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# Update on novel purinergic P2X3 and P2X2/3 receptor antagonists and their potential therapeutic applications

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**Abstract**

**Introduction**: Purinergic P2X3-P2X2/3 receptors are placed in nociceptive neurons' strategic location and show unique desensitization properties, hence they represent an attractive target for many pain related diseases. Therefore a broad interest from academic and pharmaceutical scientists has focused on the search for P2X3 and P2X2/3 receptor ligands and has led to the discovery of numerous new selective antagonists. Some of them have been studied in clinical trials for the treatment of pathological conditions such as bladder disorders, gastrointestinal and chronic obstructive pulmonary diseases.

**Areas covered**: This review provides a summary of the patents concerning the discovery of P2X3 and/or P2X2/3 receptor antagonists published between 2015 and 2019 and their potential clinical use. Thus, the structures and biological data of the most representative molecules are reported.

**Expert opinion**: The 2016 publication of the crystallographic structure of the human P2X3 receptor subtype gave an improvement of published patents in 2017. Hence, a great number of small molecules with dual antagonist activity on P2X3-P2X2/3 receptors, a favorable pharmacokinetic profile, and reasonable oral bioavailability was discovered. The most promising compounds are the phenoxy-diaminopyrimidines including gefapixant (AF-219), and the imidazo-pyridines like BLU-5937, which are in phase III and phase II clinical trials, respectively, for refractory chronic cough.

**Key words**: P2X3 and P2X2/3 receptors, P2X3 receptor antagonists, patents on P2X3 receptor antagonists, AF-219

**Article highlights**

● P2X3 and P2X2/3 receptor antagonists are a promising target to treat many pathological conditions like refractory chronic cough and pain.

● The discovery of new classes of molecules able of antagonize these receptors are able to reduce cough and avoid central nervous system side effects such as sedation, characteristic of many current antitussive drugs.

● A new class of potential antitussive drugs, the P2X3R selective antagonists imidazo-pyridines, has the advantage of not altering the taste function, a typical side effect of P2X3 and P2X2/3 receptor unselective antagonists.

● The use of P2X3R selective antagonists could represent an excellent strategy in pain management reducing the probability of adverse effects in brain, gastrointestinal, or cardiovascular tissues, effects that remain deterring aspects for many current pain drugs.

## 1. Introduction

Adenosine 5'-triphosphate (ATP) is an abundant, multifaceted molecule that provides energy to drive many processes in living cells. It is an important extracellular signaling molecule that acts as a neurotransmitter in both peripheral and central nervous systems. Moreover, ATP is involved in initiation of pain and autocrine signal, and it is an extracellular messenger of movement, distension, distress, ischemia, damage and inflammation [1].

ATP is the natural ligand of the purinergic P2X receptors (P2XRs). The main source of ATP acting on purinoceptors originates from damaged or inflamed tissues. P2XRs are cation-selective channels with high permeability to $Ca^{++}$, $Na^{+}$, and $K^{+}$ and are widely expressed in the nervous, immune, cardiovascular, skeletal, gastrointestinal, respiratory, and endocrine systems. To date, P2XRs are organized in seven distinct homotrimeric receptors, named P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, and P2X7, and in three heteromeric receptors identified as P2X2/3, P2X4/6, P2X1/5 [2,3]. Each receptor subtype can associate also to form homotrimeric complexes [4-6]. Two trans-membrane domains and a large glycosylated and disulphide-rich extracellular domain are present in each subtype [7]. The binding site for the ATP binding is located in the extracellular domain where also competitive antagonists and modulatory metal ions interact [8].

In the last years, a wide interest from academic and pharmaceutical scientists was focused in the discovery of P2X3R antagonists, supported by molecular modeling studies of P2XRs and the recently published crystallographic structure of the P2X3R subtype stabilized in the *apo*/resting state with the competitive antagonists TNP-ATP and A-317491 [9,10].

The homotrimeric P2X3 and heteromeric P2X2/3Rs are present in dorsal root ganglia on nerve terminals, spinal cord, and in neurons of the brain. Numerous studies have shown that the activation of homotrimeric P2X3Rs and heteromeric P2X2/3Rs, expressed in primary sensory neurons, contributes to acute nociceptive behavior, hyperalgesia and allodynia in rodents [11-13].

After tissue injury, it was shown an increasing ATP release and an enhance pain behaviors in rat models [14]. An increase in cytosolic ATP release produced by damaged skin cells may evoke large current P2X3R-mediated responses in dorsal root ganglion (DRG) neurons [15].

Nociceptive signals from peripheral to spinal dorsal horns in central processes of DRG are evocated by P2X3Rs transmission [16]. Moreover, P2X3R expression is potentiated during tissue injury increasing current responses, and promoting the excitability of P2X3Rs in DRG neurons [17]. This evidence was confirmed by P2X3R gene disruption, which results in a diminished sensitivity to noxious chemical stimuli and reduced pain. In particular, functional analysis of neurons from P2X2 and P2X2/3Rs knockout mice showed a minimal to no response to ATP. These data indicate that P2XRs on sensory and autonomic ganglia neurons involve almost exclusively P2X2 and P2X3R subunits [18,19]. Functional analysis of P2X2 and P2X2/3 knockout mice further demonstrated/confirmed the presence of P2X2/3Rs in sensory and autonomic ganglia neurons and primary afferent nerve fibers in the urinary bladder.

Hence, these receptors are considered attractive therapeutic targets for pain management and development of selective antagonists is currently progressing.

### 1.1. Pharmacology of P2X3 and P2X2/3 receptors

Inflammatory mediators contributing to spontaneous activity of sensory fibers and pathological hyper-responsiveness influence the neuronal expression of nociceptors and ion channels, including ATP receptors [20]. Neuropathic pain can develop subsequently to nerve injury, diabetes, herpes infections or cancer, and it is characterized by severe allodynia [21]. This type of pain is usually resistant to typical pain management, such as non-steroidal anti-inflammatory drugs and opioids. It is well known that ATP stimulates sensory nerve endings by activating P2X3Rs and/or P2X2/3Rs that are almost exclusively expressed in nociceptive neurons. In addition, P2X3Rs and its heterodimer are expressed in trigeminal ganglia sensory neurons suggesting their involvement in migraine and headache pain processing [22]. Research data suggest that activation of P2X3Rs - P2X2/3Rs by ATP, which results in pain sensation, is due to the release of glutamate, a key neurotransmitter involved in nociceptive signaling [16]. Additionally, the involvement of P2X3Rs was established in P2X3R knockout mice that showed reduced pain behavior, and the same effect was reproduced by reduction of oligonucleotides P2X3 expression or siRNA [23]. Research data suggested also a role for P2X3Rs in bladder sensation. Cockayne et al. studied voiding reflexes in wild-type and P2X3-knockout animals [24]. Knockout mice lacking the P2X3Rs showed significantly decreased micturition frequencies and increased bladder capacity. This finding underlines the role of P2X3Rs in sensing internal organ distention. ATP is released from urothelial cells in response to bladder

distention and it binds to P2X3Rs on sub-urothelial afferent neurons. This interaction activates an afferent neuronal pathway that leads to sensation of bladder fullness [24,25].

P2X3-P2X2/3Rs are involved also in other physiological and pathological conditions. In fact, there is an over expression of receptors in inflamed human colon than in normal colon [26]. Moreover, they are implicated in detection of distension or intraluminal pressure in the small intestine during the reflex contractions initiation [27] and mediate visceral hypersensitivity during acute colitis [28]. The homotrimeric and heteromeric receptors are expressed also in pulmonary neuroepithelial bodies where they are probably involved in pain transmission [29] and $pO_2$ detection [30].

For these reasons, the antagonists of homotrimeric P2X3Rs and heteromeric P2X2/3Rs are of great interest as potential drugs in a number of pain models, bladder disorders and gastrointestinal diseases.

Another approach to regulate the sensitivity of P2X3Rs to painful stimuli could be the use of partial agonists able to reduce the responsiveness of these receptors to ATP stimuli [31].

