REVIEW

Selective P2X$_7$ receptor antagonists for chronic inflammation and pain

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Abstract ATP, acting on P2X$_7$ receptors, stimulates changes in intracellular calcium concentrations, maturation, and release of interleukin-1β (IL-1β), and following prolonged agonist exposure, cell death. The functional effects of P2X$_7$ receptor activation facilitate several proinflammatory processes associated with arthritis. Within the nervous system, these proinflammatory processes may also contribute to the development and maintenance of chronic pain. Emerging data from genetic knockout studies have indicated specific roles for P2X$_7$ receptors in inflammatory and neuropathic pain states. The discovery of multiple distinct chemical series of potent and highly selective P2X$_7$ receptor antagonists have enhanced our understanding of P2X$_7$ receptor pharmacology and the diverse array of P2X$_7$ receptor signaling mechanisms. These antagonists have provided mechanistic insight into the role(s) P2X$_7$ receptors play under pathophysiological conditions. In this review, we integrate the recent discoveries of novel P2X$_7$ receptor-selective antagonists with a brief update on P2X$_7$ receptor pharmacology and its therapeutic potential.

Keywords Calcium flux · Pore formation · Pain ·
Inflammation · Interleukin-1 · Nerve injury · Microglia ·
Hyperalgesia · Neural-glial interactions

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Introduction

P2X$_7$ receptors belong to the family of ATP-sensitive ionotropic P2X receptors that are composed of seven homomeric receptor subtypes (P2X$_1$–P2X$_7$) [1]. P2X$_7$ receptors are unique among the P2X receptor family since they are activated by high concentrations of ATP (>100 μM) [2]. The P2X$_7$ subunit, previously termed the P2$_Z$ receptor [3], was initially cloned from rat [4] and human brain [5], and from human macrophages [6]. P2X$_7$ receptors are selectively expressed on cells of hematopoietic lineage including mast cells, lymphocytes, erythrocytes, fibroblasts, and peripheral macrophages [4]. Within the CNS, functional P2X$_7$ receptors are localized on microglia and Schwann cells, as well as on astrocytes [5, 7]. The existence of functional P2X$_7$ receptors on peripheral or central neurons remains controversial due to the poor specificity of both antibodies and ligands targeting the rat P2X$_7$ receptor [8]. In rat peripheral sensory ganglia (dorsal root), P2X$_7$ receptors appear to be selectively localized on glial cells, but not neurons [9].

P2X$_7$ receptor-mediated changes in intracellular potassium concentrations lead to the activation of caspase-1 and the rapid maturation and release of the proinflammatory cytokine, interleukin-1β (IL-1β) [10–13]. Increased IL-1β concentrations, in turn, trigger the induction of procaspase-I [14], nitric oxide synthase, cycloxygenase-2, and tumor necrosis factor-α (TNF-α) [15–18]. Activation of caspase-3 has also been linked to P2X$_7$ receptor activation and may underlie receptor-associated cytolytic mechanisms including pore formation [19]. Inhibitors of caspase-1 and caspase-3 effectively block P2X$_7$ receptor-mediated IL-1β release and Yo-Pro uptake, respectively [20]. Activation of P2X$_7$ receptors has also been associated with other downstream signaling pathways including phospholipase D (PLD) [21], phospholipase A$_2$ (PLA$_2$), nuclear factor

kappa B (NF-κB) [22, 23], and mitogen-activated protein kinases (MAPKs) [22, 24–27].

Unlike other members of the P2 receptor superfamily, homomeric P2X$_7$ receptors are activated by high concentrations of ATP (>100 μM) and 2′, 3′-O-(4-benzoylbenzoyl)-ATP (BzATP) which has significantly greater potency (EC$_{50}$= 20 μM) than ATP (EC$_{50}$>100 μM) [2]. It has been demonstrated that the human receptor has a lower sensitivity to agonists than the rat receptor [6]. Originally, functional P2X$_7$ receptors were thought to exist as homomers; however, recent evidence has emerged indicating that P2X$_7$ receptors may form functional heteromeric combinations with P2X$_4$ receptors [28, 29].

