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Recent Patents on Novel $P2X_7$ Receptor Antagonists and Their Potential for Reducing Central Nervous System Inflammation

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Abstract

Inflammation arises in the CNS from a number of neurodegenerative and oncogenic disorders, as well as from ischemic and traumatic brain injuries. These pathologies give rise to increased levels of extracellular adenine nucleotides which, via activation of a variety of cell surface P2 purinergic receptors, influence the inflammatory activities of responding immune cells. One P2 receptor subtype in particular, the $P2X_7$ receptor, potentiates the release of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) from macrophage-like cells. It is also thought to contribute to secondary brain injury by inducing neuronal cell death. Therefore, antagonism of this receptor could have significant therapeutic impact on all disorders, not just CNS, to which excessive inflammatory activities contribute. The use of currently available $P2X_7$ receptor antagonists for the treatment of CNS inflammation has been limited to the generally non-selective antagonists PPADS, oxidized ATP, Brilliant Blue G, suramin, calmidizolium, and KN-62. However, the recent patents and development of novel $P2X_7$ receptor antagonists, as discussed in this review, will provide new tools both for clinical and research purposes. Here we discuss compounds for which patents have been applied since 2006, from the following categories: benzamide inhibitors, bicycloheteroaryl compounds, acylhdranzine antagonists, biaromatic $P2X_7$ antagonists, heterocyclic compounds and amide derivatives, and aromatic amine antagonists.

Keywords

P2X7; antagonist; inflammation; CNS; nucleotides

INTRODUCTION

Acute traumatic and ischemic injury to the brain and spinal cord often result in irrecoverable, widespread neuronal death. Moreover, secondary injury, resulting from inflammation present hours to days after the initial insult, exacerbates the primary injury causing further CNS damage [1]. Thus, therapeutics which interfere with inflammatory processes in the CNS may have significant benefit for minimizing tissue damage and promoting neuronal survival after the primary insult. Secondary injury, consisting of an exaggerated and prolonged inflammatory response, is potentially maintained by continued cell lysis and/or the release of pro-inflammatory cytokines by neighboring glial cells [2]. In cases of CNS trauma, ischemia, neurodegenerative disease or malignant tumor growth, increased levels of extracellular nucleotides in the microenvironment are well-established [3–5]. These nucleotides and their

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Conflict of Interest
The authors do not have any conflicts of interest to declare.

metabolites, the endogenous ligands for P2 nucleotide receptors (traditionally known as purinergic receptors), may be responsible for contributing to secondary neuronal injury by inducing apoptosis and prolonging inflammation. Many of these activities of adenine nucleotides are mediated through P2X receptors, and in particular, through the activation of the $P2X_7$ receptor subtype [3,6]. In Table (1) we list a number of inflammatory disorders with which P2X receptors are associated.

The type and severity of CNS insult dictates the levels of extracellular nucleotides present in brain tissue. Because P2 receptors have different affinities for different nucleotides, not all receptors will be activated in all conditions; however, ATP is an agonist for most P2 receptors. ATP is released in the brain by several normal physiologic mechanisms [7,8], including co-release with other neurotransmitters from neurons [9–13] and release from nearby cells through membrane channels or gap junctions [11,15–17]. In this regard, ATP is necessary for propagation of calcium waves between astrocytes [14–19]. Importantly, intracellular ATP concentrations average 3–5mM in most CNS cells [20], and its release into the extracellular space from lysed and damaged cells both within and surrounding the lesion or insult can lead to abnormally high nucleotide levels in the extracellular microenvironment. For example, during hypoxia or ischemia, extracellular ATP levels have been measured in the micromolar range by tissue microdialysis [7,8], although levels in discrete areas are presumably higher still. And in tumors, extracellular ATP levels are chronically high primarily due to their release from regions of cellular necrosis within the tumor itself as well as from dead and injured cells as the tumor grows into healthy tissue. In addition, the levels and activities o f ectonucleotidases (the extracellular enzymes that degrade nucleotides) are also strongly regulated by pathological processes; their expression is greatly suppressed in gliomas [21,22] and in infarcted tissue following embolic ischemia [23]. ATP can also be released from platelets [24] and erythrocytes [25,26] that infiltrate the lesioned tissue, increasing the ways by which nucleotide levels, and their durations of action, can be influenced in the CNS. Although adenine nucleotides perform a number of important regulatory roles in the maintenance of CNS homeostasis and health, their prolonged increase can also contribute to tissue damage and pathology.