**1.2. Well known P2X3 and P2X2/3 receptor antagonists**

The trypanocidal drug suramin (8-[[4-methyl-3-[[3-[[3-[[2-methyl-5-[(4,6,8-trisulfonaphthalen-1-yl)carbamoyl]phenyl]carbamoyl]phenyl]carbamoylamino]benzoyl]amino]benzoyl]amino]naphthalene-1,3,5-trisulfonic acid) and its derivatives, consisting in high molecular weight aryl-polysulphonate molecules, were the first discovered class of P2X3R orthosteric antagonists. Modification of the natural ligand ATP led to the discovery of the potent but nonselective P2X3R antagonist TNP-ATP (2',3'-*O*-(2,4,6-trinitrophenyl)adenosine-5'-triphosphate), in which the trinitrophenyl moiety was replaced by cycloalkyl, aromatic rings, and small methylene or an isopropyl group to give simplified antagonist derivatives [32-34]

The antagonist behavior of TNP-ATP was demonstrated also in local administration and it was shown to block the pro-nociceptive effects induced by the P2X3R agonist α,β-meATP in rat [35]. The small ligand A-317491 (5-[[[(3-phenoxyphenyl)methyl][(1S)-1,2,3,4-tetrahydro-1-naphthalenyl]amino] carbonyl]-1,2,4-benzenetricarboxylic acid) was the first competitive and selective P2X3R antagonist with Ki value in the low nM range [36]. However, due to its polar nature (three carboxylate function), it shows poor oral and CNS bioavailability.

Then, a number of small molecules with diaminopyrimidine structure from Roche, i.e. RO3 (5-(2-isopropyl-4,5-dimethoxybenzyl)pyrimidine-2,4-diamine) and RO4 (5-(5-iodo-2-isopropyl-4-methoxyphenoxy)pyrimidine-2,4-diamine), were reported as potent and selective P2X3R antagonists [37,38]. These ligands are allosteric modulators of P2X3Rs with good pharmacokinetic profile. In particular, the adequate pharmacokinetic properties of RO4 make it an excellent tool for in vivo studies in animal models [39,40]. The replacement of the pyrimidine scaffold of these molecules with a purine ring led to compounds, which maintain the antagonist behavior [41,42].

In addition, other pharmaceutical Companies, such as Astrazeneca and Evotec developed some compounds, as P2X3 antagonists, with three aryl and/or heteroaryl moieties and a carboxamide function in meta arrangement on the central ring with $IC_{50}$ values in the nanomolar range [43].

Other P2X3 antagonists include MK-3901 from Merck (N-[1(R)-(5-fluoropyridin-2-yl)ethyl]-3-(5-methylpyridin-2-yl)-5-[5(S)-(2-pyridyl)-4,5-dihydroisoxazol-3-yl]benzamide), a compound which is a substituted arylamide derivative with a high potency and selectivity and good ability to penetrate the brain blood barrier. A very promising diaminopyrimidine analogue from Roche is gefapixant (AF-219 or MK-7264) (5-(2,4-diaminopyrimidin-5-yloxy)-4-isopropyl-2-methoxy-benzenesulfonamide) [44]. This compound was extensively investigated in humans for different pathologies such as chronic cough, pain associated with interstitial cystitis and cough in patients with idiopathic pulmonary fibrosis and it is at present in phase III of clinical trials for refractory chronic cough (https://clinicaltrials.gov/ct2/show/NCT03449134; https://clinicaltrials.gov/ct2/show/NCT03449147). In the last years, many efforts by pharmaceutical companies were directed toward the discovery of potent and selective P2X3R antagonists, especially versus the identification of orally active compounds able to penetrate the central nervous system. To this purpose, a Gedeon Richter Plc, Budapest-Hungary compound collection was tested. Results evidentiated a compound, the 3-(2,5-dimethoxyphenethyl)-N,N-dimethyl-4-oxo-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidine-7-carboxamide, as a potential tool for testing P2X3R inhibitory effects in vivo [45]. In this context it was synthetized compound AZ-2 (N-((R)-1-(6-fluoropyridin-3-yl)ethyl)-3-(5-methylpyridin-2-yl)-5-(5-(pyridin-3-yl)-4,5-dihydroisoxazol-3-yl)benzamide) by AstraZeneca, which showed the highest level of selectivity (about 570 fold) for P2X3 versus P2X2/3Rs and the compound N-(4-(3-(2,5-dimethoxyphenethyl)-4-oxo-3,4-dihydroquinazolin-6-yl)-3-methoxyphenyl)acetamide, an active allosteric antagonist with a

4-oxo-quinazoline, from Gedeon Richter. The latter showed an $IC_{50}$ value of 5 nM and is considered as one of the most potent P2X3R antagonist reported so far [46].

### 1.3. Patent application overview (2015 - 2019)

In this review, a set of about twenty five novel patent applications filed between 2015 – 2019 years and describing new P2X3-P2X2/3R antagonists, together with synthetic methods, pharmaceutical compositions comprising methods of using and formulating the compounds, are described. The major contributor results the pharmaceutical company Roche with ten applications in the last five years.

The new antagonists were tested in *in vitro* assays in transfected cells and *in vivo* experiments in rats or guinea pigs. The *in vitro* activity was evaluated in a functional experiment performed using FLIPR (Fluorometric Imaging Plate Reader) Assay in CHO-K1, CHO-TRex, TRex-293, and HEK-293 cells transfected with cloned rat or human P2X3 and P2X2/3R subunits. Hence, the calcium influx was determined using calcium-sensitive dye in the cells pre-incubated with the tested compound and treated with a fixed dose of the agonist α,β-meATP. The antagonist activity was expressed as $pIC_{50}$ or $pK_i$ (unless noted), calculated as the ability to counteract the effect of the agonist. The structures of the representative compounds, together with in vitro biological data, are reported in the following sections. Nomenclature is given using AUTONOM v.4.0. or ChemDraw Professional 15.0 systems.

## 2. Arylamide derivatives

The pharmaceutical industry Roche focused the attention in the P2X3R and P2X2/3R antagonists, useful for the treatment of chronic inflammatory and neuropathic pain, and genitourinary, gastrointestinal and respiratory diseases. The diseases of urinary tract include incontinence, bladder hyper-reactivity, benign prostatic hypertrophy, prostatitis, urinary urgency, overactive bladder, urethritis, prostatitis, pelvic pain syndrome, etc. In addition, the diseases associated with pain comprise inflammatory, surgical, visceral, premenstrual, and central pain, migraine or cluster headaches, nerve injury, neuritis, cancer pain, post-traumatic injury, etc. The general structure of the arylamide derivatives is represented in fig. 1 [47].

Some examples of the most active compounds, which show only small structure differences, are reported in table 1.

In particular, the stereochemistry of the carbon atom linked to the nitrogen of the amide does not play an important role in the receptor interaction. In fact, the racemic mixture **5**

and the S-enantiomer **6** exhibit not significantly different activity in both P2X3-P2X2/3Rs (8.45 and 6.53; 8.77 and 6.38, respectively). Moreover, also the position of azote in the triazol moiety does not significantly influence the activity, see compounds **1** *versus* **4** and **2** *versus* **6** [47].

Then, the same researchers reported a series of arylamides in which the triazole moiety was substituted with a tetrazole [48]. In figure 2 are showed some examples.

The antagonist activity of the compounds showed a $pIC_{50}$ values in the range 6 and 8.8 at P2X3Rs and 5.5 and 8.1 at P2X2/3Rs. Only biological data of the two following compounds are reported: **1** ($pIC_{50}$ = 8.8) results the most active at P2X3Rs while **3** ($pIC_{50}$ = 8.1) is the most potent at P2X2/3Rs. In the next year [49] a new series of indole, indazole and benzimidazole arylamides (table 2) was patented by Roche, but the activity was no better than those of the first patent [47].

Compound **2** showed the best activity at P2X3Rs with a $pK_i$ = 8.01; the substitution of the tolyl group of **2** with a flexible isobutyl chain reduced the activity (**3**; $pK_i$ = 7.3). The replacement of the indazole core di **3** with an indole (**1**; $pK_i$ = 7.17) does not affect significantly the activity, which decreased when a benzoimidazole scaffold is present (compound **4**; $pK_i$ = 6.46).

In the 2017, the pharmaceutical industry Roche presented five patents describing substituted biphenyl and phenyl-pyridine amides [50], pyridine derivatives [51], thiadiazole arylamides [52], pyrazole arylamides [53], and thiazole/oxazole arylamides [54] as P2X3R and P2X2/3R antagonists. The most representative compounds are shown in the table 3.

These latter series of derivatives return to the structure of the substituted triazole arylamides in which the triazole moiety is replaced with different open chains or five-ring heterocycles containing not only nitrogens but also other heteroatoms like oxygen and sulfur. These molecules were not found more active than those claimed in the first patent [47]. Of all the arylamides listed above, none was included in clinical practice.

