethyl-benzoylamino)-3,4-dihydro-1H-isoquinoline-2-carboxylic
acid tert-butyl ester;
N-(1,2,3,4-tetrahydro-isoquinolin-7-yl)-2-chloro-3-trifluoromethyl-benzamide
hydrochloride;
N-(1,2,3,4-tetrahydro-isoquinolin-7-yl)-2-chloro-3-trifluoromethyl-benzamide;
15 N-(4-methyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(5,6,7,8-tetrahydro-naphthalen-1-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-chroman-5-yl-2-chloro-3-trifluoromethyl-benzamide;
N-(2-oxo-1,2,3,4-tetrahydro-quinolin-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
20 N-(3-oxo-3,4-dihydro-2H-benzo[1,4]oxazin-7-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(8-chloro-quinolin-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-quinolin-3-yl-2-Chloro-3-trifluoromethyl-benzamide;
N-quinolin-3-yl-2-chloro-3-fluoro-benzamide; and
N-quinolin-3-yl-3-chloro-2-fluoro-benzamide.

25 **Experimental procedure:**

The below scheme depicts a general process for the preparation of the compound of formula (I), wherein, Ring A, R1 and R2 are as defined above and LG is a suitable leaving group such as halogen (e.g. chloro, bromo), imidazole, hydroxy, alkoxy, anhydride and the like reactive leaving groups.

Scheme**Preparation of the compound of formula (I)**

The compound of formula (I) can be prepared by using the following acid-amine coupling reactions:

(1) Acid-amine coupling reaction by activating the carboxylic group:

The compound of formula (I) is obtained by the reaction of a compound of formula-1 with a compound of formula-2 in a suitable solvent, such as dichloromethane, 1,2-dichloroethane, acetonitrile, THF, 1,4-dioxane, DMF or DMSO in the presence of suitable base such as triethylamine, diisopropylethylamine, K_2CO_3 or $NaOCH_3$ and coupling agents such as HATU, TBTU, EDCI, CDI, or ethylchloroformate. The reaction can be carried out at a temperature ranging from 0 °C to the reflux temperature of the solvent used in the reaction, preferably at room temperature. The reaction can be completed in 1 to 24 hours, preferably in 16 hours.

(2) Acid-amine coupling reaction by converting carboxylic acid to respective acid chloride:

The compound of formula-2 in chlorinating reagents like oxalyl chloride, thionyl chloride or cyanuric chloride alone or in a solvent selected from DCM, DCE, DMF or DMSO was stirred at a temperature ranging from 0 °C to 85 °C or at the reflux temperature of the solvent used preferably at r.t. The reaction can be completed in 1 to 24 hours, preferably in 1 hour. The volatiles can be evaporated under reduced pressure to obtain the corresponding acid chloride. The crude acid chloride can be dissolved in solvents like DCM or DCE, treated with bases selected from Et_3N , DIPEA, $NaHCO_3$, K_2CO_3 , etc., followed by a compound of formula-1 at a temperature ranging from 0 °C to 85 °C or at the reflux temperature of the solvent used preferably at room temperature. The reaction can be completed in 1 to 24 hours, preferably in 1 hour. The volatiles can be evaporated under reduced pressure to obtain the compound of formula (I).

Preparation of pharmaceutically acceptable salt of the compound of formula (I)

The compound of formula (I) can optionally be converted into its pharmaceutically acceptable salt by reaction with the appropriate acid or acid derivative. Suitable pharmaceutically acceptable salts will be apparent to those skilled in the art. The salts are formed with inorganic acids e.g., hydrochloric, dihydrochloric, hydrobromic, sulfuric, nitric or phosphoric acid, or organic acids e.g., oxalic, succinic, maleic, acetic, propanoic, hydroxyacetic, phenylacetic, stearic, glycolic, oleic, palmitic, lauric, mandelic, lactic, pyruvic, fumaric, citric, malic, tartaric, benzoic, p-toluic, p-toluenesulfonic, benzenesulfonic acid, methanesulfonic, trifluoromethanesulfonic, ethanesulfonic or naphthalene sulfonic acid.

10 Preparation of stereoisomers of the compound of formula (I)

The stereoisomers of the compounds of formula (I) may be prepared in one or more conventional ways presented below:

- a. One or more of the reagents may be used in their optically active form.
- b. Optically pure catalyst or chiral ligands along with metal catalyst may be employed in the reduction process. The metal catalyst may be rhodium, ruthenium, indium, and the like. The chiral ligands may preferably be chiral phosphines.
- c. The mixture of stereoisomers may be resolved by conventional methods such as forming diastereomeric salts with chiral acids or chiral amines or chiral amino alcohols, or chiral amino acids. The resulting mixture of diastereomers may then be separated by methods such as fractional crystallization, chromatography, and the like, which is followed by an additional step of isolating the optically active product from the resolved material salt.
- d. The mixture of stereoisomers may be resolved by conventional methods such as microbial resolution, resolving the diastereomeric salts formed with chiral acids or chiral bases. Chiral acids that can be employed may be tartaric acid, mandelic acid, lactic acid, camphorsulfonic acid, amino acids, and the like. Chiral bases that can be employed may be cinchona alkaloids, brucine or a basic amino acid such as lysine, arginine, and the like.

In one embodiment, the present invention relates to the compound of formula (I) or an isotopic form, a stereoisomer or a pharmaceutically acceptable salt thereof, for use in the antagonism of P2X7R.

In another embodiment, the present invention relates to a method of treating or preventing diseases or disorders mediated by the P2X7R in a patient, comprising administering to the patient in need thereof a therapeutically effective amount of the compound of formula (I) or an isotopic form, a stereoisomer or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method of treating or preventing diseases or disorders selected from CNS related disorders, acute kidney disease, endocrine or hormonal disorders, neurodegenerative disorders, psychiatric disorders, neuropathic pain, chronic pain, acute pain, cancer, headaches and inflammatory process of musculoskeletal system, comprising administering to the patient in need thereof a therapeutically effective amount of the compound of formula (I), or an isotopic form, a stereoisomer or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to the compound of formula (I) or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof for use in the treatment or prevention of diseases or disorders selected from CNS related disorders, acute kidney disease, endocrine or hormonal disorders, neurodegenerative disorders, psychiatric disorders, neuropathic pain, chronic pain, acute pain, cancer, headaches, and inflammatory process of musculoskeletal system.

In another embodiment, the present invention relates to the compound of formula (I) or an isotopic form, a stereoisomer or a pharmaceutically acceptable salt thereof, for use in the treatment of diseases or disorders mediated by P2X7R, wherein the disease or disorder is selected from the group consisting of CNS related disorders, acute kidney disease, endocrine or hormonal disorders, neurodegenerative disorders, psychiatric disorders, neuropathic pain, chronic pain, acute pain, cancer, headaches, and inflammatory process of musculoskeletal system.

In another embodiment, the present invention relates to the use of the compound of formula (I) or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment or prevention of diseases or disorders selected from CNS related disorders, acute kidney disease, endocrine or hormonal disorders, neurodegenerative disorders, psychiatric disorders, neuropathic pain, chronic pain, acute pain, cancer, headaches, and inflammatory process of musculoskeletal system.

In another embodiment, the present invention relates to the pharmaceutical composition comprising a therapeutically effective amount of the compound of formula (I) or

an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable excipients, for use in the treatment or prevention of diseases or disorders selected from CNS related disorders, acute kidney disease, endocrine or hormonal disorders, neurodegenerative disorders, psychiatric disorders, neuropathic pain, chronic pain,
5 acute pain, cancer, headaches, and inflammatory process of musculoskeletal system.

In some embodiments, the CNS related disorders are selected from Alzheimer's disease, Parkinson's disease, Huntington's disease, spinal cord injury, cerebral ischemia, head trauma, meningitis, post-traumatic stress disorder, and sleep disorder associated with neuropathic pain.

10 In some embodiments, the acute kidney disease is renal ischemia-reperfusion injury.

In some embodiments, the endocrine or hormonal disorder is polycystic ovary syndrome.

In some embodiments, the neurodegenerative disorder is selected from amyotrophic lateral sclerosis, multiple sclerosis, and HIV-induced neuroinflammation.

15 In some embodiments, the psychiatric disorder is selected from major depression, dysthymia, mania, bipolar disorder such as bipolar disorder type I and bipolar disorder type II, cyclothymic disorder, rapid cycling, ultradian cycling, hypomania, schizophrenia, schizophreniform disorders, schizoaffective disorders, personality disorders, attention disorders with or without hyperactive behaviour, delusional disorders, brief psychotic
20 disorders, shared psychotic disorders, psychotic disorder due to a general medical condition, substance-induced psychotic disorders, psychotic disorder not otherwise specified, anxiety disorders such as generalized anxiety disorder, panic disorders, post-traumatic stress disorder, impulse control disorders, phobic disorders, dissociative states and moreover in smoke, drug addiction or alcoholism. In some embodiments, the psychiatric disorder is selected from
25 bipolar disorders, psychosis, anxiety or addiction.

In some embodiments, the neuropathic pain is selected from diabetic neuropathy, sciatica, non-specific lower back pain, multiple sclerosis pain, fibromyalgia, HIV-related neuropathy, and neuralgia.

In some embodiments, the chronic pain is selected from chronic pain caused by
30 inflammation or an inflammatory-related condition, osteoarthritis, rheumatoid arthritis, acute injury or trauma, upper back pain or lower back pain resulting from systematic, regional or primary spine disease such as radiculopathy, bone pain due to osteoarthritis, osteoporosis, bone metastasis or unknown reasons, pelvic pain, spinal cord injury-associated pain, cardiac

chest pain, non-cardiac chest pain, central post-stroke pain, myofascial pain, sickle cell pain, cancer pain, geriatric pain, pain caused by headache, gout, and fibrosis.