ATP is postulated to serve as a “danger signal” to alert and recruit immune cells to the site of tissue damage [27,28] following injury. However, this protective measure often goes awry, as chronic extracellular nucleotide elevation activates the $P2X_7$ receptor which is thought to contribute to secondary neuronal damage. Of all known P2 receptors, the $P2X_7$ receptor requires the highest nucleotide levels for activation [29], and because of its unique functional properties (described below), it has become a prime therapeutic target for decreasing secondary brain injury. We discuss below its characteristics, and activities which make it a good therapeutic target for minimizing secondary brain damage. In addition, we provide a description of novel $P2X_7$ antagonists that have been recently developed/patented.

THE P2 RECEPTOR FAMILY & $P2X_7$-DEPENDENT CYTOLYSIS

The P2 nucleotide receptors are plasma membrane proteins that are subdivided into two classes, P2Y and P2X, based primarily on agonist specificity and predicted transmembrane topologies. P2Y receptors (currently 8 members: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$), are hetero-trimeric G-protein coupled, seven transmembrane spanning proteins for which both ATP and UTP are ligands. P2X receptors, (7 members known: $P2X_1$–$P2X_7$), are trimeric, ATP-gated cation channels [30]. The $P2X_7$ receptor is exceptional in that its activation requires concentrations of nucleotides in the millimolar range as opposed to the micromolar levels sufficient to activate other P2 receptor family members [29]. Moreover, it was long thought that the $P2X_7$ receptor was the only P2X receptor that did not heterotrimerize with other P2X subunits [29]. However, recently, it was shown that $P2X_7$ receptor subunits can functionally interact with $P2X_4$ subunits in macrophages [31]. The heteromeric $P2X_4$/$P2X_7$

receptor appears to have pharmacologic properties of both homomeric receptor subtypes [31]. For example, channel conductance through the heteromeric receptor is sensitive to both ivermectin, a positive $P2X_4$ modulator, and a $P2X_4$ antagonist TNP-ATP. Conversely, the $P2X_7$ antagonist BBG also blocks the heteromeric receptor, suggesting that $P2X_7$ antagonists may be able to interfere with both homo- and heteromeric $P2X_7$ receptor function. This increases the therapeutic utility of $P2X_7$ receptor antagonists since $P2X_4$ receptors have also been implicated in neuropathic pain [32]. However, in both microglia and macrophages, very recently, it was shown that the preferential assembly of $P2X_4$ and $P2X_7$ receptors is homomeric, and that the predominant interactions between $P2X_4$ and $P2X_7$ receptor subunits occur between trimeric receptor complexes, not within individual complexes [33,34], so the pharmacologic profile of these complexes is not yet clear. Additional studies are needed.

Another distinguishing characteristic of this receptor is its ability to mediate reversible permeablization of the plasma membrane [35], provided ATP stimulation is of short duration. $P2X_7$ activation evokes currents carried primarily by Ca^{2+}, K^+, and Na^+ cations [36–45]. Prolonged activation of $P2X_7$ receptors causes the formation of a large, irreversible, non-selective pore [46,47] capable of allowing molecules less than 1 kDa in size to enter the cell. Dyes such as ethidium bromide, propidium iodide, and quinolinium fluorescent molecules such as YO-PRO-1 [35,48,49] are commonly used to study the $P2X_7$ associated pore, which is now thought to result from an interaction between the $P2X_7$ receptor and pannexin [50,51], a recently discovered hemi-channel protein that can function in an unpaired conformation. The important role of pannexins in transmitting signals for apoptosis and necrosis in ischemia has been recently reviewed [52].

Prolonged $P2X_7$ receptor activation causes necrotic lysis, membrane permeablization and subsequent loss of cellular homeostasis, similar to that of bacterial toxins or soluble immune factors such as complement or perforin. However, $P2X_7$ receptors can also induce a more organized form of cellular apoptosis including characteristic membrane blebbing, cell shrinkage, nuclear condensation and DNA fragmentation [53]. Whether a cell undergoes necrosis or apoptosis in response to $P2X_7$ activation likely depends on the cell type and the duration and dose of ATP to which it is exposed. The $P2X_7$ receptor is therefore thought to be central to the development of secondary brain and spinal cord injury when levels of extracellular ATP are chronically elevated.