### 3. Diaminopyrimidine derivatives

In 2015, the Roche pharmaceutical company presented an EU [55] patent regarding diaminopyrimidine derivatives, which have been previously reported in a US patent [56], as antagonists of the P2X3Rs and/or P2X2/3Rs for the treatment of genitourinary and pain-related diseases. The research then continued by Broka et al. and the use of the compounds was extended to the treatment of respiratory and gastrointestinal diseases [57,58]. In figure 3 are reported some diaminopyrimidine compounds.

Among these derivatives, potent P2X3Rs antagonists were found (i.e. 4-(2,4-diamino-pyrimidin-5-yloxy)-2-iodo-5-isopropyl-phenol which exhibited a $pIC_{50}$ = 8.3 and 5-(2,4-diaminopyrimidin-5-yloxy)-4-isopropyl-2-methoxy-benzenesulfonamide, called gefapixant or AF-219). Some diaminopyrimidines entered in clinical trials; among them, gefapixant (AF-219), originated from Roche and developed by Merck, is an orally active small molecule with an $IC_{50}$ of ~30 nM and 100-250 nM at hP2X3 homotrimeric and hP2X2/3 heteromeric receptors, respectively. It is a potent and very selective allosteric antagonist with $IC_{50}$ values >>10,000 nM at other recombinant homotrimeric P2XRs like hP2X1, hP2X2, hP2X4, rP2X5 and hP2X7 channels [59].

Some diaminopyrimidine derivatives were developed by Afferent Pharmaceuticals as agents for treating hypertension heart failure, dyspnea, sleep apnea and altering carotid body tonicity or activity [60].

In 2018, the same Company reported a patent with new crystalline forms of gefapixant or a salt thereof, especially the citrate and tartrate salts [61]. These salts can be provided stably and constantly for the standpoint of the manufacturing process and are useful in the potential treatment of P2X3 and/or P2X2/3 mediated conditions.

In the last years, Afferent Pharmaceuticals is involving in the research of cures for respiratory diseases like acute or sub-acute cough, urge to cough, and chronic cough. Chronic cough affects considerably on patients' daily-life activities and until now there are no very effective treatments.

P2X3Rs are involved in sensitization of the cough reflex that produces chronic cough. This finding is supported by preclinical studies proposing that P2X3R expression in the vagal afferent nerves of the airways contributes to hypersensitization of sensory neurons. P2X3Rs could mediate cough reflex sensitization, leading to chronic cough [62].

Since, elevated airway levels of ATP were reported in lung pathological conditions, the inhibition of P2X3Rs expressed on airway sensory afferent nerves can produced benefit [63]. Recently, the diaminopyrimidine gefapixant [60] showed to reduce cough frequency in a small placebo-controlled, crossover study of patients with unresolved chronic cough. The compound at high doses (600 mg bd) is highly effective at attenuating the cough related symptoms, representing the most promising therapeutic tool in development [64]. In fact, it is at present in phase III of clinical trials for the treatment of refractory cough. Other known diaminopyrimidine derivatives were patented as agents for the treatment of related cough diseases [65-66].

In this context, Patara Pharma, LLC developed some new pharmaceutical products, obtained combining cromolyn (5,5'-(2 hydroxypropane-l, 3-diyl) bis(oxy)bis(4-oxo-4H-chromene-2-carboxylic acid) with a P2X3 and/or P2X2/3R antagonist, endowed with diaminopyrimidine structure. Cromolyn is a well-known mast cell stabilizer that prevent mast cell degranulation. The synergistic effect of this combination reduce of lung disease symptoms improving side effect profile, tolerability, patient compliance, and efficacy. [67]

**4. Pyrazole, oxazole, pyridine, and pyrimidine derivatives**

From 2015, researchers from Merck Pharmaceuticals reported several molecules inhibiting P2X3Rs in two patents [68, 69]. The first patent describes the synthesis of novel pyrazole and oxazole P2X3R antagonists, which were tested *in vitro* in cell lines stable transfected with the human P2X3 and P2X2/3Rs, and *in vivo* in pain model rats such as spinal nerve ligation model, visceral pain model, etc. Many of them show a P2X3R $IC_{50}$ less than 200 nM and some are depicted in table 4. In the second patent they described pyridine and pyrimidine derivatives as P2X3R antagonists (table 4) [69].

Another patent, by Gwangju Institute of Science and Technology, reports some pyridine derivatives useful for management of neuropathic pain with the activity at P2X3Rs in the nanomolar range [70]. The corresponding research paper was also published [71] and the most interesting of the series is the 3-hydroxy-5-methyl-6-(3-phenoxybenzyl)-2-propylisonicotinic acid that showed an $IC_{50}$ of 295 nM. This antagonist shows also high efficacy in antiallodynic effects in spinal nerve ligation rats.

**5. Pyrazolo-pyrimidine and imidazo-pyridine derivatives**

Asana Biosciences, a biopharmaceutical company focused on the discovery and development of novel targeted therapies in inflammation/immunology and oncology, was involved in 2018 by Thomson and coworkers in the research of novel P2X3-P2X2/3R modulators with pyrazolo-pyrimidine and imidazo-pyridine structures reported in two patents [72,73]. They described also the methods of pharmaceutical formulation and administration of the compounds. Below are depicted some of the most active and/or selective molecules. In particular, some pyrazolo-pyrimidines of the first patent such as **1** and **2**, showed an $IC_{50}$ between 1 and 100 nM at P2X3Rs and >10,000 nM at P2X2/3Rs, resulting very selective (fig. 4). Among the imidazo-pyrimidine derivatives, reported in the second patent, none reached the P2X3 versus P2X2/3Rs selectivity of the above

mentioned pyrazolo-pyrimidines; **3** and **4** are examples of the most active compounds ($IC_{50}$ between 1 and 100 nM) at both receptors.

Researchers of Biopharmaceutical Company BELLUS Health are developing novel imidazo-pyridine derivatives for the treatment of chronic cough. Their purpose was to overcome the loss of taste response, an unpleasant side effect that occurs during the treatment of chronic cough with P2X3 and P2X2/3R modulators. Hence, Garceau et al. developed and patented a new series of selective homotrimeric versus heteromeric P2X2/3R antagonists [74,75]. These molecules showed a selectivity from 10 to >10,000 folds for P2X3 *versus* P2X2/3Rs. Moreover, the therapeutically effective amount of antagonists that are at least 10 fold selective for P2X3 versus P2X2/3Rs was determined. The most selective compounds are showed in table 5

Among novel P2X3R antagonists, the stereochemistry seems to plays a role in the interaction of P2X3Rs; in fact all the compounds having a stereo center with (S) configuration are more active than (R) ones at P2X3Rs. The most selective of the series is compound **2** bearing a propyonyl substituent in 4-position of morpholine group. Experiments with compound **1** were performed in animal models using gefapixant as reference antagonist. The latter exhibited an $IC_{50}$ of 158 nM and 241 nM at P2X3Rs and P2/3X3Rs, respectively. Results demonstrated a comparable antitussive effects in cough response models. On the contrary, in a two bottles taste study, gefapixant altered taste function, while compound **1** was ineffective, demonstrating the advantage deriving from the use of a selective homotrimeric P2X3 antagonist.

Among them, a potent selective and non-competitive homotrimeric P2X3R antagonist named BLU-5937 (**6**) is now in clinical trial phase II for the treatment of refractory chronic cough https://clinicaltrials.gov/ct2/show/NCT03979638?term=BLU-5937&rank=1.

## 6. Conclusion

A consistent number of patent applications regarding the synthesis and pharmaceutical compositions comprising methods of using and formulating of P2X3 and P2X2/3R antagonists were filled in the last five years, reaching its maximum in 2017 with the Roche Pharmaceutical Company as the major applicant. A great number of molecules belonging to different chemical classes were reported. Some of them, which are diaminopyrimidine derivatives previously discovered, reached the clinical trials. In particular, gefapixant was studied in patients affected by a number of pathologies as bladder pain syndrome,

idiopathic pulmonary fibrosis, osteoarthritis of the knee and it is at present in phase III clinical trial for refractory chronic cough.

Recently, the research, focused on selective homotrimeric P2X3R antagonists, led to the discovery of molecules, which avoid the loss of taste response given by the use of unselective P2X3-P2X2/3R antagonists in animal models of chronic cough. In particular, compound BLU-5937 is in phase II clinical trial for refractory chronic cough. Furthermore, novel potential applications in peripheral nociceptive processes of somatic and visceral pain, such as endometriosis pain, have also been reported in recent papers suggesting the potential role of P2X3Rs in various signaling conditions.