In some embodiments, the acute pain is selected from acute injury, illness, sport-medicine injuries, musculoskeletal sprains and strains, pain related to surgery such as open heart or bypass surgery, post-operative pain, kidney stone pain, gallbladder pain, gallstone pain, obstetric pain or dental pain.

In some embodiments, the cancer is selected from the prostate cancer, breast cancer, lung cancer, ovarian cancer, pancreatic cancer, intestinal cancer, colon cancer, gastric cancer, skin cancer, brain tumor, leukemia, or lymph cancer.

In some embodiments, the headache is selected from migraine, tension type headache, transformed migraine, evolutive headache, and cluster headache.

The compounds of the invention inhibit inflammatory processes affecting all body systems. Therefore, are useful in the treatment of inflammatory processes of the musculoskeletal system. In some embodiments, the inflammatory process of the musculoskeletal system include, but are not limited to, arthritic conditions such as ankylosing spondylitis, cervical arthritis, fibromyalgia, gout, juvenile rheumatoid arthritis, lumbosacral arthritis, osteoarthritis, osteoporosis, psoriatic arthritis, rheumatic disease; disorders affecting skin and related tissues such as eczema, psoriasis, dermatitis and inflammatory conditions.

In another embodiment, the present invention relates to the pharmaceutical compositions comprising the compound of formula (I) or an isotopic form, a stereoisomer or a pharmaceutically acceptable salt thereof, and pharmaceutically acceptable excipients. To use the compound of formula (I) or their stereoisomers and a pharmaceutically acceptable salt thereof in therapy, they will normally be formulated into a pharmaceutical composition in accordance with standard pharmaceutical practice.

The pharmaceutical compositions of the present invention may be formulated in a conventional manner using one or more pharmaceutically acceptable excipients are well known in the art.

In another embodiment, the compounds of the invention may be formulated in the form of pills, tablets, coated tablets, capsules, powder, granules, pellets, patches, implants, films, liquids, semi-solids, gels, aerosols, emulsions, elixirs and the like. Such pharmaceutical compositions and processes for preparing the same are well known in the art.

The following abbreviations are used herein:

	AUC	:	Area under curve
	Boc	:	tert-Butyloxycarbonyl
	CaCl ₂	:	Calcium chloride
5	CDI	:	1,1'-Carbonyldiimidazole
	CO ₂	:	Carbon dioxide
	cIC ₅₀	:	Corrected Half maximal inhibitory concentration
	C _{max}	:	Maximum concentration
	DCE	:	1,2-Dichloroethane
10	DCM	:	Dichloromethane
	DIPEA	:	N,N-Diisopropylethylamine
	DMF	:	N,N-Dimethylformamide
	DMSO	:	Dimethyl sulfoxide
	DMAP	:	4-Dimethylaminopyridine
15	EDCI	:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
	EtOAc	:	Ethyl acetate
	Et ₃ N	:	Triethyl amine
	H ₂ O	:	Water
	h	:	Hour(s)
20	HATU	:	1-[Bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-Oxide Hexafluorophosphate
	HEPES	:	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
	IC ₅₀	:	Half maximal inhibitory concentration
	IPA	:	Isopropanol
25	K ₂ CO ₃	:	Potassium carbonate
	KCl	:	Potassium chloride
	MeOH	:	Methanol
	NaCl	:	Sodium chloride
	NaOH	:	Sodium hydroxide
30	NaHCO ₃	:	Sodium bicarbonate
	Na ₂ SO ₄	:	Sodium sulfate
	NaOCH ₃	:	Sodium methoxide

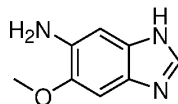
	NH ₄ Cl	:	Ammonium chloride
	NCS	:	N-Chlorosuccinimide
	pIC ₅₀	:	A negative logarithmic expression of the half maximal inhibitory concentration (IC ₅₀)
5	r.t	:	Room temperature (25 °C to 30 °C)
	SnCl ₂	:	Stannous chloride
	TBTU	:	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
	THF	:	Tetrahydrofuran
10	t _{1/2}	:	Half-life time
	<i>i.m</i>	:	<i>Intramuscular</i>
	μL	:	Microliter
	μM	:	Micromolar
	ng/mL	:	Nanograms per <i>milliliter</i>
15	<i>p.o</i>	:	Per oral
	<i>s.c</i>	:	Subcutaneous

EXAMPLES

Most of the aromatic amines used in the preparation of the compounds were obtained from commercial vendors. For those amines not commercially available, synthesis was carried out following the experimental procedures detailed below, using appropriate materials and conditions.

Preparation of Intermediates:

Intermediate-1-1: Synthesis of 6-methoxy-3H-benzoimidazol-5-ylamine



Intermediate-1-1

Step-1: Synthesis of N-(2-methoxy-4-nitro-phenyl)-acetamide

To the stirred solution of 2-methoxy-4-nitro-phenylamine (1.5 g, 8.9 mmol) in DCM (36.0 mL) cooled to 0 °C, acetic anhydride (1.26 mL, 11.4 mmol) was added. The reaction mixture was gradually warmed to r.t. and was stirred for 16 h. Upon completion of the reaction, the

reaction mixture was diluted with water and DCM and the two layers were separated. The organic layer was washed with brine solution, dried over anhydrous Na₂SO₄ and evaporated under reduced pressure to obtain *N*-(2-methoxy-4-nitro-phenyl)-acetamide (1.72 g) in 92% yield. ¹H - NMR (400 MHz, CDCl₃): δ 8.58 (d, *J* = 8.8 Hz, 1H), 7.97 (bs, 1H), 7.92 (dd, *J* = 2.0 Hz, 9.2 Hz, 1H), 7.74 (d, *J* = 1.6 Hz, 1H), 4.00 (s, 3H), 2.26 (s, 3H). Mass (m/z): 211.1(M+H)⁺.

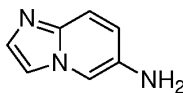
Step-2: Synthesis of *N*-(2-methoxy-4,5-dinitro-phenyl)-acetamide

To a stirred solution of *N*-(2-methoxy-4-nitro-phenyl)-acetamide (1.72 g, 8.1 mmol) obtained in the above step cooled to 0 °C, fuming nitric acid (3.30 mL) was added. The reaction mixture was stirred for an additional 1 h at 0 °C. The reaction mixture was diluted with ice-cold water and extracted with EtOAc. The combined organic layer was washed with water, brine solution, dried over anhydrous Na₂SO₄ and evaporated under reduced pressure to obtain *N*-(2-Methoxy-4,5-dinitro-phenyl)-acetamide (1.62 g) in 81% yield. ¹H - NMR (400 MHz, CDCl₃): δ 9.03 (s, 1H), 7.96 (bs, 1H), 7.40 (s, 1H), 4.07 (s, 3H), 2.28 (s, 3H). Mass (m/z): 254.3 (M-H)⁺.

Step-3: Synthesis of 6-methoxy-3H-benzoimidazol-5-ylamine

To the stirred solution of *N*-(2-methoxy-4,5-dinitro-phenyl)-acetamide (0.3 g, 1.2 mmol) in formic acid (3.6 mL) at r.t, SnCl₂·2H₂O (2.36 g, 10.5 mmol) was added. The reaction mixture was heated to reflux for 4 h. The volatiles were evaporated under reduced pressure. The residue was diluted with 10% HCl solution (10.0 mL) and refluxed the contents for 1 h. The reaction mixture was cooled to 0 °C, basified with 2.5 N NaOH solution to pH 8.0. The contents were extracted with EtOAc and the combined organic layer was dried over anhydrous Na₂SO₄ and evaporated under reduced pressure to obtain 6-methoxy-3H-benzoimidazol-5-ylamine (0.14 g) in 72% yield. ¹H - NMR (400 MHz, CDCl₃): δ 7.82 (s, 1H), 7.10 (s, 1H), 6.91 (s, 1H), 3.95 (s, 3H). Mass (m/z): 164 (M+H)⁺.

Intermediate-1-2: Synthesis of 6-amino-imidazo[1,2-a]pyridine



Intermediate-1-2

Step-1: Synthesis of 6-nitro-imidazo[1,2-a]pyridine

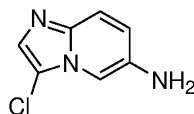
To a stirred solution of 2-amino-5-nitro-pyridine (2.0 g, 14.4 mmol) in ethanol (28.0 mL) at r.t, a 55% aqueous solution of chloroacetaldehyde (4.1 mL) was added. The reaction mixture

was gradually heated to reflux for 6 h. The volatiles were removed under reduced pressure, the crude product was triturated with EtOAc. The combined organic layer was evaporated under reduced pressure to obtain 6-nitro-imidazo[1,2-a]pyridine (2.84 g) in quantitative yield. ¹H - NMR (400 MHz, DMSO-d₆): δ 10.09 (s, 1H), 8.41 (s, 1H), 8.35 (d, *J* = 9.6 Hz, 1H), 8.17 (s, 1H), 8.01 (d, *J* = 10 Hz, 1H). Mass (m/z): 164.0 (M+H)⁺.