$P2X_7$ INFLAMMATORY RESPONSE

$P2X_7$ nucleotide receptors are widely distributed. They are found on hematopoietic cells [54], taste bud cells [55], immune cells such as macrophages and microglia [5,56,57], as well as in certain populations of neurons, such as spinal cord motor neurons [58–60]. Within the brain there is also some evidence for $P2X_7$ receptor expression in both oligodendrocytes [61] and astrocytes [62], although aspects of this remain controversial [63]. Several studies indicate a role for this receptor in regulating immune cell inflammatory responses in the CNS [44,64–66]. For example, $P2X_7$ receptors are up-regulated on microglia at the site of ischemic damage after middle cerebral artery occlusion [67]. They are also up-regulated in microglia and astrocytes around β-amyloid plaques in Alzheimer disease [68], and in reactive astrocytes from brain autopsy sections of multiple sclerosis lesions [69]. Moreover, $P2X_7$ receptors are also involved in superoxide generation [68], and the processing and release of mature cytokines including interleukin (IL)-1α, IL-1β and IL-18 [5,38,69,70] from macrophages and microglia. The processing and release of IL-1β is the best studied of these $P2X_7$ receptor cytokine effects, which is now known to involve the rapid activation of caspase-1 [71,72], in both an inflammasome-dependent and inflammasome-independent manner, depending upon the cell type [73,74]. Inhibiting $P2X_7$ receptor activities in hippocampal slice cultures strongly decreases IL-1β levels following LPS treatment [75], and their antagonism *in vivo* also

decreases LPS-induced neuron damage [76]. In a study that used cortical trauma instead of LPS to induce inflammation *in vivo*, a $P2X_7$ receptor antagonist was also beneficial [77]. These studies and others, support the utility of $P2X_7$ receptor antagonists in treating brain inflammation.

It should be mentioned here that a role for $P2X_7$ receptors in promoting neuropathic pain has also been suggested. Because $P2X_7$ receptor activation promotes mature IL-1β release, and IL-1β is implicated in promoting hyperalgesia [78–80], compounds that antagonize these receptors may also be beneficial for treating neuropathic pain [81,82]. Indeed $P2X_7$ receptor deletion [83] and pharmacologic antagonists have recently been shown to decrease pain in different rodent models [82,84–87].

LABORATORY AND MEDICINAL APPLICATIONS

At present, available pharmacological ligands for the $P2X_7$ receptor suffer from poor receptor specificity [35]. For example, BzATP, the most potent known agonist for $P2X_7$ receptors [88], more strongly activates $P2X_1$, $P2X_4$ and $P2Y_{11}$ receptor subtypes than $P2X_7$ [29,58,89–91]; this lack of pharmacologic specificity, also true of $P2X_7$ receptor antagonists, complicates the therapeutic use of these ligands. Therefore, the aim of new drug discovery in this regard, is to develop $P2X_7$ antagonists with greater functional selectivity, higher affinities and lower IC_{50} values, to identify more potent antagonists with fewer side effects. It is necessary to note here however, that it is probable that $P2X_7$ antagonists will have differential effects depending on the cell type targeted and the disease state for which it they are used.

Currently available $P2X_7$ receptor antagonists can be classified into four main groups [29]. The first group contains ions, such as calcium, copper, magnesium, zinc, and protons, all of which inhibit ATP-evoked currents through the $P2X_7$ receptor channel. The second group (Fig. (1)) includes generic or non-selective P2X receptor antagonists such as suramin, pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (PPADS), and Brilliant Blue G (BBG). Third, are the compounds that contain two large organic cations, i.e., the isoquinoline KN-62 (1-(*N*,*O*-bis[5-isoquinolinesulfonyl]-*N*-methyl-L-tyrosyl)-4-phenylpiperazine) and the imidazole calmidazolium (Fig. (1)) which function to block currents through $P2X_7$ receptors and thereby prevent their activity. Finally, monocolonal antibodies are used to block $P2X_7$ receptor function *in vitro*.

Most compounds known to inhibit at least some $P2X_7$-mediated CNS inflammatory responses fall within the second and third classification groups: the non-selective P2 receptor antagonists and the calmidizolium/imidazole classes Fig. (1). All of these compounds, except oATP, are competitive antagonists, but their clinical use is complicated by a lack of information about their pharmacokinetic and pharmacodynamic properties since they were designed as scientific tools for the study of P2X receptors, often *in vitro*, and not as therapeutics. Therefore, when used *in vivo*, they are subject to degradation by ectonucleotidases and other enzymes that metabolize them and change or reduce their functionality, situations with which these agents were not originally designed to contend. Additionally, the current, commercially available antagonists display a great deal of species variability. For example, KN-62, first developed as a specific cell-permeable inhibitor of the autophosphorylation of Ca2+/calmodulin-dependent protein kinase II (CAMK II) [92], inhibits dye uptake and calcium mobilization after activation of human $P2X_7$ receptors, but it shows no inhibition at rat $P2X_7$ receptors [93]. In contrast, BBG has been shown to be significantly more effective at inhibiting rat $P2X_7$ receptors than human [94]. Prior to the development of the compounds by Abbott Laboratories including A-740003, A-438079 and most recently, A-839977 (Fig. (2) [81]), BBG was the most specific $P2X_7$ antagonist available; it has nanomolar affinity at rat $P2X_7$ receptors, but it also inhibits $P2X_2$ and some P2Y receptors in the low micromolar range [48,95,96].