## 7. Expert opinion

The interest of P2X3 and P2X2/3Rs modulation in neuropathic pain is increased in the last years though the first hypothesis of these receptors presence was in 1978 [76] and their involvement in the initiation of pain was in 1996 [77]. Treating pain by inhibiting ATP activation of these receptors represents an exciting new approach and P2X3R antagonists could be a new class of drugs that can meet the significant unmet needs in pain management.

In fact, since the P2X3Rs are expressed mainly in primary C-fiber afferent neurons and pre-synaptically in central terminals of afferent neurons, the use of selective P2X3R antagonists reduces the probability of adverse effects in the brain, gastrointestinal, or cardiovascular tissues, effects which remain deterring aspects for many current pain drugs. This has elevated challenge of P2X3R ligands investigation producing many reports by researchers and has piqued the interest of pharmaceutical companies. In the last five years, about twenty patent applications have been filed; some of them regarding the development of previously synthesized P2X3 and P2X2/3R antagonists, while others report the discovery of novel compounds. A pivotal finding was the publication in 2009 of the first crystal structure of a P2XR [78] consisting in P2X4R in *apo*-state and subsequent publication in 2016 of the human P2X3R subtype in the *apo*-ATP and competitive antagonist-bound states [10]. It is worthwhile to note that in silico techniques and molecular modeling played a key role for the identification of novel chemotypes that led to an improvement of the newly synthesized antagonists and related patents in the 2017 year. In general these molecules are low water soluble polisubstituted heterocyclic analogues; the production of their salts improved water solubility and made them suitable for oral administration. An important application of P2X3 and P2X2/3R antagonists is due

to their antitussive effect, especially in the refractory chronic cough. This pathology is difficult to treat as effective antitussives to control cough are currently limited. The optimal drug to treat cough should reduce but not completely suppress it and avoid central nervous system side effects such as sedation. In this sense the P2X3 and P2X2/3R antagonists are very promising. In fact, some of them, like gefapixant, were studied or are at present in clinical trials for refractory chronic cough. An improvement of the antitussive properties seems to derive by the production of novel chemotypes such as imidazo-pyridines, which are P2X3R selective antagonists and do not bind the P2X2/3Rs. The advantage of these ligands is that, contrarily to the unselective agents, they do not alter the taste function. In fact, after appropriate stimulation, the ATP released by the cells of taste buds activates the P2X2, P2X3, and P2X2/3Rs on the taste nerves. Genetic ablation of the P2X2/3Rs involves the total loss of taste in animal model, confirming that the heteromeric P2X2/3R inhibition is the principal responsible of the taste loss. Hence, selective P2X3R inhibitors, that do not bind the heteromeric P2X2/3Rs, do not cause this undesired side effect.

We think that compounds from novel imidazo-pyridines represent new opportunities for pharmacological research and we are confident that compounds like BLU-5937, which is in phase II clinical trial for refractory chronic cough, will be drugs for this disease in the future.

**Author contributions**

G Marucci: manuscript preparation. D Dal Ben: collection of the data. M Buccioni: collection of the data. A Martí Navia: preparation of figures and tables. A Spinaci: preparation of figures and tables. R Volpini: manuscript preparation and revision. C Lambertucci contributed to the English language revision.

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## Figures

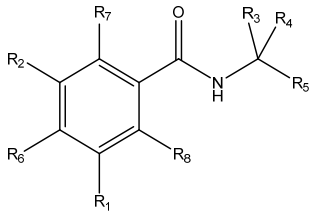
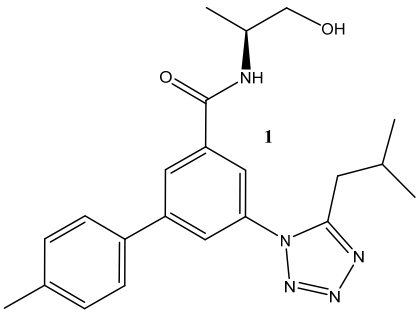
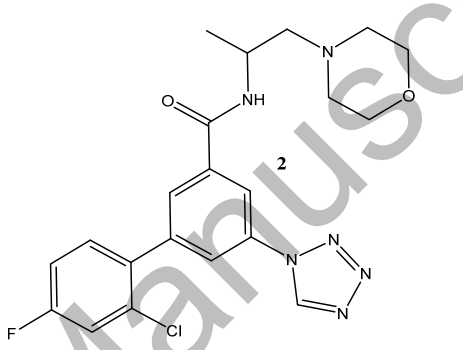