There is growing evidence to indicate a pathophysiological role for P2X$_7$ receptors in inflammatory responses [13]. For example, detailed analysis of an independently generated P2X$_7$ receptor knockout (KO) mouse revealed that P2X$_7$ (-/-) mice show a disruption of ATP-induced processing of pro-IL-1β in macrophages [12]. Using a monoclonal antibody-induced model of collagen arthritis, these investigators also demonstrated that P2X$_7$ (-/-) mice show a decreased incidence and severity of arthritis in this model as compared to wild-type control mice [30]. Although published data are lacking, a P2X$_7$ antagonist from Astra-Zeneca, AZ9056 (structure not disclosed), is reportedly under clinical development for the treatment of rheumatoid arthritis and inflammatory bowel disease [31].

A more recent study has demonstrated that P2X$_7$ knockout mice show reduced pain sensitivity following both complete Freund's adjuvant-induced inflammation and partial injury of the sciatic nerve [32]. These data were consistent with the mechanistic role of P2X$_7$ receptors in modulating IL-1β release and the ability of IL-1β to alter pain sensitivity in experimental models. It has been shown, for example, that increased IL-1 levels are associated with enhanced nociceptive signaling in a concentration-related fashion [33, 34]. The P2X$_7$ receptor has also been linked with release of the excitatory neurotransmitter glutamate, well-known for its involvement in pain transmission. In hippocampal slices from P2X$_7$ KO mice, ATP-evoked release of [^{3}H]glutamate was virtually absent, in contrast to the robust neurotransmitter release observed with wild-type controls [35]. Under electrical stimulation, [^{3}H] glutamate release was also significantly attenuated in the KO animals, although some level of residual release was present. These observations are consistent with results implicating the participation of P2X$_7$ in excitatory amino acid release from murine astrocytes [36]. The potential role of P2X$_7$ in modulating glutamate release thus provides an additional rationale for the reduced nociceptive responses of the P2X$_7$ KO mice under chronic inflammation or nerve injury conditions. In view of the participation of P2X$_7$ in the release of IL-1β and glutamate, the localization of P2X$_7$

receptors on glial cells, and the growing appreciation for the role of activated glia in promoting the development and maintenance of pathological pain [37–39], it is tempting to regard the P2X$_7$ receptor as a central player in this neuroimmune interface.

Complementing the genetic data above are recent studies using receptor-selective antagonists indicating a specific role for P2X$_7$ receptor activation in pain signaling [40–42]. Similar to the nociceptive phenotype of mice lacking P2X$_7$ receptors [32] or lacking both isoforms of IL-1 [43], systemic administration of P2X$_7$ receptor-selective antagonists (e.g., A-740003 (**5**), A-438079 (**6**), Fig. 2) produced dose-dependent antinociceptive effects in models of neuropathic [40, 41] and inflammatory pain [41]. These data are also consistent with an independent study of the Astra-Zeneca adamantane P2X$_7$ antagonist (**3**), renamed GSK314181A, that showed dose-dependent antinociception in an inflammatory pain model [42]. Collectively, the foregoing results illustrate the potential role of the P2X$_7$ receptor in modulating IL-1β, and perhaps glutamate, to reduce nociception in neuropathic pain models.

Medicinal chemistry advances

The landscape of P2X$_7$ medicinal chemistry has evolved considerably over the last 5 years. The historically known P2X$_7$ antagonists such as KN-62, PPADS, o-ATP and Brilliant Blue G (see Fig. 1) have gradually given way to a plethora of new molecules that encompass a diversity of structural types (Fig. 2). The cyclic imides (**1**), adamantane amides (**2** and **3**), diarylimidazolines (**4**), cyanoguanidines (**5**), and substituted aryltetrazoles (**6**) exemplify those that have been the subject of recent publications [44–46]. Many other recent advances have only been described in the patent literature [44] or are the subject of forthcoming reports (vide infra). All of these new entries in the field appear to possess better drug-like characteristics in terms of the "rule of 5" and may offer more opportunity for the development of viable therapeutic agents.