Step-2: Synthesis of 6-amino-imidazo[1,2-a]pyridine

To a stirred solution of 6-nitro-imidazo[1,2-a]pyridine (1.51 g, 9.3 mmol) in a 1:2 mixture of water (12.0 mL) and ethanol (24.0 mL) at r.t, iron powder (1.96 g, 35.1 mmol) and NH₄Cl (0.73 g, 13.9 mmol) was added. The reaction mixture was then refluxed for 2 h. The volatiles were removed under reduced pressure and the crude product was triturated with 1:9 mixture of MeOH and DCM. The combined organic layer was dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure to obtain 6-amino-imidazo[1,2-a]pyridine (1.1 g) in 87% yield. ¹H - NMR (400 MHz, DMSO-d₆): δ 8.09 (s, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.48 (s, 1H), 7.44 (s, 1H), 6.63 (d, *J* = 8.5 Hz, 1H), 4.21 (bs, 2H). Mass (m/z): 134.0 (M+H)⁺.

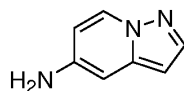
Intermediate-1-3: Synthesis of 3-chloro-6-amino-imidazo[1,2-a]pyridine



Intermediate-1-3

To a stirred solution of 6-amino-imidazo[1,2-a]pyridine (120.0 mg, 0.9 mmol) in 1,2-dichloroethane (4.0 mL), N-chlorosuccinimide (134.0 mg, 1.0 mmol) was added and the reaction mixture was refluxed for 2 h. The reaction mass was cooled to r.t, and diluted with DCM and water. The two layers were separated, the organic layer was washed with brine solution and dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure to obtain 3-chloro-6-amino-imidazo[1,2-a]pyridine (0.15 g) in 97% yield. ¹H - NMR (400 MHz, DMSO-d₆): δ 8.09 (s, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.44 (s, 1H), 6.63 (d, *J* = 8.5 Hz, 1H), 4.21 (bs, 2H). Mass (m/z): 167.2, 169.1 (M+H)⁺.

Intermediate-1-4: Synthesis of pyrazolo[1,5-a]pyridin-5-ylamine



Intermediate-1-4

Step-1: Synthesis of tert-butyl pyridin-4-yl-carbamate

To the stirred solution of 4-aminopyridine (2.0 g, 27.4 mmol) in THF (42.0 mL) at r.t, Et₃N (4.5 mL, 32.6 mmol), DMAP (0.52 g, 4.3 mmol) were added followed by dropwise addition of Boc anhydride (5.36 mL, 18.6 mmol). The reaction mixture was stirred at r.t, for 16 h. The volatiles were removed under reduced pressure to obtain a crude mixture which was diluted with DCM, and washed with dilute HCL (0.1N, 2x50 mL), aqueous NaHCO₃, and brine solution. The organic layer was dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure to obtain tert-butyl pyridin-4-yl-carbamate (3.0 g) in 74% yield. ¹H - NMR (400 MHz, CDCl₃): δ 8.44 (d, *J* = 5.6 Hz, 2H), 7.31 (d, *J* = 5.6 Hz, 2H), 6.86 (bs, 1H), 1.52 (s, 9H). Mass (m/z): 195.2 (M+H)⁺.

Step-2: Synthesis of 1-amino-4-tert-butoxycarbonylamino-pyridinium-2,4,6-trimethylbenzenesulfonate

To the stirred solution of amino 2,4,6-trimethylbenzene-1-sulfonate (0.56 g, 2.6 mmol) in DCM (20.0 mL) cooled at 0 °C, tert-butyl pyridin-4-yl-carbamate (0.5 g, 2.6 mmol) portion wise was added. The reaction mixture was gradually warmed to r.t, and stirred for 16 h. The volatiles were removed under reduced pressure to obtain the above titled compound (1.2 g) in quantitative yield. δ ¹H - NMR (400 MHz, DMSO-d₆): δ 9.12 (d, *J* = 5.6 Hz, 2H), 7.98 (bs, 1H), 7.67 (d, *J* = 5.6 Hz, 2H), 6.94 (s, 2H), 2.40 (s, 9H), 2.10 (bs, 2H), 1.53 (s, 9H). Mass (m/z): 210.1 (M+H)⁺.

Step-3: Synthesis of ethyl-5-tert-butoxycarbonylamino-pyrazolo[1,5-a]pyridine-3-carboxylate

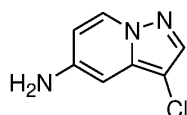
To the stirred solution of step-2 compound as obtained above (1.2 g, 2.6 mmol) in DMF (7.0 mL) cooled to 0 °C, K₂CO₃ (0.8 g, 5.8 mmol) followed by ethyl propiolate (0.3 mL, 3.0 mmol) were added. The reaction mixture was stirred at r.t, for 16 h. The reaction mixture was diluted with water and EtOAc. The two layers were separated and the organic layer was washed with brine solution, dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure to obtain the above titled compound (0.3 g) in 34% yield. ¹H - NMR (400 MHz, CDCl₃): δ 8.39 (d, *J* = 7.6 Hz, 1H), 8.32 (s, 1H), 7.96 (d, *J* = 1.6 Hz, 1H), 7.30 (d, *J* = 6.8 Hz, 1H), 6.76 (s, 1H), 4.39-4.34 (q, 2H), 1.54 (s, 9H), 1.42 (t, 3H). Mass (m/z): 306.1 (M+H)⁺.

Step-4: Synthesis of pyrazolo[1,5-a]pyridin-5-ylamine

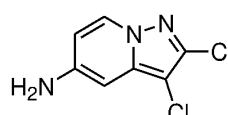
The stirred solution of step-3 compound as obtained above (0.29 g, 0.95 mmol) in aqueous H₂SO₄ (40% w/v, 1.2 mL) was refluxed for 4 h. The reaction mixture was cooled to r.t, diluted with ice cold water, basified with 20% aqueous NaOH solution, and extracted with EtOAc.

The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to obtain the above titled compound (0.06 g) in 86% yield. ^1H - NMR (400 MHz, CDCl_3): δ 8.24 (d, $J = 7.2$ Hz, 1H), 7.99 (s, 1H), 6.58 (d, $J = 1.6$ Hz, 1H), 6.24 (dd, $J = 2.0$ Hz, 7.2 Hz, 1H), 6.13 (s, 1H), 3.82 (bs, 2H). Mass (m/z): 134.1 ($\text{M}+\text{H}$) $^+$.

5 **Intermediate-1-5 and 1-6: Synthesis of 3-chloro-pyrazolo[1,5-a]pyridin-5-ylamine and 2,3-dichloro-pyrazolo[1,5-a]pyridin-5-ylamine**



Intermediate-1-5



Intermediate-1-6

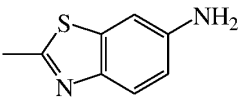
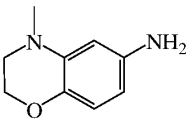
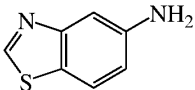
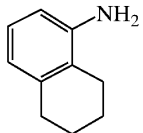
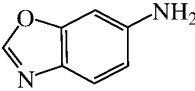
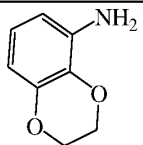
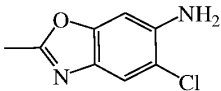
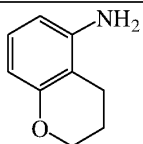
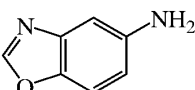
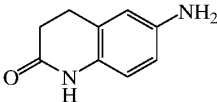
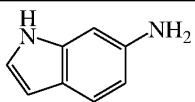
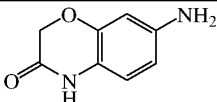
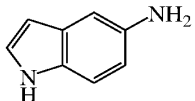
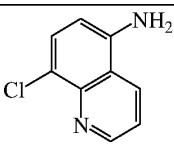
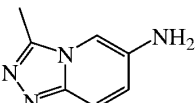
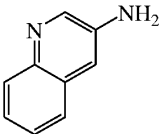
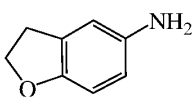
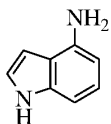
To the stirred solution of pyrazolo[1,5-a]pyridin-5-ylamine (0.07 g, 0.5 mmol) in DCE (4.0 mL), NCS (75 mg, 0.55 mmol) was added. The reaction mixture was refluxed for 3 h upon which the TLC revealed the absence of starting material. The volatiles were removed under reduced pressure and the crude product which contained two compounds was purified by silica gel to obtain intermediate-1-5 (37 mg) and intermediate-1-6 (58 mg).

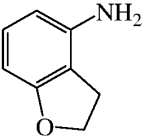
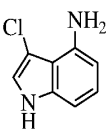
Intermediate-1-5: ^1H - NMR (400 MHz, CDCl_3): δ 8.27 (d, $J = 8.5$ Hz, 1H), 7.82 (s, 1H), 6.70 (s, 1H), 6.42 (s, 1H), 4.01 (bs, 2H). Mass (m/z): 167.2, 169.1 ($\text{M}+\text{H}$) $^+$.

15 **Intermediate-1-6:** ^1H - NMR (400 MHz, CDCl_3): δ 8.23 (d, $J = 8.2$ Hz, 1H), 6.70 (s, 1H), 6.32 (s, 1H), 4.01 (bs, 2H). Mass (m/z): 201.2, 203.1 ($\text{M}+\text{H}$) $^+$.