Nonetheless, treatment of secondary neuronal injury using the less selective P2 receptor antagonists PPADS and oATP have shown promise. When administered one hour after injury, both compounds significantly reduced motor neuron cell death and improved recovery of motor function in rats subjected to spinal cord injury using a weight drop impact model [3]. Indeed, the idea that administering a P2X receptor antagonist as a means of preventing secondary injury after spinal cord injury or ischemia resulting from stroke was patented in 2005 (US20050164975A1). In this patent, the authors showed that $P2X_7$ antagonism, in a microenvironment containing high concentrations of extracellular nucleotides (i.e. after a weight drop trauma), is beneficial in preventing further CNS damage [97].

Although there are no clinical trials currently underway to treat inflammation in CNS related disorders, there are ongoing trials using $P2X_7$ receptor antagonists to reduce inflammation and pain in peripheral pathologies. In this regard, Evotec recently announced its launch of a new research program aimed at developing novel $P2X_7$ receptor antagonists for the treatment of inflammation in diseases such as rheumatoid arthritis, irritable bowel syndrome, chronic obstructive pulmonary disease, as well as pain; their compounds have progressed to late stage pre-clinical development so far. (Please see Table (1) for a list of central and peripheral disorders in which P2X receptors are implicated). For the treatment of rheumatoid arthritis, Pfizer has an ongoing clinical trial with a $P2X_7$ receptor antagonist to prevent IL-1β and IL-18 release. Another clinical trial using a $P2X_7$ receptor antagonist is being conducted by GlaxoSmithKline to assess the benefit of treating inflammatory pain with a $P2X_7$ antagonist [98]. Lastly, the AstraZeneca compound AZD9056 is currently in phase 2 clinical trials for the treatment of rheumatoid arthritis [98], suggesting that this drug may have significant therapeutic benefit for peripheral inflammatory disorders. Targeting $P2X_7$ receptors for the treatment of inflammation in the CNS or periphery is still in its infancy, so this remains a relatively unexplored and pharmacologically lucrative area.

In this regard, 220 patent applications have been filed with the United States Patent and Trademark Office since 2001 related to $P2X_7$ receptor modulation of the inflammatory response. Among these, 150 have been filed within the last three years, indicating the rapidity with which this area of focus is expanding [99]. Among these 150 patents, at least 16 directly address the regulation of CNS inflammation by $P2X_7$ receptor antagonists (Table (2)). This does not include the many other patents examining regulation of inflammation via $P2X_7$ receptors in other, peripheral organ systems. In an effort to focus the following discussion, we will summarize the patent applications since 2006 which provide a means to attenuate the CNS inflammatory response by antagonizing $P2X_7$ receptors and their subsequent release of pro-inflammatory cytokines. Very nice comprehensive discussions on the medicinal chemistry aspects of current $P2X_7$ receptor antagonists and their therapeutic uses have been recently presented [84,100,101]. The novel $P2X_7$ antagonists to be discussed here fall into 6 major classes based on chemical structure. Each will be discussed below in succession: 1) benzamide inhibitors, 2) bicycloheteroaryl compounds, 3) acylhdranzine antagonists, 4) biaromatic antagonists, 5) heterocyclic compounds and amide derivatives and lastly, 6) aromatic amines.

1) Benzamide Inhibitors—Pfizer filed three separate patents US20070142329A1, US20070281939A1, and US20060217430A1 in 2006 and 2007, related to the use of three similar compounds. Interestingly, the benzamide structure is similar to that of the potent $P2X_7$ receptor agonist BzATP, consistent with their suggested targeting of $P2X_7$ receptors [102–104]. These drugs are structurally related, but differ primarily in modifications/ substitutions to their structures in the R1, R2 and R3 groups as indicated in the summary of invention claims. In cultured human monocytes, these compounds were found to be efficacious for inhibiting $P2X_7$-dependent pore activity (as assessed by YO-PRO and ethidium bromide uptake) and blockade of IL-1β release.

2) Bicycloheteroaryl Compounds—This class of compounds contains a lipophilic group attached to an amide, linked to an aromatic heterocycle bearing another, often polar or H-bonding group. The increase in lipophilicity of these compounds was often due to the addition of an adamantine group, which also serves to increase drug absorption into the blood stream. In four patents US20080039478A1, US20070225324A1, US20070197565A1 and US20060217448A1, all of which are derivatives of the bicycloheteroaryl compounds, the inventors show the ability of these compounds to inhibit IL-1β release in the human monocytic cell line THP-1 and in peripheral blood mononuclear cells (PBMCs); the EC_{50} values varied depending on the compound examined [105–108]. Each patent provides a table classifying the percent inhibition of IL-1β release relative to untreated cells. Compounds demonstrating inhibition of IL-1β release by 75% or greater were considered to have significant therapeutic potential; multiple drugs with similar structures displayed different capacities to inhibit IL-1β release. Each patent also examined other readouts of $P2X_7$ receptor inhibition such as the ability to reduce neuropathic pain, delay progression of experimental autoimmune encephalomyelitis (EAE), and block $P2X_7$-dependent pore formation and related ion channel currents. These readouts provide additional means by which to assess the breadth of $P2X_7$ receptor functions modulated by these antagonists. Because the lipophilicity of these drugs is increased, they possess outstanding promise for delivery to the CNS, where the ability to cross the blood barrier is often a challenge.