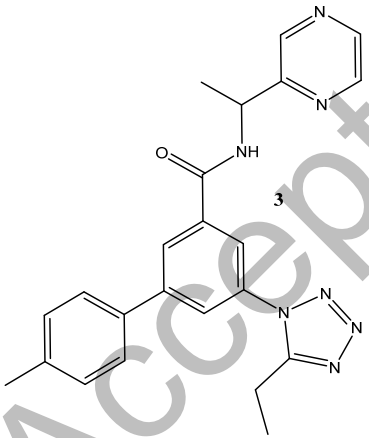

**Figure 1. General structure of arylamide derivatives**



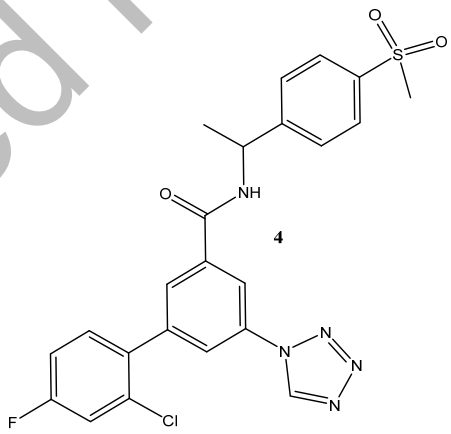

**5-( 5-isobutyl-tetrazol-1-yl)-4'-methyl-biphenyl-3-carboxylic acid ((S )-2-hydroxy-1-methyl-ethyl)-amide**




**2'-chloro-4'-fluoro-5-tetrazol-1-yl-biphenyl]-3-carboxylic acid-(1-mehyl-2-morpholin-4-yl-ethyl)-amide**

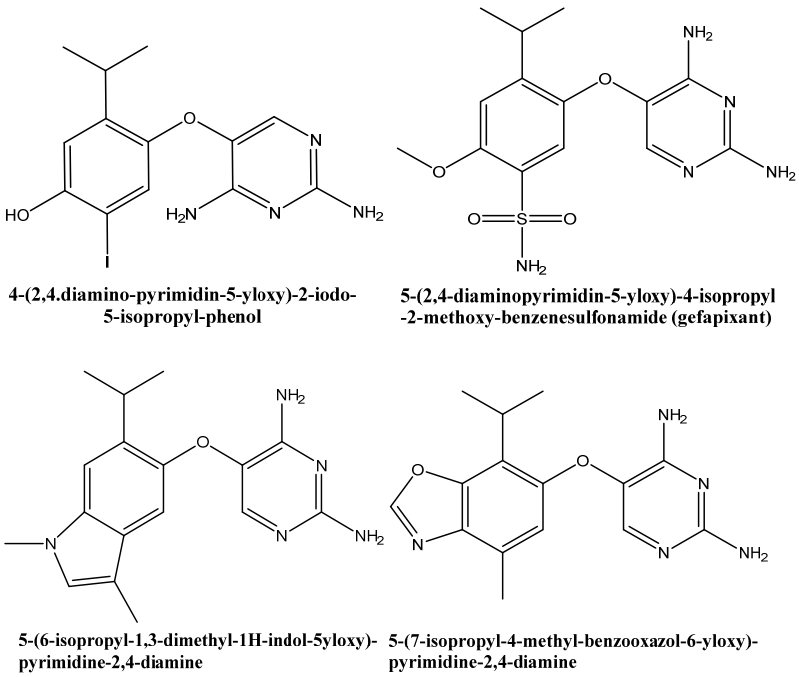



**5-( 5-ethyl-tetrazol-1-yl)-4'-methyl-biphenyl-3-carboxylic acid (1-pyrazin-2-yl-ethyl)-amide**




**2'-chloro-4'-fluoro-5-tetrazol-1-yl-biphenyl-3-carboxylic acid-[1-(4-methanesulfonyl-phenyl)-ethyl]-amide**

**Figure 2. Tetrazole-substituted arylamide derivatives**



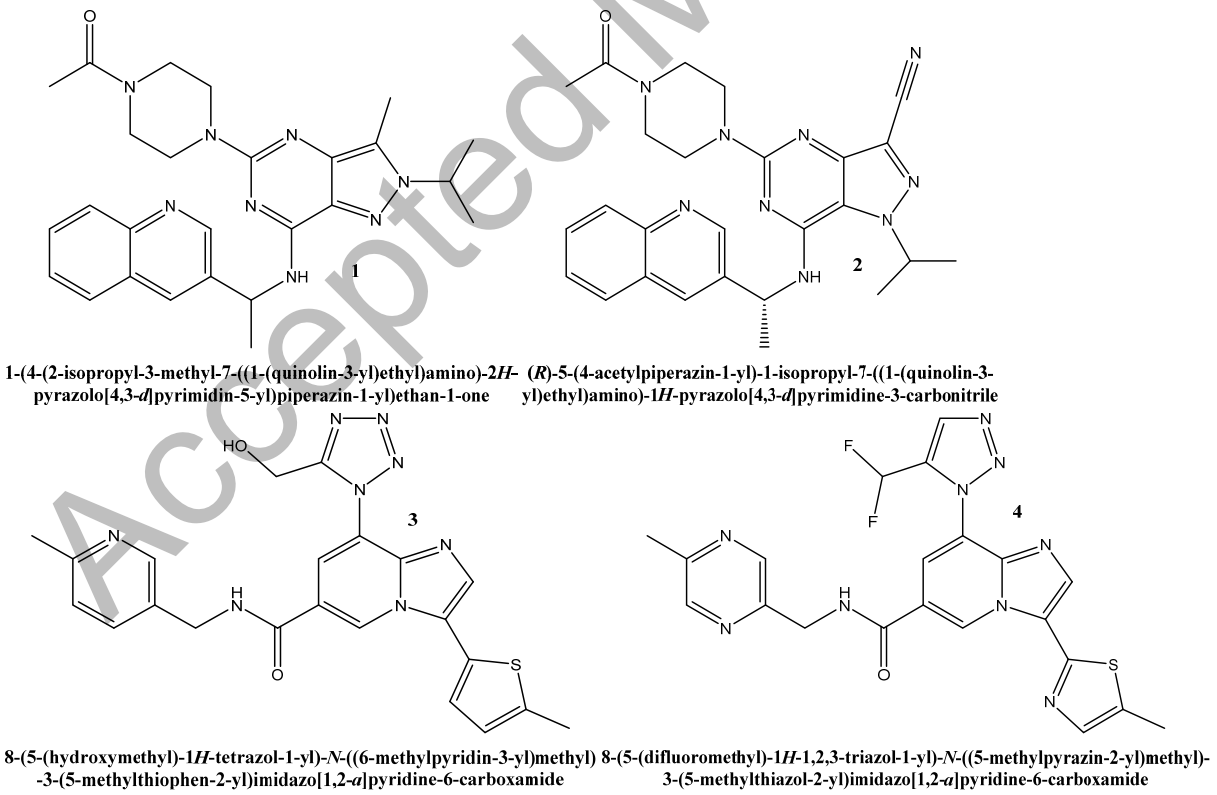
4-(2,4.diamino-pyrimidin-5-yloxy)-2-iodo-5-isopropyl-phenol

5-(2,4-diaminopyrimidin-5-yloxy)-4-isopropyl-2-methoxy-benzenesulfonamide (gefapixant)

5-(6-isopropyl-1,3-dimethyl-1H-indol-5yloxy)-pyrimidine-2,4-diamine

5-(7-isopropyl-4-methyl-benzooxazol-6-yloxy)-pyrimidine-2,4-diamine

**Figure 3. Example of diaminopyrimidine compounds**



1-(4-(2-isopropyl-3-methyl-7-((1-(quinolin-3-yl)ethyl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)piperazin-1-yl)ethan-1-one

(*R*)-5-(4-acetylpiperazin-1-yl)-1-isopropyl-7-((1-(quinolin-3-yl)ethyl)amino)-1*H*-pyrazolo[4,3-*d*]pyrimidine-3-carbonitrile

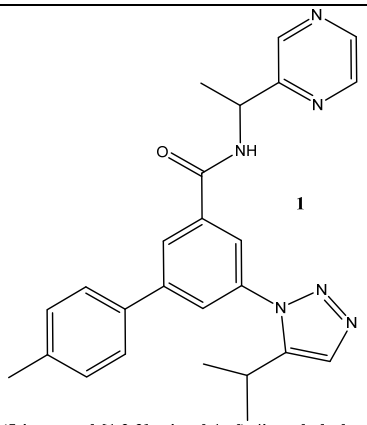
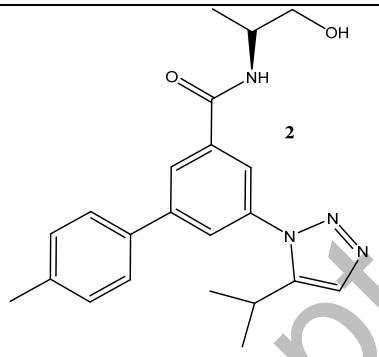
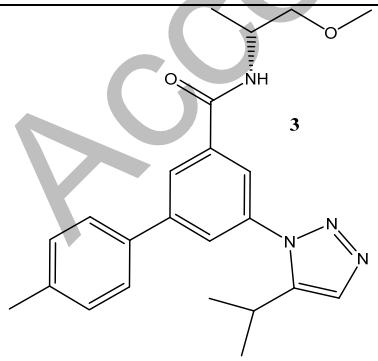
8-(5-(hydroxymethyl)-1*H*-tetrazol-1-yl)-*N*-((6-methylpyridin-3-yl)methyl)-3-(5-methylthiophen-2-yl)imidazo[1,2-*a*]pyridine-6-carboxamide

8-(5-(difluoromethyl)-1*H*-1,2,3-triazol-1-yl)-*N*-((5-methylpyrazin-2-yl)methyl)-3-(5-methylthiazol-2-yl)imidazo[1,2-*a*]pyridine-6-carboxamide

**Figure 4. Representative pyrazolo-pyrimidine and imidazo-pyridine derivatives**

## Tables

### Table 1. Structures and biological data of triazole-arylamide derivatives

| Structure | P2X3Rs | P2X2/3Rs |
|---|---|---|
|  **1** 5-(5-isopropyl-[1,2,3]-triazol-1-yl)-4'-methyl-phenyl-3-carboxylic acid-(1-pyrazin-2-yl-ethyl)-amide | 8.10 | 6.50 |
|  **2** 5-(5-isopropyl-[1,2,3]triazol-1-yl)-4'-methyl-biphenyl-3-carboxylic acid ((*S*)-2-hydroxy-1-methyl-ethyl)-amide | 8.43 | 6.51 |
|  **3** 5-(5-isopropyl-[1,2,3]triazol-1-yl)-4'-methyl-biphenyl-3-carboxylic acid ((*R*)-2-methoxy-1-methyl-ethyl)-amide | 8.13 | 6.49 |

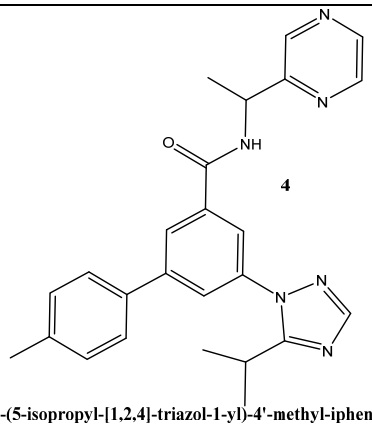
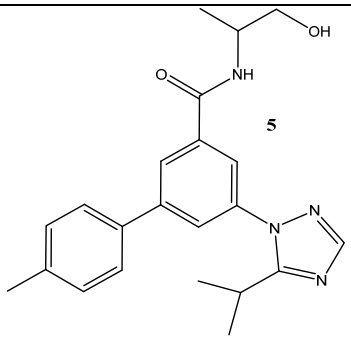
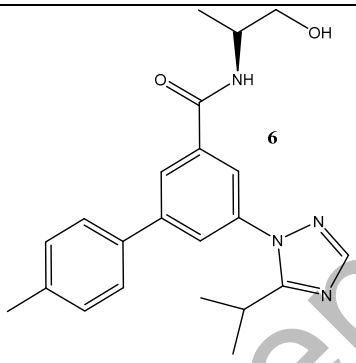
| | | |
|---|---|---|
|  **4**<br>**5-(5-isopropyl-[1,2,4]-triazol-1-yl)-4'-methyl-iphenyl-3-carboxylic acid-(1-pyrazin-2-yl-ethyl)-amide** | 8.51 | 7.62 |
|  **5**<br>**5-(5-isopropyl-[1,2,4]triazol-1-yl)-4'-methyl-biphenyl-3-carboxylic acid (-2-hydroxy-1-methyl-ethyl)-amide** | 8.45 | 6.53 |
|  **6**<br>**5-(5-isopropyl-[1,2,4]triazol-1-yl)-4'-methyl-biphenyl-3-carboxylic acid ((S)-2-hydroxy-1-methyl-ethyl)-amide** | 8.77 | 6.38 |