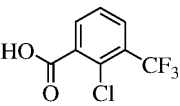
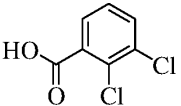
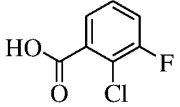
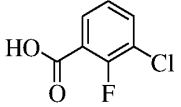
The following intermediates 1-7 to 1-30 were obtained from the commercial vendors:

Intermediate	Structure	Intermediate	Structure
1-7		1-19	
1-8		1-20	

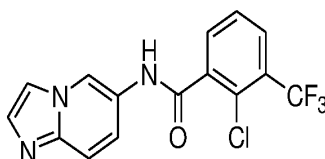
1-9		1-21	
1-10		1-22	
1-11		1-23	
1-12		1-24	
1-13		1-25	
1-14		1-26	
1-15		1-27	
1-16		1-28	
1-17		1-29	

1-18		1-30	
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The following intermediates 2-1 to 2-4 were obtained from the commercial vendors:

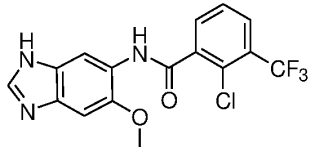
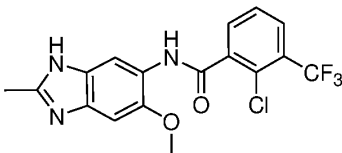
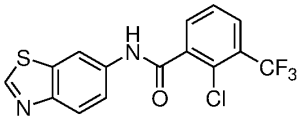
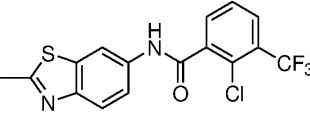
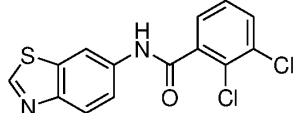
Intermediate	Chemical structure	Intermediate	Chemical structure
2-1		2-3	
2-2		2-4	

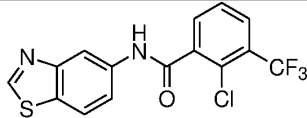
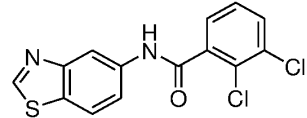
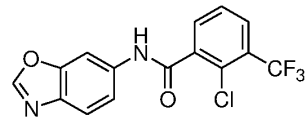
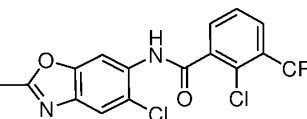
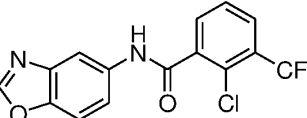
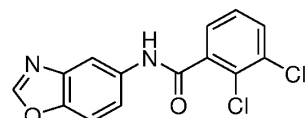
Example 1: *N*-imidazo[1,2-*a*]pyridin-6-yl-2-chloro-3-trifluoromethyl-benzamide

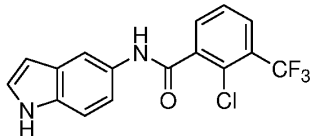
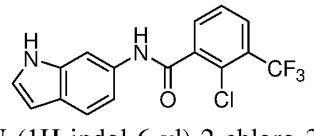
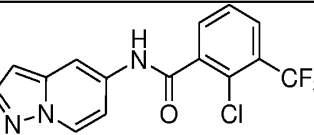
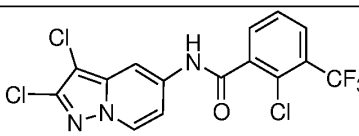
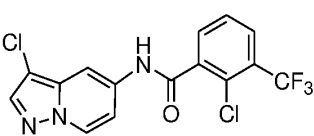
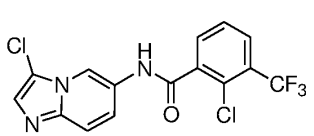


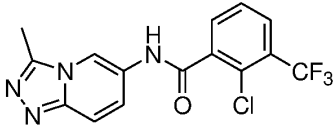
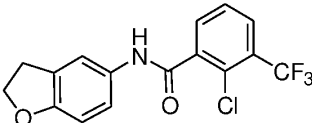
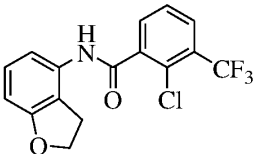
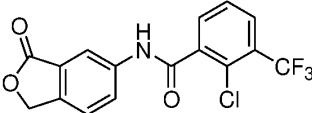
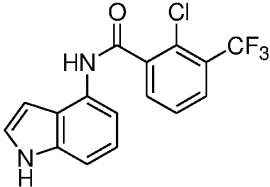
- 5 To the stirred solution of 6-amino-imidazo[1,2-*a*]pyridine (Intermediate-1-2; 1.08 g, 8.12 mmol) in DCM (32.5 mL) cooled to 0 °C, DIPEA (3.11 mL, 17.9 mmol), 2-chloro-3-trifluoromethyl-benzoic acid (Intermediate-2-1; 2.07 g, 8.96 mmol) and TBTU (2.86 g, 8.90 mmol) were sequentially added. The reaction temperature was gradually brought to r.t, and the reaction mixture was stirred for 16 h before being diluted with DCM and water. The two
- 10 layers were separated, the aqueous layer was extracted once with DCM and the combined organic layer was washed with brine solution. The resultant organic layer was dried over anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude mass was purified by silica gel column chromatography to obtain Example 1 (1.88 g) in 68% yield.
- 15 ¹H - NMR (400 MHz, CDCl₃): δ 9.32 (s, 1H), 8.03 (bs, 1H), 7.85-7.81 (m, 2H), 7.63-7.58 (m, 3H), 7.53 (t, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 9.2 Hz, 1H). Mass (m/z): 339.9, 342.0 (M+H)⁺.

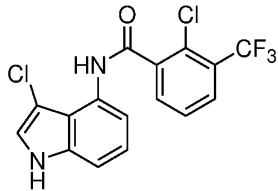
The below Examples 2 to 24 were prepared by following the experimental procedure of preparation as described in Example 1 using suitable intermediates (synthesized above or commercially procured) with some non-critical variations.

Example No.	Chemical name and Structure	Characterization data
2	 N-(6-methoxy-3H-benzoimidazol-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 7.96 (d, <i>J</i> = 8.0 Hz, 1H), 7.72-7.71 (m, 2H), 7.62-7.59 (m, 2H), 7.10 (s, 1H), 3.97 (s, 3H). Mass (m/z): 370.1, 372.2 (M+H) ⁺ .
3	 N-(6-methoxy-2-methyl-3H-benzoimidazol-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.75 (s, 1H), 8.48 (bs, 1H), 7.86-7.83 (m, 2H), 7.53 (t, <i>J</i> = 8.0 Hz, 1H), 7.19 (s, 1H), 3.92 (s, 3H), 2.55 (s, 3H). Mass (m/z): 384.1, 386.1 (M+H) ⁺ .
4	 N-Benzothiazol-6-yl-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.97 (s, 1H), 8.70 (s, 1H), 8.12 (d, <i>J</i> = 8.8 Hz, 1H), 7.86-7.84 (m, 2H), 7.80 (bs, 1H), 7.55 (t, <i>J</i> = 8.0 Hz, 1H), 7.44 (d, <i>J</i> = 8.0 Hz, 1H). Mass (m/z): 357.0, 359.1 (M+H) ⁺ .
5	 N-(2-methyl-benzothiazol-6-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.54 (d, <i>J</i> = 1.2 Hz, 1H), 7.91 (d, <i>J</i> = 8.8 Hz, 2H), 7.83 (d, <i>J</i> = 7.6 Hz, 2H), 7.51 (t, <i>J</i> = 7.6 Hz, 1H), 7.37 (dd, <i>J</i> = 1.6 Hz, 8.4 Hz, 1H), 2.83 (s, 3H). Mass (m/z): 371.2, 373.1 (M+H) ⁺ .
6	 N-Benzothiazol-6-yl-2,3-dichloro-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 10.87 (s, 1H), 9.31 (s, 1H), 8.66 (d, <i>J</i> = 0.8 Hz, 1H), 8.08 (d, <i>J</i> = 8.8 Hz, 1H), 7.80 (d, <i>J</i> = 7.6 Hz, 1H), 7.71 (dd, <i>J</i> = 1.2 Hz, 8.8 Hz, 1H), 7.62 (d, <i>J</i> = 7.2 Hz, 1H), 7.53 (t, <i>J</i> = 7.6 Hz, 1H). Mass (m/z): 323.1, 325.2, 327.2 (M+H) ⁺ .

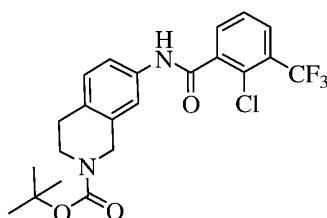
7	 N-Benzothiazol-5-yl-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 10.91(s, 1H), 9.41 (s, 1H), 8.58 (d, <i>J</i> = 1.2 Hz, 1H), 8.15 (d, <i>J</i> = 8.8 Hz, 1H), 8.02-7.95 (m, 2H), 7.74-7.69 (m, 2H). Mass (m/z): 356.9, 359 (M+H) ⁺ .
8	 N-Benzothiazol-5-yl-2,3-dichloro-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 10.83 (s, 1H), 9.41 (s, 1H), 8.57 (s, 1H), 8.14 (d, <i>J</i> = 8.8 Hz, 1H), 7.80 (d, <i>J</i> = 7.6 Hz, 1H), 7.74 (d, <i>J</i> = 8.4 Hz, 1H), 7.63 (d, <i>J</i> = 6.8 Hz, 1H), 7.53 (t, <i>J</i> = 7.6 Hz, 1H). Mass (m/z): 323, 325, 327 (M+H) ⁺ .
9	 N-Benzoxazol-6-yl-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.37 (s, 1H), 8.10 (s, 1H), 7.87-7.83 (m, 3H), 7.76 (d, <i>J</i> = 8.8 Hz, 1H), 7.53 (t, <i>J</i> = 7.6 Hz, 1H), 7.29-7.27 (m, 1H). Mass (m/z): 340.9, 343.0 (M+H) ⁺ .
10	 N-(5-chloro-2-methylbenzoxazol-6-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.80 (s, 1H), 8.33 (bs, 1H), 7.89-7.86 (m, 2H), 7.71 (s, 1H), 7.55 (t, <i>J</i> = 8.0 Hz, 1H), 2.66 (s, 3H). Mass (m/z): 389, 390.9, 392.6 (M+H) ⁺ .
11	 N-Benzoxazol-5-yl-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.13 (s, 2H), 7.86-7.83 (m, 2H), 7.77 (s, 1H), 7.66-7.59 (m, 2H), 7.53 (t, <i>J</i> = 8.0 Hz, 1H). Mass (m/z): 340.9, 343.0 (M+H) ⁺ .
12	 N-Benzoxazol-5-yl-2,3-dichloro-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.11(s, 2H), 7.99 (bs, 1H), 7.65 (d, <i>J</i> = 8.8 Hz, 1H), 7.58-7.56 (m, 3H), 7.33 (t, <i>J</i> = 7.6 Hz, 1H). Mass (m/z): 307, 309 (M+H) ⁺ .