3) Acylhdranzine antagonists—Similar to those discussed above, patent US20060276505A1, filed by Abbott Laboratories, also bases the utility of selected acyl hydrazide and N'-quinoline acyl hydrazide compounds to decrease inflammation in animal models, on their ability to inhibit IL-1β release. These studies were performed both in the THP-1 monocytic cell line and *in vivo* [109]. The greatest inhibition of IL-1β release was demonstrated by the 3-chloro-1-adamantyl variation of the quinoline derived acyl hydrazide (summarized by Nelson *et al.* [110]). Additional acyl compounds were tested using a murine model in which IL-1β levels were decreased and latency of paw withdrawal was increased, indicating effective reductions in tactile allodynia, and hyperalgesia when tested using the Ching and CFA models. Antagonist activity at both the human and rat $P2X_7$ receptor was shown to be similar for one quinoline derived acyl hydrazide compound derivative (1-(4 methoxyphenyl)cyclohexyl).

4) Biaromatic P2X7 Antagonists—Another variation of $P2X_7$ receptor antagonists is presented within patent US20080146612A1, where inventors use a substituted biaromatic group [111]. The addition of the cyclohexylmethyl or cyclohephylmethal groups to the biaromatic-amide derivatives allows for high $P2X_7$ receptor antagonist activity as assessed by their ability to inhibit BzATP-induced $P2X_7$-dependent pore activity as assessed by ethidium bromide uptake. Only compounds able to significantly inhibit dye uptake were included in the patent, however, other measurements of $P2X_7$ receptor function were not presented.

5) Heterocyclic Compounds & Amide Derivatives—The heterocyclic compounds in patents US20080132550A1 and US20080009541A1 both target $P2X_7$ ion channel function. The compound in US20080132550A1 was evaluated for its ability to antagonize the $P2X_7$ receptor using pore formation and Ca^{2+} influx in HEK293 cells expressing recombinant human $P2X_7$ receptors [112]. Of note, this patent was the only one to examine the ability to prevent ischemic brain damage following a 2 hour ischemic episode and 24 hour recovery period. In addition to direct examination of the ischemic brains, functional tests including elicited forelimb placing, postural reflex and shoulder push resistance were performed.

Particularly interesting is patent US20080009541A1, which was designed to specifically block the binding of ATP to the ligand binding domain of the $P2X_7$ receptor [113]. This heterocylic amide derivative is unlike any of the other $P2X_7$ receptor antagonists discussed up to this point,

which do not target specific domains or motifs in the $P2X_7$ receptor protein. The targeting ability of the compound patented was tested both *in vivo* and *in vitro*. The first model employed the use of human recombinant $P2X_7$ receptor-expressing HEK cells. They tested the ability of the bi-aromatic compound to modulate intracellular calcium levels after BzATP treatment. In a second model, they tested its ability to reduce acute inflammatory and neuropathic pain in rats. The ability to directly antagonize the binding of ATP to $P2X_7$ receptors provides for a range of applications. In addition to their therapeutic potential, these drugs will likely also have use in the laboratory due to the limited availability of drugs that show specificity towards the $P2X_7$ receptor [114]. Of the $P2X_7$ targeting drugs currently available, all exert effects on other P2X receptors, if not some P2Y receptors as well. For example, oATP, suramin, BBG and PPADS inhibit most other P2X and P2Y receptors. In addition, many of these non-selective antagonists also block certain ectonucleotidases, a number of intracellular enzymes, and a variety of other ligand-gated ion channels [29,115]. The recently developed heterocyclic amine, A-839977 (Fig. (2)), by Abbott Laboratories appears to be the most specific drug available to date, but further study is needed to confirm this. The availability of these heterocyclic compounds in the future is sure to be an excellent tool for therapeutic treatment of ischemic injury as well as basic $P2X_7$ receptor research.