**Table 2.** Some examples of indole, indazole and benzimidazole arylamide derivatives

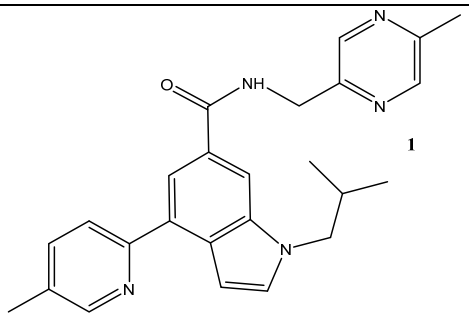
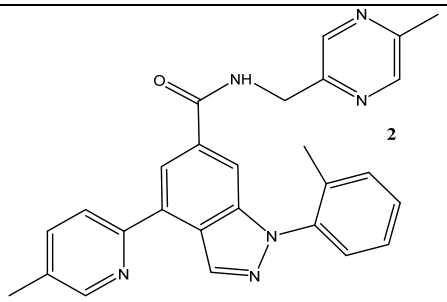
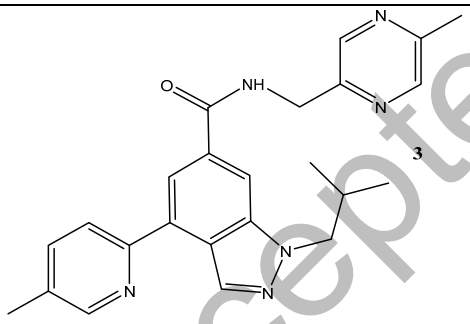
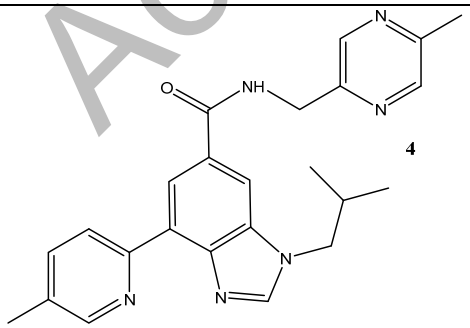
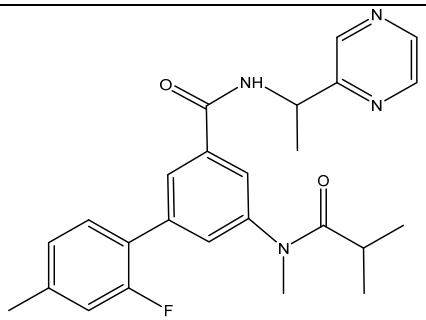
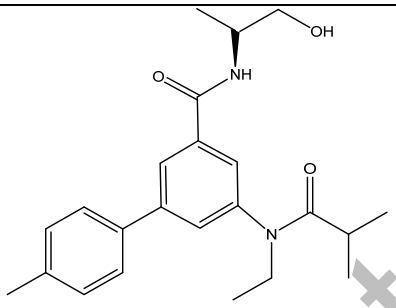
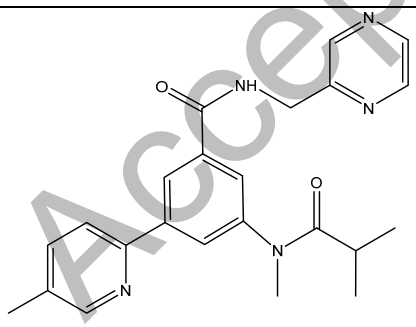
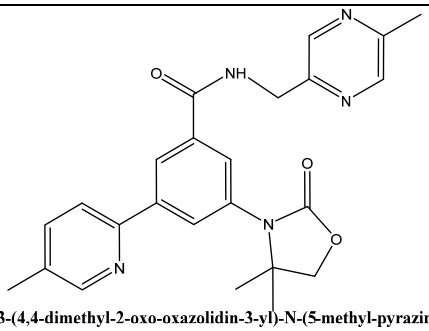
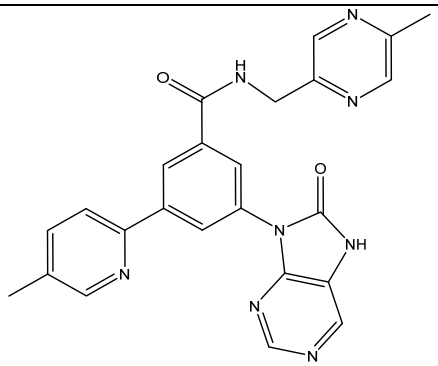
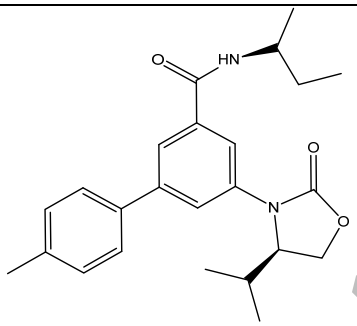
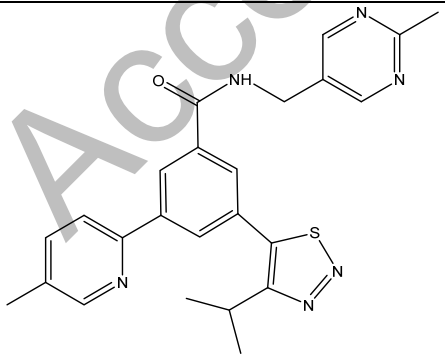
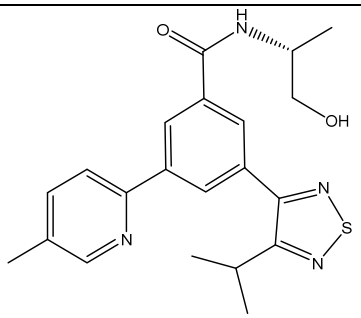
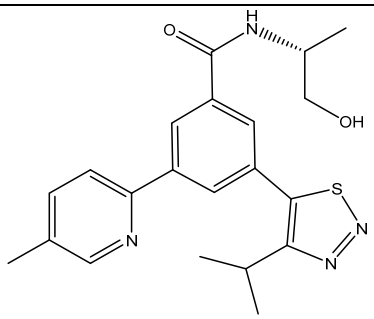
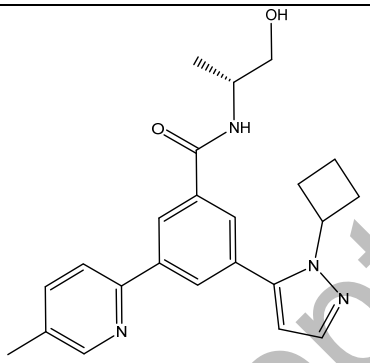
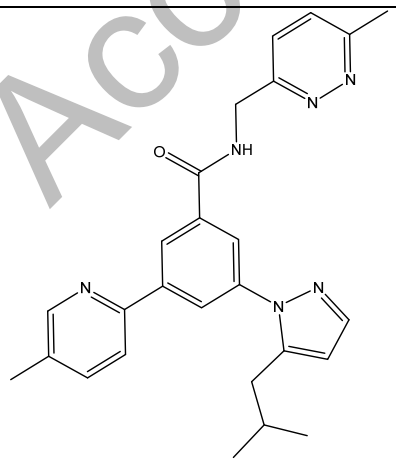
| Structure | P2X3Rs | P2X2/3Rs |
|---|---|---|
|  1-isobutyl-4-(5-methyl-pyridin-2-yl)-1H-indole-6-carboxylic acid (5-methyl-pyrazin-2-ylmethyl)-amide | 7.17 | 5.92 |
|  4-(5-methyl-pyridin-2-yl)-1-o-tolyl-1H-indazole-6-carboxylic acid (5-methyl-pyrazin-2-ylmethyl)-amide | 8.01 | 7.26 |
|  1-isobutyl-4-(5-methyl-pyridin-2-yl)-1H-indazole-6-carboxylic acid (5-methyl-pyrazin-2-ylmethyl)-amide | 7.3 | 5.78 |
|  1-isobuyl-7-(5-methyl-pyridin-2-yl)-3H-benzoimidazole-5-carboxylic acid (5-methyl-pyrazin-2-ylmethyl)-amide | 6.46 | |