13	 N-(1H-indol-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 11.06 (s, 1H), 10.42 (s, 1H), 7.99-7.95 (m, 2H), 7.90 (d, <i>J</i> = 7.2 Hz, 1H), 7.69 (t, <i>J</i> = 7.6 Hz, 1H), 7.37-7.29 (m, 3H), 6.42 (s, 1H). Mass (m/z): 338.9, 340.9 (M+H) ⁺ .
14	 N-(1H-indol-6-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 11.06 (s, 1H), 10.52 (s, 1H), 8.08 (s, 1H), 7.98 (d, <i>J</i> = 7.6 Hz, 1H), 7.91 (d, <i>J</i> = 7.6 Hz, 1H), 7.69 (t, <i>J</i> = 8.0 Hz, 1H), 7.48 (d, <i>J</i> = 8.4 Hz, 1H), 7.31 (t, <i>J</i> = 2.0 Hz, 1H), 7.15-7.12 (dd, <i>J</i> = 0.8 Hz, 8.4 Hz, 1H), 6.38 (s, 1H). Mass (m/z): 339.6, 341.3 (M+H) ⁺ .
15	 N-pyrazolo[1,5-a]pyridin-5-yl-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.47 (d, <i>J</i> = 7.6 Hz, 1H), 8.18 (s, 1H), 7.95 (d, <i>J</i> = 1.6 Hz, 1H), 7.85-7.82 (m, 3H), 7.53 (t, <i>J</i> = 7.6 Hz, 1H), 6.83 (d, <i>J</i> = 6.8 Hz, 1H), 6.50 (s, 1H). Mass (m/z): 339.9, 342.1 (M+H) ⁺ .
16	 N-(2,3-dichloro-pyrazolo[1,5-a]pyridin-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 10.79 (s, 1H), 8.75 (d, <i>J</i> = 7.6 Hz, 1H), 8.23 (s, 1H), 8.02 (d, <i>J</i> = 8.0 Hz, 1H), 7.91 (d, <i>J</i> = 7.6 Hz, 1H), 7.73 (t, <i>J</i> = 8.0 Hz, 1H), 7.36 (d, <i>J</i> = 7.2 Hz, 1H). Mass (m/z): 407.9, 409.9 (M+H) ⁺ .
17	 N-(3-chloro-pyrazolo[1,5-a]pyridin-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 11.10 (s, 1H), 8.70 (d, <i>J</i> = 7.2 Hz, 1H), 8.23 (s, 1H), 8.12 (s, 1H), 8.04 (d, <i>J</i> = 7.6 Hz, 1H), 7.98 (d, <i>J</i> = 7.2 Hz, 1H), 7.74 (t, <i>J</i> = 7.6 Hz, 1H), 7.02 (d, <i>J</i> = 6.4 Hz, 1H). Mass (m/z): 372.0, 374.0 (M+H) ⁺ .
18	 N-(2-chloro-pyrazolo[1,5-a]pyridin-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 9.16 (s, 1H), 7.87-7.85 (m, 3H), 7.61-7.57 (m, 2H), 7.54 (t, <i>J</i> = 8.0 Hz, 1H), 7.07 (d, <i>J</i> = 9.6 Hz, 1H). Mass (m/z): 373.9, 375.6 (M+H) ⁺ .

	N-(3-chloro-imidazo[1,2-a]pyridin-6-yl)-2-chloro-3-trifluoromethyl-benzamide	
19	 N-(3-methyl-[1,2,4]triazolo[4,3-a]pyridin-6-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 9.49 (s, 1H), 7.86 (d, <i>J</i> = 7.6 Hz, 3H), 7.68 (d, <i>J</i> = 9.2 Hz, 1H), 7.54 (t, <i>J</i> = 7.6 Hz, 1H), 7.4 (d, <i>J</i> = 9.6 Hz, 1H), 2.61 (s, 3H). Mass (m/z): 355.0, 357.0 (M+H) ⁺ .
20	 N-(2,3-dihydro-benzofuran-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 7.81-7.80 (m, 2H), 7.64 (s, 1H), 7.50-7.46 (m, 2H), 7.16 (d, <i>J</i> = 8.4 Hz, 1H), 6.78 (d, <i>J</i> = 8.4 Hz, 1H), 4.62 (t, <i>J</i> = 8.4 Hz, 2H), 3.27 (t, <i>J</i> = 8.4 Hz, 2H). Mass (m/z): 342.1, 344.0 (M+H) ⁺ .
21	 N-(2,3-dihydro-benzofuran-4-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 7.86 (t, <i>J</i> = 6.8 Hz, 2H), 7.53-7.47 (m, 2H), 7.34 (d, <i>J</i> = 8.0 Hz, 1H), 7.19 (t, <i>J</i> = 8.0 Hz, 1H), 6.71 (d, <i>J</i> = 8.0 Hz, 1H), 4.66 (t, <i>J</i> = 8.8 Hz, 2H), 3.26 (t, <i>J</i> = 8.4 Hz, 2H). Mass (m/z): 341.9, 343.9 (M+H) ⁺ .
22	 N-(3-oxo-1,3-dihydro-isobenzofuran-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.12-8.08 (m, 2H), 7.87-7.82 (m, 3H), 7.55-7.51 (m, 2H), 5.33 (s, 2H). Mass (m/z): 356.1, 358.2 (M+H) ⁺ .
23	 2-Chloro-N-(1H-indol-4-yl)-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 11.16 (s, 1H), 10.40 (s, 1H), 7.98 (d, <i>J</i> = 7.6 Hz, 1H), 7.92 (d, <i>J</i> = 7.6 Hz, 1H), 7.70 (t, <i>J</i> = 7.6 Hz, 1H), 7.64 (d, <i>J</i> = 7.6 Hz, 1H), 7.29 (bs, 1H), 7.24 (d, <i>J</i> = 8.0 Hz, 1H), 7.10 (t, <i>J</i> = 8.0 Hz, 1H), 6.70 (bs, 1H). Mass (m/z): 338.9, 340.9 (M+H) ⁺ .

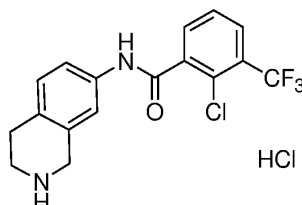
24	 2-Chloro-N-(3-chloro-1H-indol-4-yl)-3-trifluoromethylbenzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 11.51 (s, 1H), 10.30 (s, 1H), 7.99 (d, <i>J</i> = 8.0 Hz, 1H), 7.96 (d, <i>J</i> = 8.0 Hz, 1H), 7.73 (t, <i>J</i> = 7.6 Hz, 1H), 7.51 (bs, 1H), 7.37 (d, <i>J</i> = 8.0 Hz, 1H), 7.20 (t, <i>J</i> = 7.6 Hz, 1H), 7.09 (d, <i>J</i> = 7.6 Hz, 1H). Mass (m/z): 371.1, 373.0 (M+H) ⁺ .
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Example 25: 7-(2-chloro-3-trifluoromethyl-benzoylamino)-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester



- 5 To a stirred solution of 2-chloro-3-trifluoromethyl-benzoic acid (Intermediate-2-1; 1.96 g, 8.75 mmol) in DCE (17.5 mL) cooled to 0 °C under nitrogen atmosphere, thionyl chloride (1.25 mL, 17.5 mmol) was added. The reaction mixture was gradually raised to r.t. and then refluxed for 4 h. After completion of the reaction, volatiles were evaporated to obtain crude acid chloride. The crude acid chloride obtained was dissolved in DCM (18.0 mL) under
- 10 nitrogen atmosphere at 0 °C, Et₃N (3.6 mL, 26.3 mmol) and a solution of intermediate 1-20 (2.2 g, 8.8 mmol) in DCM (18.0 mL) were added. The reaction mixture was gradually warmed to r.t. and stirred for 16 h. After completion of the reaction, the reaction mixture was diluted with water and DCM. The separated organic layer was washed with brine and dried over anhydrous Na₂SO₄ and the solvent was evaporated under a vacuum to obtain the crude product
- 15 which was purified by silica gel column chromatography using a mixture of hexane and EtOAc to obtain Example 25 (2.6 gm) in 65% yield. ¹H - NMR (400 MHz, CDCl₃): δ 7.86-7.71 (m, 3H), 7.52-7.41 (m, 2H), 7.17-7.09 (m, 1H), 6.57 (bs, 1H), 4.58 (s, 2H), 3.70-3.60 (m, 2H), 2.88-2.76 (m, 2H), 1.47 (s, 9H). Mass (m/z): 453.5, 455.2 (M-H)⁺.