6) Aromatic Amine Antagonists—Lastly, Abbott Laboratories has three patents filed for aromatic amines that inhibit $P2X_7$ receptors US20070259920A1, US20070105842A1 and US20080076924A1. These three patents describe drugs to be used for the treatment of a variety of CNS-related inflammatory diseases [116–118]. Compared to the other compounds mentioned above, these drugs are structurally distinct because their basic structures contain aromatic amine functional groups. Again, as with many of the other $P2X_7$ antagonists discussed above, these drugs were shown to inhibit IL-1β release *in vitro* and exert anti-nociceptive effects *in vivo* as a measure of their antagonist activities at $P2X_7$ receptors.

CURRENT & FUTURE DEVELOPMENTS

Evidence of a role for $P2X_7$ receptors in neuroinflammation and neurodegeneration is becoming increasingly clear, both *in vivo* and *in vitro* [54]. Antagonists of these receptors may therefore be effective for the treatment of inflammation associated with progressive, neurodegenerative conditions; a recent review by Prof. Burnstock provides an in-depth summary of many currently available P2 receptor agonists and antagonists, and their potential uses in the treatment of CNS disorders [119]. In light of these new tools, it is important to keep in mind that expected antagonist effects *in vivo* may be different from those in *in vitro* pre-clinical studies due to the variable extracellular environments encountered in different disease states. For example, $P2X_7$ inhibition studies by suramin, KN-62, oATP, PPADS, and BBG have IC_{50} values that vary by 10- to 20- fold based on the agonist used, and the extracellular conditions to which they are exposed [48,96,120]. Thus, when comparing the efficacy of different $P2X_7$ receptor antagonists, one must take care not to directly compare absolute IC_{50} values from study to study, as many factors may influence the reported values. Another caveat in the search for effective $P2X_7$ antagonists is the consideration of target specificity. The ability to target a specific receptor without cross-reactivity will allow for focused treatment with minimal side effects. Lastly, many of the studies presented in the above patent applications dealt primarily with the ability of these novel antagonists to decrease cytokine release from human monocytes following LPS stimulation. Although LPS is an important tool for manipulating CNS inflammation in animal models, $P2X_7$ antagonism in a more physiological setting of neurodegenerative or ischemic inflammation may not be identical. However, the patents discussed here do represent a promising start and a significant advancement in the discovery of tools useful for both research and clinical inquiries into the role of $P2X_7$ receptors in CNS inflammation. These antagonists will also assuredly have beneficial effects on the many other disease states influenced by aberrant $P2X_7$ receptor activation as well.

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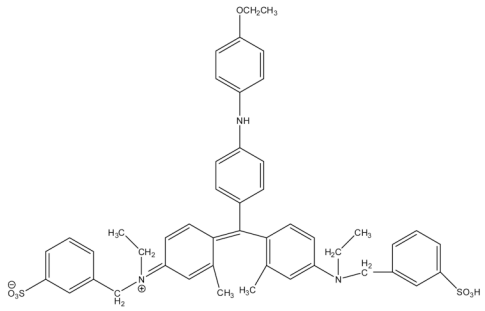
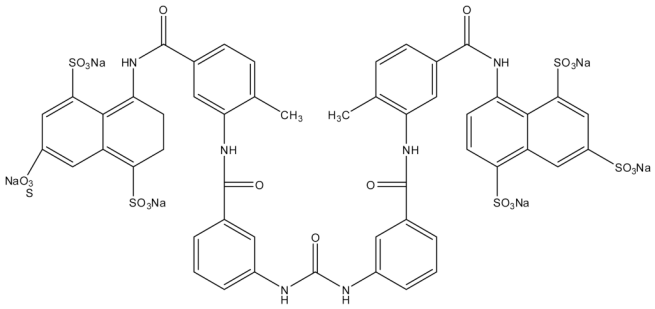
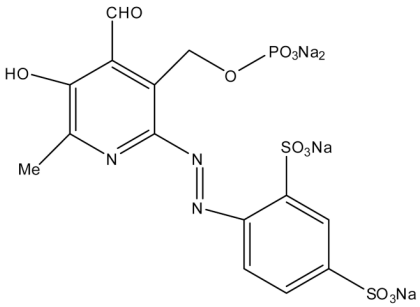