**Table 3.** Substituted biphenyl and phenyl-pyridine amides, pyridine derivatives, thiadiazole arylamides, pyrazole arylamides, thiazole/aoxazole arylamides,

| Structure | P2X3Rs | P2X2/3Rs |
|---|---|---|
| **Biphenyl and phenyl-pyridine amides** | | |
|  2'-fluoro-5-(isobutyryl-methyl- amino)-4'methyl-biphenyl-3-carboxylic acid (1-pyrazin-2-yl-ethyl)-amide | 8.25 | |
|  5-(ethyl-isobutyryl- amino)-4'-methyl-biphenyl-3-carboxylic acid ((S)-2-hydroxy-1-methyl-ethyl)-amide | 8.38 | |
|  3-(isobutyryl-methyl- amino)-N-(pyrazin-2-ylmethyl)-5-(-5-methyl-pyridin-2-yl)-benzamide | 8.36 | |
| **Substituted pyridines** | | |

| | | |
|---|---|---|
|  <br>3-(4,4-dimethyl-2-oxo-oxazolidin-3-yl)-N-(5-methyl-pyrazin-2-ylmethyl)-5-(5-methyl-pyridin-2-yl)-benzamide | 8.45 | 7.13 |
|  <br>N-(5-methyl-pyrazin-2-ylmethyl)-3-(5-methyl-pyridin-2-yl)-5-(8-oxo-7,8-dihydro-purin-9-yl)-benzamide | 8.5 | 7.45 |
|  <br>5-((R)-4-isopropyl-2-oxo-oxazolidin-3-yl)-4'-methyl-biphenyl-3-carboxylic acid ((S)-2-hydroxyl-1-methyl-ethyl)-amide | 8.66 | 7.1 |
| **Thiadiazole-substituted arylamides** | | |
|  <br>3-(4-isopropyl-[1,2,3]thiadiazol-5-yl)-5-(5-methyl-pyridin-2-yl)-N-(2-methyl-pyrimidin-5-ylmethyl)-benzamide | 8.05 | 7.47 |

| | | |
|---|---|---|
|  <br>**N-((R)-2-hydroxy-1-methyl-ethyl)-3-(4-isopropyl-[1,2,3]thiadiazol-3-yl)-5-(5-methyl-pyridin-2-yl)-benzamide** | 8.16 | 7.14 |
|  <br>**N-((R)-2-hydroxy-1-methyl-ethyl)-3-(4-isopropyl-[1,2,3]thiadiazol-5-yl)-5-(5-methyl-pyridin-2-yl)-benzamide** | 8.37 | 6.67 |
| **Pyrazole arylamides** | | |
|  <br>**3-(2-cyclobutyl-2H-pyrazol-3-yl)-N-((S)-2-hydroxy-1-methyl-ethyl)-5-(5-methyl-pyridin-2-yl)-benzamide** | 8.67 | |
|  <br>**3-(5-isobutyl-pyrazol-1-yl)-N-(6-methyl-pyridazin-3-ylmethyl)-5-(5-methyl-pyridin-2-yl)-benzamide** | | 7.67 |

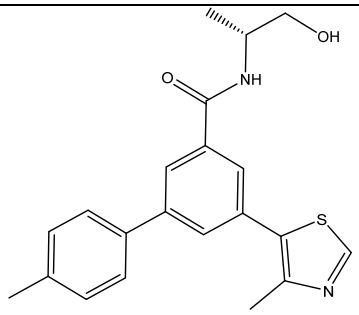
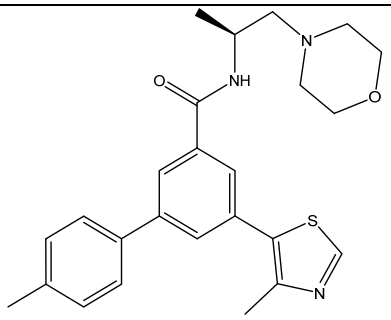
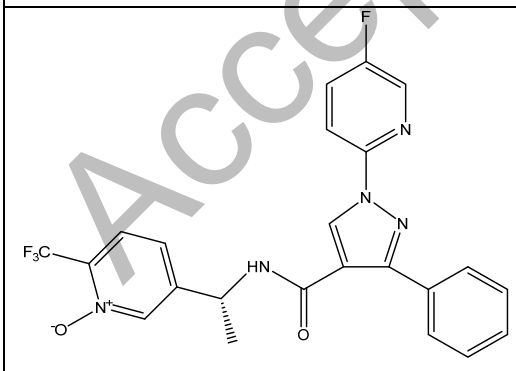
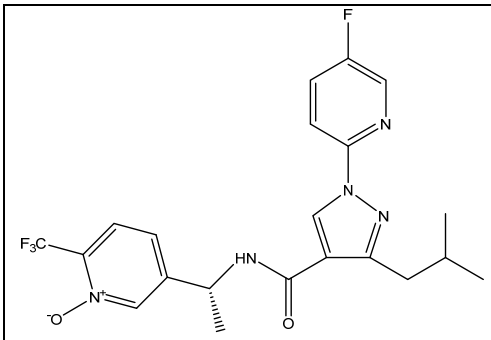
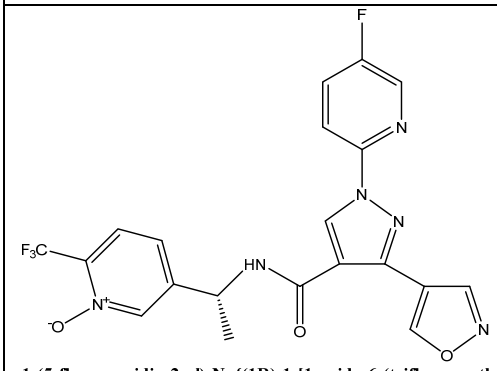
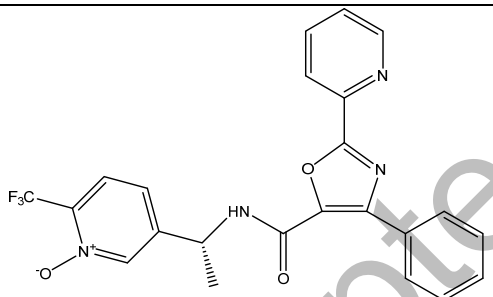
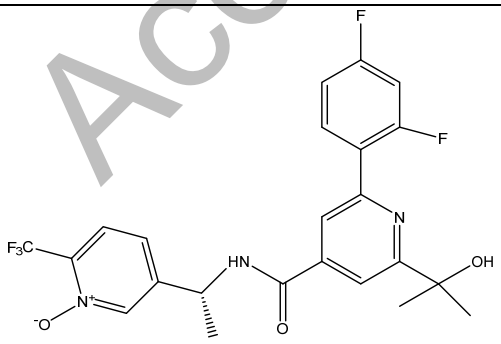
| Thiazole/oxazole arylamides | | |
|---|---|---|
|  <br>(S )-4'-methyl-5-( 4-methyl-thiazol-5-yl)-biphenyl-3-carboxylic acid (2-hydroxy-1-methyl-ethyl)-amide | 8.24 | |
|  <br>(R)-4'-methyl-5-(4-methyl-thiazol-5-yl)-biphenil-3-carboxylic acid (1-methyl-2-morpholin-4-yl-ethyl)-amide | | 7.3 |