Example 26: N-(1,2,3,4-tetrahydro-isoquinolin-7-yl)-2-chloro-3-trifluoromethyl-benzamide hydrochloride

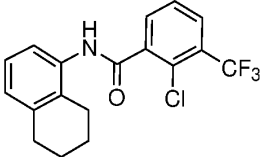
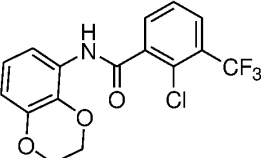
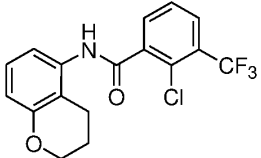
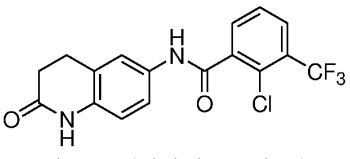
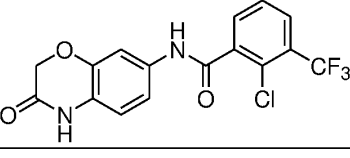


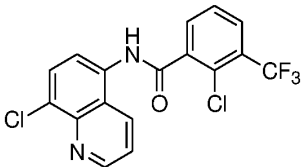
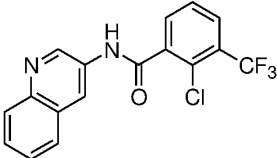
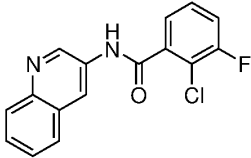
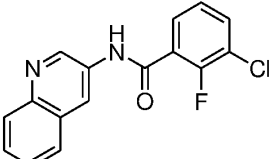
To the stirred solution of Example 25 (50.0 mg, 0.11 mmol) in IPA (0.5 mL), cooled to 0 °C, dry HCl (3.0 M, 0.5 mL) was added. The reaction mixture was warmed to r.t and stirred for 6 h. The volatiles were removed under reduced pressure to obtain a crude mass which was triturated with copious amounts of pentane, mixture of pentane and ether, and ether. The hydrochloride salt thus obtained dried under reduced pressure to obtain the compound of Example 26 (24.0 mg) in 89% yield. ¹H - NMR (400 MHz, DMSO-d₆): δ 9.35-9.10 (m, 2H), 8.0 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.70-7.62 (m, 2H), 7.47 (d, *J* = 8.00 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 4.32-4.27 (m, 2H), 4.20-4.15 (m, 1H), 3.0-2.94 (m, 2H), 2.80-2.72 (m, 1H). Mass (m/z): 355.0, 357.0 (M+H)⁺.

A small quantity of above obtained hydrochloride salt (10.0 mg) was dissolved in 1:1 mixture of 1N NaOH solution and EtOAc. The two layers were separated, the organic layer was dried over anhydrous Na₂SO₄ and the solvent was removed to obtain free base of Example 26, i.e., N-(1,2,3,4-tetrahydro-isoquinolin-7-yl)-2-chloro-3-trifluoromethyl-benzamide (7.2 mg): ¹H - NMR (400 MHz, CDCl₃): δ 7.81 (t, *J* = 7.0 Hz, 2H), 7.52 (bs, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.41 (s, 1H), 7.29-7.27 (m, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 3.59 (bs, 2H), 2.91 (t, *J* = 6.0 Hz, 2H), 2.70 (t, *J* = 6.0 Hz, 2H). Mass (m/z): 355.2, 357.1 (M+H)⁺.

The below Examples 27 to 36 were prepared by following the experimental procedure of preparation as described in the Examples 25 and 26 using suitable intermediates with some non-critical variations.

Example No.	Chemical name and Structure	Characterization data
27		¹ H - NMR (400 MHz, CDCl ₃): δ 7.81-7.79 (m, 2H), 7.49 (t, <i>J</i> = 7.6 Hz, 1H), 7.42 (bs, 1H), 7.15 (d, <i>J</i> = 1.6 Hz, 1H), 6.76-6.69 (m,

	N-(4-methyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-2-chloro-3-trifluoromethyl-benzamide	2H), 4.29 (t, $J = 4$ Hz, 2H), 3.30 (t, $J = 4.4$ Hz, 2H), 2.93 (s, 3H). Mass (m/z): 371.0, 373.1 (M+H) ⁺ .
28	 N-(5,6,7,8-tetrahydro-naphthalen-1-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 7.86 (m, 3H), 7.52 (t, $J = 7.6$ Hz, 1H), 7.40 (s, 1H), 7.21 (t, $J = 7.6$ Hz, 1H), 7.01 (d, $J = 7.6$ Hz, 1H), 2.82 (t, $J = 6.0$ Hz, 2H), 2.68 (t, $J = 5.6$ Hz, 2H), 1.86-1.78 (m, 4H). Mass (m/z): 354.0, 356.1 (M+H) ⁺ .
29	 N-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.07 (d, $J = 8.0$ Hz, 2H), 7.83 (d, $J = 7.2$ Hz, 2H), 7.50 (t, $J = 7.6$ Hz, 1H), 6.90 (t, $J = 8.0$ Hz, 1H), 6.72 (d, $J = 8.4$ Hz, 1H), 4.31 (d, $J = 8.8$ Hz, 4H). Mass (m/z): 358.0, 360 (M+H) ⁺ .
30	 N-chroman-5-yl-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 7.87 (t, $J = 8.4$ Hz, 2H), 7.52 (t, $J = 7.6$ Hz, 1H), 7.46 (d, $J = 8.0$ Hz, 1H), 7.39 (bs, 1H), 7.19 (t, $J = 8.0$ Hz, 1H), 6.76 (d, $J = 8.0$ Hz, 1H), 4.19 (t, $J = 5.2$ Hz, 2H), 2.74 (t, $J = 6.4$ Hz, 2H), 2.08-2.03 (m, 2H). Mass (m/z); 356.0, 358.1 (M+H) ⁺ .
31	 N-(2-oxo-1,2,3,4-tetrahydro-quinolin-6-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 7.86-7.78 (m, 2H), 7.68 (bs, 1H), 7.61 (s, 1H), 7.54 (bs, 1H), 7.52 (t, $J = 8.0$ Hz, 1H), 7.33 (d, $J = 7.2$ Hz, 1H), 6.76 (d, $J = 8.4$ Hz, 1H), 3.02 (t, $J = 7.2$ Hz, 2H), 2.65 (t, $J = 7.6$ Hz, 2H). Mass (m/z); 369.3, 371.4 (M+H) ⁺ .
32	 N-(2-oxo-1,2,3,4-tetrahydro-isoquinolin-6-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 10.68 (s, 1H), 10.58 (s, 1H), 7.98 (d, $J = 7.6$ Hz, 1H), 7.88 (d, $J = 7.2$ Hz, 1H), 7.68 (t, $J = 7.6$ Hz,

	N-(3-oxo-3,4-dihydro-2H-benzo[1,4]oxazin-7-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H), 7.39 (d, <i>J</i> = 1.2 Hz, 1H), 7.26-7.23 (dd, <i>J</i> = 1.2 Hz, 8.0 Hz, 1H), 6.88 (d, <i>J</i> = 8.4 Hz, 1H), 4.56 (s, 2H). Mass (m/z): 369.2, 371.1 (M+H) ⁺ .
33	 N-(8-chloro-quinolin-5-yl)-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 10.93 (s, 1H), 9.08 (d, <i>J</i> = 3.2 Hz, 1H), 8.64 (d, <i>J</i> = 8.4 Hz, 1H), 8.10 (d, <i>J</i> = 7.2 Hz, 1H), 8.05-8.03 (m, 2H), 7.91 (d, <i>J</i> = 8.0 Hz, 1H), 7.75-7.72 (m, 2H). Mass (m/z): 384.9, 386.9 (M+H) ⁺ .
34	 N-quinolin-3-yl-2-chloro-3-trifluoromethyl-benzamide	¹ H - NMR (400 MHz, DMSO-d ₆): δ 11.18 (s, 1H), 8.98 (s, 1H), 8.87 (s, 1H), 8.04-7.98 (m, 4H), 7.75-7.68 (m, 2H), 7.63 (t, <i>J</i> = 7.2 Hz, 1H). Mass (m/z): 351.9, 354.1 (M+H) ⁺ .
35	 N-quinolin-3-yl-2-chloro-3-fluoro-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.91 (s, 1H), 8.82 (d, <i>J</i> = 1.6 Hz, 1H), 8.20 (bs, 1H), 8.08 (d, <i>J</i> = 8.4 Hz, 1H), 7.87 (d, <i>J</i> = 8.0 Hz, 1H), 7.69 (t, <i>J</i> = 7.6 Hz, 1H), 7.61-7.56 (m, 2H), 7.42-7.37 (m, 1H), 7.34 (t, <i>J</i> = 8.4 Hz, 1H). Mass (m/z): 300.4, 302.7 (M+H) ⁺ .
36	 N-quinolin-3-yl-3-chloro-2-fluoro-benzamide	¹ H - NMR (400 MHz, CDCl ₃): δ 8.90 (s, 1H), 8.86 (d, <i>J</i> = 2.4 Hz, 1H), 8.62 (bs, 1H), 8.11-8.07 (m, 2H), 7.87 (d, <i>J</i> = 8.0 Hz, 1H), 7.69-7.62 (m, 2H), 7.59 (t, <i>J</i> = 7.6 Hz, 1H), 7.33 (t, <i>J</i> = 8.0 Hz, 1H). Mass (m/z): 300.4, 302.7 (M+H) ⁺ .