A common characteristic of many P2X$_7$ antagonists is that of disparate potency between the human and rat P2X$_7$ receptors. The absence of activity at a rodent P2X$_7$ receptor is, from a preclinical standpoint, an obvious impediment to determining the potential therapeutic applications of P2X$_7$ antagonism. KN-62 is a prime example of this phenomenon, having potent activity at the human receptor but lacking activity at the rat receptor (rP2X$_7$ pIC$_{50}$<4; hP2X$_7$ pIC$_{50}$ ~ 6.7) [46]. The more recently disclosed AZ116453743 (**1**) [47] similarly shows a significant reduction in potency at the rat receptor relative to human (rP2X$_7$ pIC$_{50}$<5; hP2X$_7$ pIC$_{50}$ ~ 8). As will be described below, however, strides are being made in identifying structural types that possess

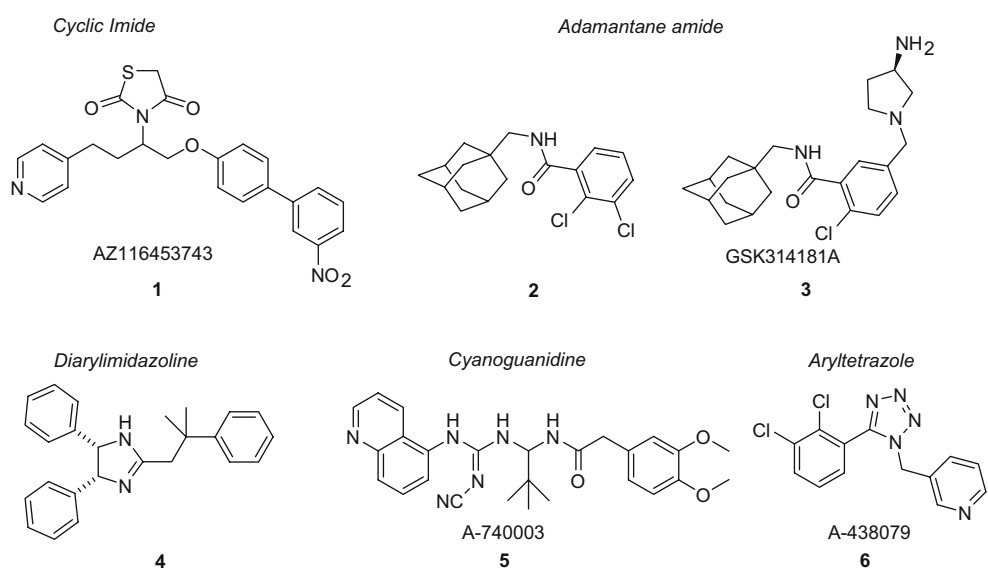**Fig. 1** Historical P2X$_7$ antagonists

PPADS Tetrasodium salt

KN62

oxidized ATP

Brilliant Blue G
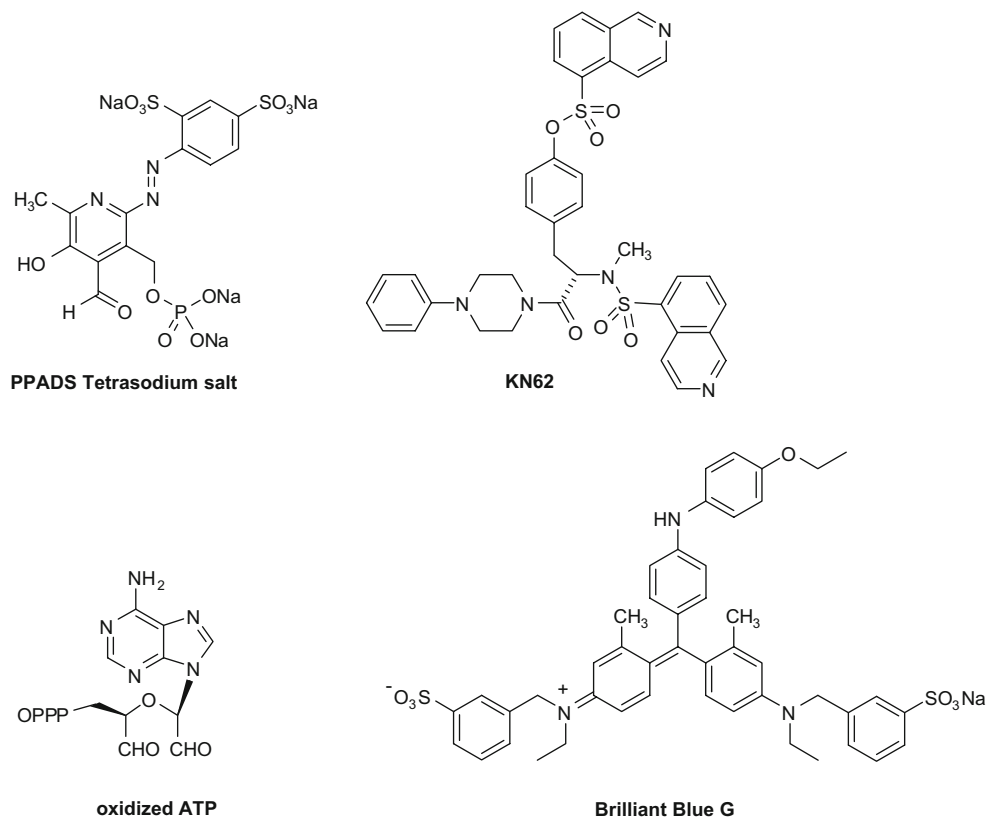
similar potencies across species. These studies have led to the novel finding that selective, competitive P2X$_7$ antagonists are efficacious in models of neuropathic and inflammatory pain as well as inflammatory peritonitis.