**Table 4.** Examples of pyrazole, oxazole, pyridine, and pyrimidine derivatives

| Structure | P2X3Rs ($IC_{50,}$ nM) |
|---|---|
| **Pyrazole and oxazole derivatives** | |
|  <br>1-(5-fluoropyridin-2-yl)-N- {(1R)-1-[1-oxido-6-(trifluoromethyl) pyridin-3yl]ethyl}-3-phenyl-1H-pyrazole-4-carboxamide | 53 |

| | |
|---|---|
|  <br>**1-(5-fluoropyridin-2-yl)-N- {(1R)-1-[1-oxido-6-(trifluoromethyl) pyridin-3yl]ethyl}-3-isopropyl-1H-pyrazole-4-carboxamide** | 34 |
|  <br>**1-(5-fluoropyridin-2-yl)-N- {(1R)-1-[1-oxido-6-(trifluoromethyl) pyridin-3yl]ethyl}-3-isoxazole-1H-pyrazole-4-carboxamide** | 125 |
|  <br>**(*R*)-5-(1-(2-(5-methylpyridin-2-yl)-4-phenyloxazole-5-carboxamido)ethyl)-2-(trifluoromethyl)pyridine 1-oxide** | 84 |
| **Pyridine and pyrimidine derivatives** | |
|  <br>**2-(2,4-difluorophenyl)-6-( 1-hydroxy-1-methylethyl}-N-{(1 R)-1-(1-oxido-6-(trifluoromethyl}pyridin-3-yllethyl}isonicotinamide** | 23 |

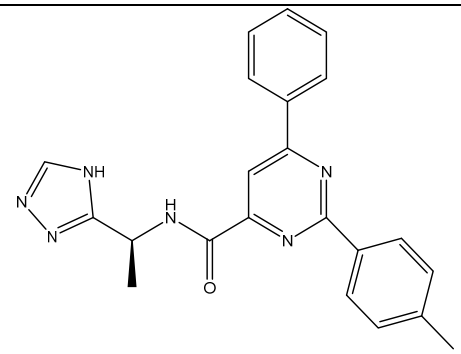
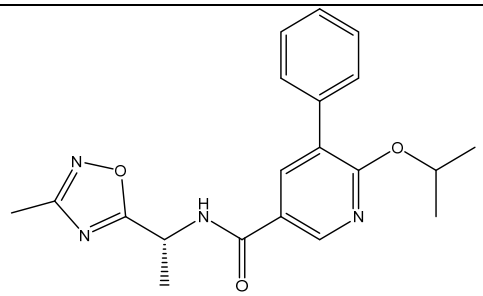
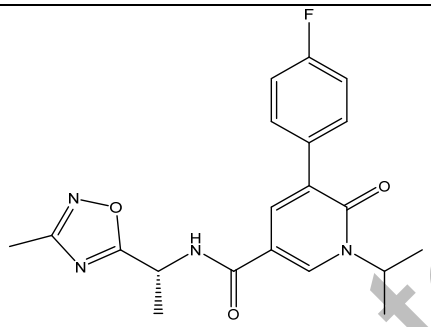
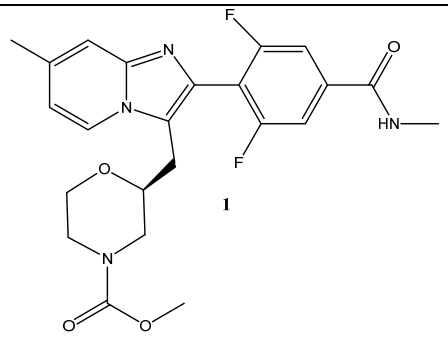
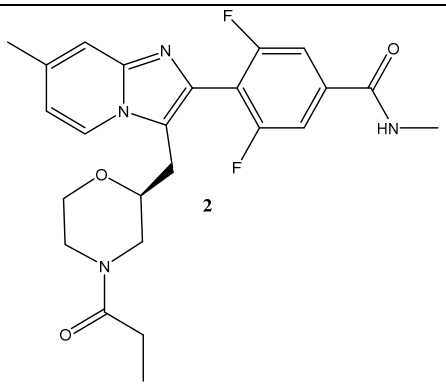
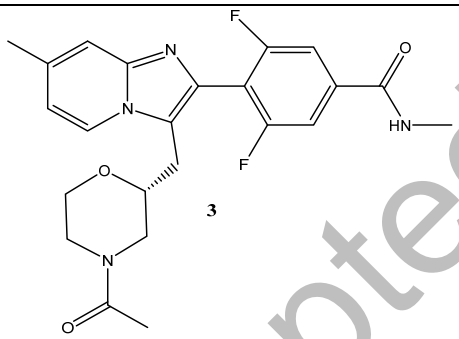
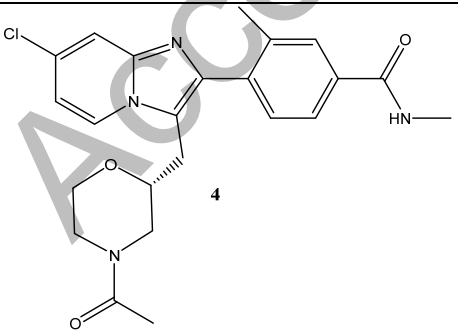
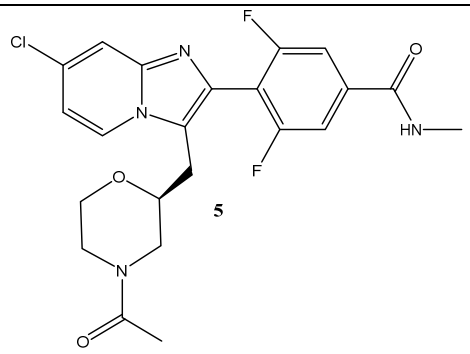
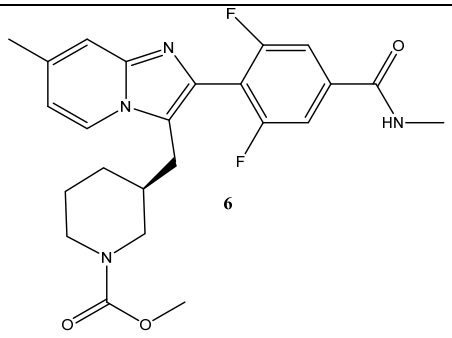
| | |
|---|---|
|  <br>(*S*)-*N*-(1-(4*H*-1,2,4-triazol-3-yl)ethyl)-6-phenyl-2-(*p*-tolyl) pyrimidine-4-carboxamide | 165 |
|  <br>(*R*)-6-isopropoxy-*N*-(1-(3-methyl-1,2,4-oxadiazol-5-yl)ethyl)-5-phenylnicotinamide | 42 |
|  <br>(*R*)-5-(4-fluorophenyl)-1-isopropyl-*N*-(1-(3-methyl-1,2,4-oxadiazol-5-yl)ethyl)-6-oxo-1,6-dihydropyridine-3-carboxamide | 48 |

**Table 5.** P2X3R selective imidazo-pyridine antagonists

| Structure | P2X3R ($IC_{50}$ nM) | P2X2/3R ($IC_{50}$ µM) | Selectivity |
|---|---|---|---|

| | | | |
|---|---|---|---|
|  <br>**1**<br>**methyl (*S*)-2-((2-(2,6-difluoro-4-(methylcarbamoyl)phenyl)-7-methylimidazo[1,2-*a*]pyridin-3-yl)methyl)morpholine-4-carboxylate** | 11 | >30 | >2,700 |
|  <br>**2**<br>**(*S*)-3,5-difluoro-*N*-methyl-4-(7-methyl-3-((4-propionyl morpholin-2-yl)methyl)imidazo[1,2-*a*]pyridin-2-yl)benzamide** | 3 | >30 | > 10,000 |
|  <br>**3**<br>**(*R*)-4-(3-((4-acetylmorpholin-2-yl)methyl)-7-methylimidazo [1,2-*a*]pyridin-2-yl)-3,5-difluoro-*N*-methylbenzamide** | 127 | >30 | > 236 |
|  <br>**4**<br>**(*R*)-4-(3-((4-acetylmorpholin-2-yl)methyl)-7-chloroimidazo[ 1,2-*a*]pyridin-2-yl)-*N*,3-dimethylbenzamide** | 42 | >30 | >714 |

| | | | |
|---|---|---|---|
|  <br>**5**<br>**(*S*)-4-(3-((4-acetylmorpholin-2-yl)methyl)-7-chloroimidazo[1,2-*a*]pyridin-2-yl)-3,5-difluoro-*N*-methylbenzamide** | 39 | >30 | >770 |
|  <br>**6**<br>**methyl (S)-3-((2-(2,6-difluoro-4-(methylcarbamoyl)phenyl)-7-methylimidazo[1,2-a]pyridin-3-yl)methyl)piperidine-1-carboxylate (BLU-5937)** | 25 | >24 | >960 |