Example 37: In-vitro functional ethidium bromide accumulation assay

Compounds were tested for in vitro biological activity at the P2X7 receptor in ethidium accumulation assay.

Studies were performed using NaCl assay buffer of the following composition: NaCl (140 mM), HEPES (10 mM), N-methyl-D-glucamine (5 mM), KCl (5.6 mM), D-glucose (10 mM), CaCl₂ (0.5 mM) (pH 7.4). HEK293 cells expressing human recombinant P2X7R (B'SYS HEK 293 P2X7R (RRID: CVCL_C0YM)), were grown in poly-D-lysine coated 96 well plates (Greiner) for 24 hours at 37 °C with 5% CO₂. The cells were washed twice with 350 µl of assay buffer before addition of 50 µl of test compound. The cells were then incubated at room temperature for 30 min before addition of Benzoylbenzoyl-ATP (BzATP) and ethidium (100 µM final assay concentration). Incubations were continued for 8 to 10 min and were terminated by addition of 25 µl of 1.3 M sucrose containing 5 mM of the P2X7R antagonist reactive black 5 (Aldrich). Cellular accumulation of ethidium was determined by measuring fluorescence (excitation wavelength of 535 nm and emission wavelength of 625 nm) from below the plate with SpectraMax iD5 (Molecular Devices). Antagonist IC₅₀ values for blocking BzATP responses were determined using iterative curve fitting techniques in GraphPad Prism software, and cIC₅₀ values are calculated using the Cheng-Prusoff equation for functional assay; $cIC_{50} = IC_{50}/(1+[Agonist]/EC_{50})$, values are tabulated in Table-1.

The known P2X7R compound GSK1482160, used as an internal control had cIC₅₀ value of 1.8 nM, consistent with previous reports (Gao et al., Bioorg Med Chem Lett. 2015; 25:1965–1970; Han et al., Nucl Med Commun. 2017; 38:372–382).

Table-1: cIC₅₀ and IC₅₀ values of compounds of the invention

Example No.	cIC ₅₀ /IC ₅₀ (nM)	Example No.	cIC ₅₀ /IC ₅₀ (nM)	Example No.	cIC ₅₀ /IC ₅₀ (nM)
1	170/1388	13	1.3/23	25	1043/6563
2	>10000	14	49/669	26	60/375
3	57/1243	15	259/2473	27	4.6/45
4	12/119	16	>10000	28	0.9/9.3
5	31/196	17	554/4516	29	4.2/38
6	11/57	18	36/426	30	1.4/37.7
7	94/894	19	165/1931	31	11/150
8	225/1162	20	7.7/70	32	27/277

9	9.4/128	21	7.2/78.8	33	2.6/28.4
10	1.8/13	22	572/5925	34	45.2/378.4
11	145/792	23	0.2/2.4	35	746.1/6247
12	522/5449	24	7.7/75	36	1067/8936

Example 38: Rodent Pharmacokinetic Study

Male Wistar rats (260 ± 50 g) were used as experimental animals. Animals were housed individually in polypropylene cage. Two days prior to study, male Wistar rats were anesthetized with isoflurane for surgical placement of jugular vein catheter. Rats were randomly divided for oral (3 mg/kg) and intravenous (1 mg/kg) dosing ($n = 3/\text{group}$) and fasted overnight before oral dosing (*p.o.*). However, food and water were provided *ad libitum* to the rats allocated for intravenous dosing.

At pre-determined time points, blood samples were collected through jugular vein and replenished with an equivalent volume of normal saline. Collected blood was transferred into a labeled eppendorf tube containing 10 μL of heparin as an anticoagulant. Typically blood samples were collected at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post dose. Blood samples were centrifuged at 4000 rpm for 10 min. Plasma was separated and stored frozen at -80°C until analysis. The concentrations of the test compounds were quantified in plasma by qualified LC-MS/MS method using suitable extraction technique. The test compounds were quantified in the calibration range around 1-1000 ng/mL in plasma. Study samples were analyzed using calibration samples in the batch and quality control samples spread across the batch.

Pharmacokinetic parameters C_{max} , AUC_t , $t_{1/2}$, Clearance and Bioavailability (F) were calculated using standard non-compartmental model by using Phoenix WinNonlin 6.0.4 version software package.

Table-2: Pharmacokinetic profile of the test compounds*

Example No.	ROA	C_{max} (ng/mL)	AUC_{0-t} (ng.hr/mL)	$t_{1/2}$ (hr)	Clearance (mL/min/kg)	F (%)
3	oral (gavage)	483 ± 138	1667 ± 228	1.3 ± 0.6	-	66 ± 9
	intravenous (bolus)	-	846 ± 75	1.5 ± 0.3	19 ± 2	
4	oral (gavage)	101 ± 30	199 ± 28	1.2 ± 0.3	-	12 ± 2

	intravenous (bolus)	-	544 ± 82	1.4 ± 0.4	31 ± 5	
7	oral (gavage)	961 ± 344	11293 ± 4320	2.3 ± 0.0	-	102 ± 39
	intravenous (bolus)	-	3693 ± 1344	2.3 ± 0.4	4 ± 1	
9	oral (gavage)	355 ± 179	437 ± 159	1.6 ± 0.6	-	37 ± 13
	intravenous (bolus)	-	393 ± 124	1.3 ± 0.5	45 ± 14	
21	oral (gavage)	70 ± 39	218 ± 192	3.3 ± 3.8	-	15 ± 13
	intravenous (bolus)	-	484 ± 86	1.5 ± 0.4	34 ± 5	
23	oral (gavage)	245 ± 33	479 ± 7	1.0 ± 0.2	-	45 ± 1
	intravenous (bolus)	-	355 ± 52	1.4 ± 0.2	47 ± 6	
24	oral (gavage)	131 ± 40	244 ± 54	1.6 ± 0.3	-	31 ± 7
	intravenous (bolus)	-	264 ± 10	1.8 ± 1	62 ± 2	
26	oral (gavage)	75 ± 12	877 ± 134	4.3 ± 0.5	-	111 ± 17
	intravenous (bolus)	-	264 ± 85	3.8 ± 1.2	57 ± 13	

*Fasted male Wistar rats were used; Vehicle used is water for injection for both oral and i.v. routes. Values are mean ± S.D, n=3 animals/time point.

Example 39: Rodent Brain Penetration Study

Male Wistar rats (260 ± 40 g) were used as experimental animals. Three animals were housed in each cage. Animals were given water and food *ad libitum* throughout the experiment and maintained on a 12 h light/dark cycle.

Brain penetration was determined in discrete manner in rats. One day prior to dosing, male Wistar rats were acclimatized and randomly grouped according to their body weight. At each time point (0.50, 1 and 2 h), n = 3 animals were used.

The test compounds were suitably preformulated and administered orally at (free base equivalent) 3 mg/kg. Blood samples were removed via cardiac puncture under isoflurane anesthesia. The animals were sacrificed to collect brain tissue. Plasma was separated and brain samples were homogenized. Plasma and brain homogenates were stored frozen at -20 °C until

analysis. The concentrations of the test compounds in plasma and brain were determined using LC-MS/MS method.

The test compounds were quantified in plasma and brain homogenate by qualified LC-MS/MS method using suitable extraction technique in the calibration range of 1-500 ng/mL.

- 5 Study samples were analyzed using calibration samples in the batch and quality control samples spread across the batch. Extent of brain-plasma ratio ($C_{\text{brain}}/C_{\text{plasma}}$) was calculated.

Table-3: Blood-Brain Penetration data of the test compounds

Example No.	Rat Brain Penetration ($C_{\text{brain}}/C_{\text{plasma}}$) at 3 mg/kg, <i>p.o.</i> @ 1 h
3	0.89 ± 0.32
4	1.54 ± 0.13
7	0.11 ± 0.03
9	1.53 ± 0.62
21	3.67 ± 0.16
23	2.43 ± 0.48
24	5.03 ± 1.05
26	2.59 ± 0.69

Example 40: Diabetic neuropathic pain

- Rats with body weight 280-300g were dosed with Streptozotocin 50 mg/kg, *i.p.* to induce diabetic neuropathy. Blood glucose levels were recorded on day-3 post streptozotocin injection and animals which shows blood glucose levels of >260 mg/dL were considered diabetic. After 3 to 6 weeks after induction, paw withdrawal thresholds (PWTs) were measured on left paw using Von Frey monofilaments. The rats with PWTs ≤ 4g, were selected. After selection, the rats were randomized based on PWTs to different treatment groups. On the next day, rats were administered vehicle or test compounds. The PWTs were determined at 30, 60 and 120 minutes time points post dosing. Data were expressed as 50% withdrawal threshold in grams.
- 10
15

Table-4: Diabetic neuropathic pain assay data of the test compounds

Example No.	Minimum Efficacy Dose	Paw withdrawal thresholds (PWT) in grams	Inference
4	50 mg/kg, <i>i.p.</i>	13.5	Active
9	30 mg/kg, <i>i.p.</i>	15	Active
24	50 mg/kg, <i>i.p.</i>	8.5	Active

Example 41: Chronic constriction injury (CCI)

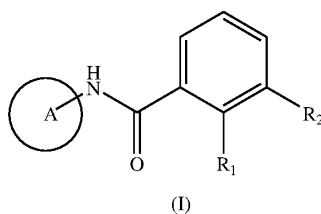
Rats with body weight 150-180g subjected to CCI surgery. These rats were anaesthetized with avertin 200 mg/kg, *i.p.* The rat's body temperature was maintained at 37 °C using thermoregulatory pad. The upper thigh region hair was removed using chromini cordless clipper. A small incision was made in the thigh region, sciatic nerve was exposed. Using chromic cat-gut no-4, four loose ligatures were tied around the sciatic nerve just 1 mm above the trifurcation of the nerve. The opened tissue was closed using silk thread no-5, skin was sutured back using cotton thread no-5. The gentamycin 8.8 mg/kg, *s.c.* was administered, povidone-iodine solution was applied over the sutures. These rats were kept in the home cage for 3 weeks for recovery and development of neuropathic pain. After 3 weeks of CCI surgery PWTs were recorded on left paw (ipsilateral paw) of rats using Von Frey monofilaments. Rats which showed PWTs less than or equal to 4g were selected for the study. Selected rats were randomized into different groups and were administered test compound or vehicle. PWTs of rats were determined at 30, 60, 120 min post treatment. Data were expressed as 50% withdrawal threshold in grams.