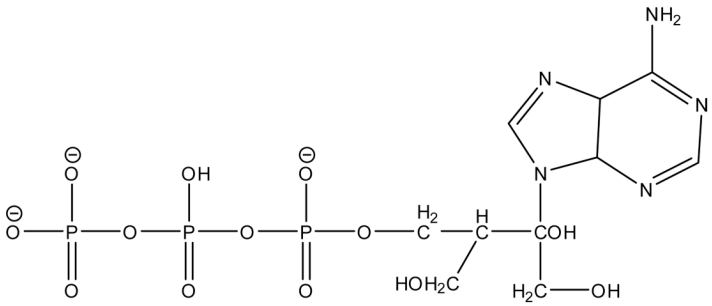
Brilliant Blue G



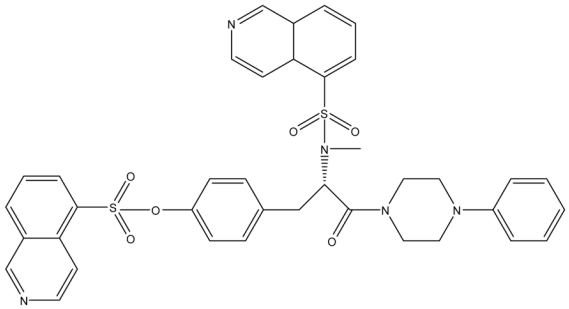
Suramin



PPADS



oATP



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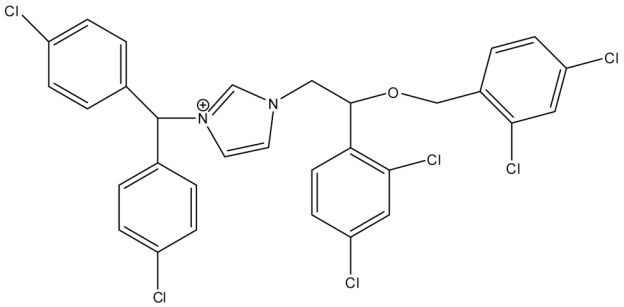
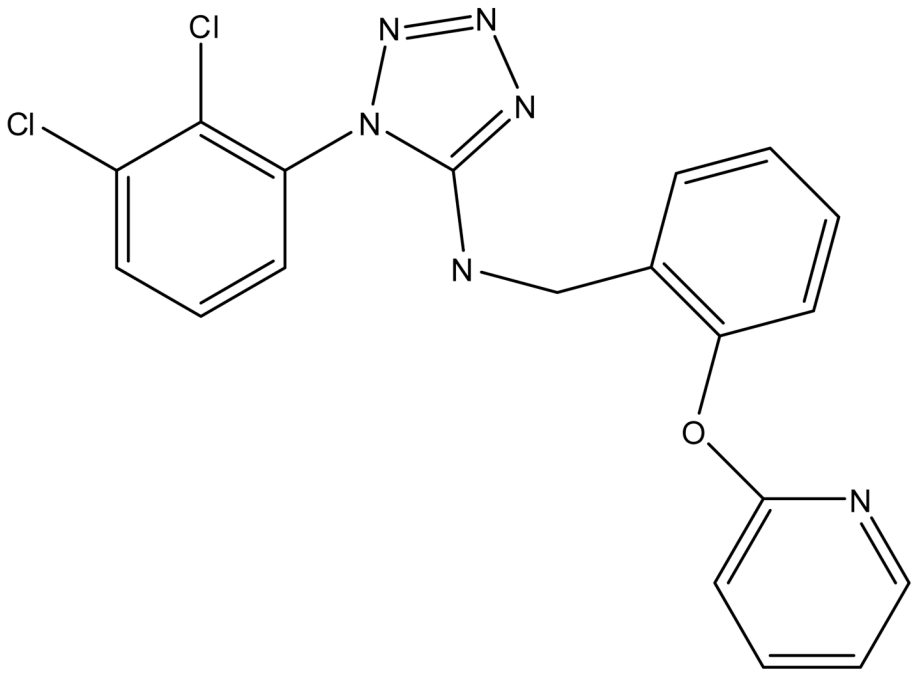


Calmidazolium

Figure 1.
General $P2X_7$ Antagonists



1-(2,3-dichlorophenyl)-N-[2-(pyridin-2-yloxy)benzyl]-1H-tetrazol-5-amine.

Figure 2.
Specific $P2X_7$ Antagonist A-839977

Table 1

P2X Related Diseases

Disease	P2X Receptor Involvement	Citation
Alzheimer's Disease	$P2X_7$ receptors up regulated around β-amyloid plaques $P2X_7$ receptors stimulate excess superoxide production	[68,121–123]
Asthma	Found loss of function P2X7 genotype related to virus-induced loss of asthma control	[124]
Amyotrophic Lateral Sclerosis	$P2X_7$ increases in microglia in end stage SOD1 animals Blocking $P2X_4$ extends the life span of SOD1 mice High $P2X_4$ levels in degenerating motor neurons in spinal cord ventral horns	[68,125–127]
Chaga's Disease	$P2X_7$ receptor associated cell permeabilization	[128]
Chlamydia	Role for $P2X_7$ in disease resistance	[129,130]
Chronic Heart Failure	Upregulation of $P2X_6$	[131]
Diabetes	Increased $P2X_7$ receptor-induced pore formation and apoptosis in retinal microvasculature	[132]
Epilepsy	$P2X_{2,4}$ decreased in hippocampus of seizure prone gerbils Evidence of microglial activation after status epilepticus	[133,134]
Erectile Dysfunction	$P2X_1$ knockout mice show a loss of sympathetic co-transmission in the vas deferens	[135,136]
Inflammatory Bowl Disease	Upregulation of $P2Y_6$ in T lymphocytes and increase in $P2X_7$ induced cytokine expression	[137–139]
Interstitial Cystitis	Upregulation of $P2X_{2,3}$ in urotheilal cells	[140,141]
Ischemia	$P2X_7$ antagonists reduce infarct size	[142]
Major Depressive Disorder	Single-nucleotide polymorphism in $P2X_7$ significantly associated	[143]
Migraine	P2X receptors involved in vasodilation phase	[144]
Multiple Sclerosis	$P2X_{5,6}$ absent in white and grey matter in the frontal cortex of MS tissue $P2X_7$ deficient mice are more susceptible than wild type	[61,125,145–147]
Neuroblastoma	$P2X_7$ mediates proliferation	[148]
Neuropathic Pain	$P2X_7$ receptor antagonism reduces pain $P2X_4$ receptor activation increases microglial activation and pain levels $P2X_4$ receptor antagonists reduce pain $P2X_4$ deficient mice lack mechanical hyperalgesia	[81,82,149–151]
Parkinson's Disease	Disrupted cells stimulate $P2X_7$ dependent cell death leading to pathogenesis of the disease Augmented $P2X_{1,3,4,6}$ proteins in lesions	[128,152–154]
Polycystic Kidney Disease	Purinergic signaling increases cyst expansion	[137]
Rheumatoid Arthritis	$P2X_7$ activation increases leukocyte function and cartilage damage Use of a $P2X_7$ antagonist reduces inflammation and associated pain	[155,156]
Tuberculosis	$P2X_7$ loss of function polymorphism increase susceptibility for TB reactivation	[157]

Table 2

Major Patents/Applications Since 2006 Targeting $P2X_7$ Receptors

Patent Number	Compound Class	Authors	Corporate Affiliation
US20070142329A1 [102]	Benzamide Inhibitor	Dombroski, M.A.; Duplantier, A.J.; Subramanyam, C.	Pfizer Inc.
US20070281939A1 [103]	Benzamide Inhibitor	Dombroski, M.A.; Duplantier, A.J.	Pfizer Inc.
US20060217430A1 [104]	Benzamide Inhibitor	Dombroski, M.A.; Duplantier, A.J.; Subramanyam, C.	Pfizer Inc.
US20080039478A1 [105]	Bicycloheteroaryl Compounds	Kelly, M.G.; Kincaid, J.	Evotec
US20070225324A1 [106]	Bicycloheteroaryl Compounds	Kelly, M.G.; Kincaid, J.; Fang, Y.; He, J.; Cao, Y.; Kaub, C.; Gowlugari, S.; Wang, Z.	Evotec
US20070197565A1 [107]	Bicycloheteroaryl Compounds	Kelly, M.G.; Kincaid, J.	Evotec
US20060217448A1 [108]	Bicycloheteroaryl Compounds	Kelly, M.G.; Kincaid, J.	Evotec
US20060276505A1 [109]	Acylhydrazine Antagonist	Nelson, D.W.; Jarvis, M.F.; Carroll, W.A.	Abbott Laboratories
US20080146612A1 [111]	Biaromatic P2X7 Antagonist	Thompson, T.; Willis, P.	Astrazeneca AB
US20080132550A1 [112]	Heterocyclic Ion Channel Blocker	Shum, P.; Gross, A.; Ma, L.; McGarry, D.G.; Merriman, G.H.; Rampe, D.; Ringheim, G.; Sabol, J.S.; Volz, F.A.	Aventis Pharmaceuticals Inc.
US20080009541A1 [113]	Amide Derivative	Chambers, L.J.; Gleave, R.; Senger, S.; Walter, D.S.	GlaxoSmithKline
US20070259920A1 [116]	Aromatic Amine Antagonists	Carroll, W.A.; Perez-Medrano, A.; Li, T.	Abbott Laboratories
US2007015842A1 [117]	Aromatic Amine Antagonists	Carroll, W.A.; Florjancic, A.S.; Perez-Medrano, A.; Peddi, S.	Abbott Laboratories

Patent Number	Compound Class	Authors	Corporate Affiliation
US20080076924A1 [118]	Aromatic Amine Antagonists	Betschmann, P.; Carroll, W.A.; Ericsson, A.M.; Fix-Stenzel, S.R.; Friedman, M.; Hirst, G., C.; Josephsohn, N.S.; Li, B.; Perez-Medrano, A.; Morytko, M.J.; Rafferty, P.; Chen, H.	Abbott Laboratories