Table-5: Chronic constriction injury assay data of the test compounds

Example No.	Minimum Efficacy Dose	Paw withdrawal thresholds (PWT) in grams	Inference
4	50 mg/kg, <i>i.p.</i>	10.5	Active
9	50 mg/kg, <i>i.p.</i>	12.5	Active

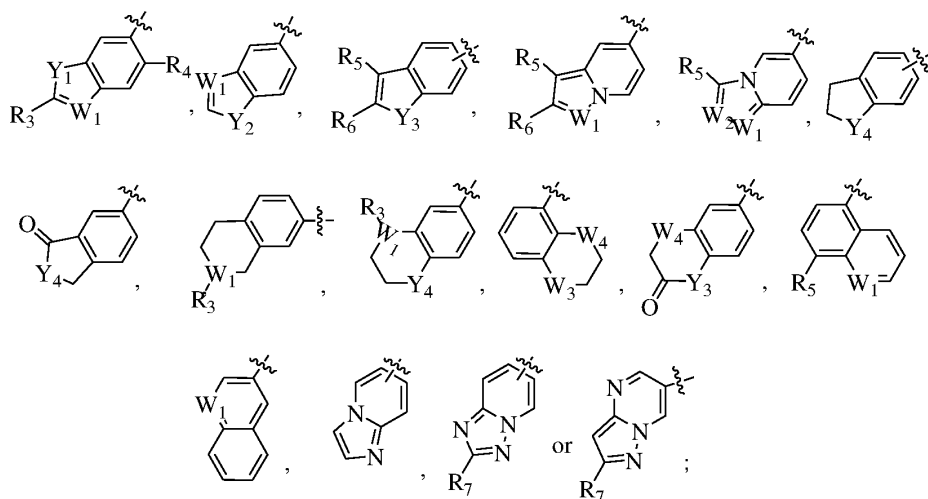
We claim

1. A compound of formula (I), or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof,



5 wherein,

Ring A is selected from,



wherein,

10 “” represents the point of attachment;

R₁ is selected from halogen;

R₂ is selected from CF₃ or halogen;

R₃ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl, -(C₁₋₆)-alkyl-O-(C₁₋₆)-alkyl, -C(O)O-(C₁₋₆)-alkyl or cyano;

15 R₄ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

R₅ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

R₆ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

R₇ is selected from hydrogen, halogen, -(C₁₋₆)-alkyl, -O-(C₁₋₆)-alkyl or cyano;

W₁ is N or CH;

- W₂ is N or CH;
W₃ is CH₂ or O;
W₄ is CH₂, N or O;
Y₁ is CH₂, NH, O or S;
5 Y₂ is CH₂, NH, O or S;
Y₃ is NH or CH₂; and
Y₄ is CH₂, NH, O or S.
2. The compound as claimed in claim 1, wherein the compound is selected from:
- 10 N-imidazo[1,2-*a*]pyridin-6-yl-2-chloro-3-trifluoromethyl-benzamide;
N-(6-methoxy-3H-benzoimidazol-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(6-methoxy-2-methyl-3H-benzoimidazol-5-yl)-2-chloro-3-trifluoromethyl-
benzamide;
N-Benzothiazol-6-yl-2-chloro-3-trifluoromethyl-benzamide;
15 N-(2-methyl-benzothiazol-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-Benzothiazol-6-yl-2,3-dichloro-benzamide;
N-Benzothiazol-5-yl-2-chloro-3-trifluoromethyl-benzamide;
N-Benzothiazol-5-yl-2,3-dichloro-benzamide;
N-Benzooxazol-6-yl-2-chloro-3-trifluoromethyl-benzamide;
20 N-(5-chloro-2-methyl-benzooxazol-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-Benzooxazol-5-yl-2-chloro-3-trifluoromethyl-benzamide;
N-Benzooxazol-5-yl-2,3-dichloro-benzamide;
N-(1H-indol-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(1H-indol-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
25 N-pyrazolo[1,5-*a*]pyridin-5-yl-2-chloro-3-trifluoromethyl-benzamide;
N-(2,3-dichloro-pyrazolo[1,5-*a*]pyridin-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-chloro-pyrazolo[1,5-*a*]pyridin-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-chloro-imidazo[1,2-*a*]pyridin-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-methyl-[1,2,4]triazolo[4,3-*a*]pyridin-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
30 N-(2,3-dihydro-benzofuran-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(2,3-dihydro-benzofuran-4-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-oxo-1,3-dihydro-isobenzofuran-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
2-Chloro-N-(1H-indol-4-yl)-3-trifluoromethyl-benzamide;

- 2-Chloro-N-(3-chloro-1H-indol-4-yl)-3-trifluoromethyl-benzamide;
7-(2-chloro-3-trifluoromethyl-benzoylamino)-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester;
N-(1,2,3,4-tetrahydro-isoquinolin-7-yl)-2-chloro-3-trifluoromethyl-benzamide
5 hydrochloride;
N-(1,2,3,4-tetrahydro-isoquinolin-7-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(4-methyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(5,6,7,8-tetrahydro-naphthalen-1-yl)-2-chloro-3-trifluoromethyl-benzamide;
10 N-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-chroman-5-yl-2-chloro-3-trifluoromethyl-benzamide;
N-(2-oxo-1,2,3,4-tetrahydro-quinolin-6-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-(3-oxo-3,4-dihydro-2H-benzo[1,4]oxazin-7-yl)-2-chloro-3-trifluoromethyl-benzamide;
15 N-(8-chloro-quinolin-5-yl)-2-chloro-3-trifluoromethyl-benzamide;
N-quinolin-3-yl-2-Chloro-3-trifluoromethyl-benzamide;
N-quinolin-3-yl-2-chloro-3-fluoro-benzamide; and
N-quinolin-3-yl-3-chloro-2-fluoro-benzamide.
or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof.
- 20 3. A pharmaceutical composition comprising the compound of formula (I) or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof as claimed in claim 1 or claim 2 and pharmaceutically acceptable excipients.
4. The pharmaceutical composition as claimed in claim 3, for use in the treatment of disease or disorder selected from CNS related disorders, acute kidney diseases,
25 endocrine or hormonal disorders, neurodegenerative disorders, psychiatric disorders, neuropathic pain, chronic pain, acute pain, cancer, headaches, or inflammatory process of musculoskeletal system.
5. A compound of formula (I) or an isotopic form, a stereoisomer, or a pharmaceutically acceptable salt thereof as claimed in claim 1 or claim 2, for use in the treatment of diseases or disorders mediated by P2X7 receptor, wherein the disease or disorder
30 selected from the group consisting of CNS related disorders, acute kidney disease,

endocrine or hormonal disorders, neurodegenerative disorders, psychiatric disorders, neuropathic pain, chronic pain, acute pain, cancer, headaches and inflammatory process of musculoskeletal system.

- 5 6. The compound for use as claimed in claim 5, wherein the neuropathic pain is selected from diabetic neuropathy, sciatica, non-specific lower back pain, multiple sclerosis pain, fibromyalgia, HIV-related neuropathy, or neuralgia.
- 10 7. The compound for use as claimed in claim 5, wherein the chronic pain is selected from chronic pain caused by inflammation or an inflammatory-related condition, osteoarthritis, rheumatoid arthritis, acute injury or trauma, upper back pain or lower back pain resulting from systematic, regional or primary spine disease such as radiculopathy, bone pain due to osteoarthritis, osteoporosis, bone metastasis or unknown reasons, pelvic pain, spinal cord injury-associated pain, cardiac chest pain, non-cardiac chest pain, central post-stroke pain, myofascial pain, sickle cell pain, cancer pain, geriatric pain, pain caused by headache, gout, or fibrosis.
- 15 8. The compound for use as claimed in claim 5, wherein the acute pain is selected from acute injury, illness, sport-medicine injuries, musculoskeletal sprains and strains, pain related to surgery such as open heart or bypass surgery, post-operative pain, kidney stone pain, gallbladder pain, gallstone pain, obstetric pain or dental pain.
- 20 9. The compound for use as claimed in claim 5, wherein the headache is selected from migraine, tension type headache, transformed migraine, evolutive headache, or cluster headache.
- 25 10. The compound for use as claimed in claim 5, wherein the inflammatory process of the musculoskeletal system includes arthritic conditions such as ankylosing spondylitis, cervical arthritis, fibromyalgia, gout, juvenile rheumatoid arthritis, lumbosacral arthritis, osteoarthritis, osteoporosis, psoriatic arthritis, rheumatic disease; disorders affecting skin and related tissues such as eczema, psoriasis, dermatitis or inflammatory conditions.