Adamantane carboxamides

There has been a significant amount of patent activity around the adamantane amide pharmacophore [44] in addition to the early literature report that provided some

initial insight into the structure-activity relationships (SAR) of this series at the human P2X$_7$ receptor [48]. Very recently, a second study was published that expanded upon the previous findings and also revealed some SAR trends for this series at the rat P2X$_7$ receptor [49]. Reflecting a common trend in the field of P2X$_7$ ligands, the potency of **2** at rat P2X$_7$ (pIC$_{50}$=6.1) was found to be approximately tenfold less than that at the human receptor (pIC$_{50}$=7.2), as measured by Ca^{2+} flux in a FLIPR instrument [50]. On the other hand, GSK314181A (**3**) was reported to be potent at

Fig. 2 Classes of small molecule P2X$_7$ antagonist

Cyclic Imide

AZ116453743

1

Adamantane amide

2

GSK314181A

3

Diarylimidazoline

4

Cyanoguanidine

A-740003

5

Aryltetrazole

A-438079

6

the rat P2X$_7$ receptor (pIC$_{50}$=9.0) as measured using a whole cell patch clamp technique [42] (human data not reported). In a pattern that will be seen repeated across different series of P2X$_7$ antagonists, it was observed that the presence of an *ortho* substituent on the aromatic ring of the benzamide portion gives rise to substantially increased potency. The absence of an *ortho* substituent such as a halogen or a methyl group is accompanied by an attendant drop-off in activity. Compound **2** was among the most potent compounds reported (P2X$_7$ pA2=8.8). Although chain extension from methylene to ethylene was tolerated between the amide and the adamantane, excision of the methylene resulted in a large decrease in activity. Similarly, replacement of the amide NHCO group with NMeCO (*N*-methylamide) and NHCH$_2$ (aminomethyl) was also not tolerated. Further investigation of the linker portion between the phenyl and adamantane revealed that reversing the connectivity of the amide also provided analogs with potent P2X$_7$ activity such as **7** (P2X$_7$ pA2=8.3) and **8** (P2X$_7$ pA2= 7.4) shown in Fig. 3. Many of the published adamantane amide analogs were found to possess poor metabolic stability and pharmacokinetic characteristics; however, the indazole-containing compound **8** (P2X$_7$ pA2=7.4) was improved in this regard, albeit with a corresponding reduction in potency.

Numerous patent applications have disclosed additional structural variations on the adamantane amides with the apparent thrust of activities being to improve the solubility and pharmacokinetic characteristics of this highly lipophilic class of molecules [44]. The limited data available from these sources suggest that this pharmacophore is amenable to incorporation of solubilizing amine-containing functionality with retention of robust P2X$_7$ activity. GSK314181A (**3**) represents a prime example of this structural type as evidenced by the presence of the substituted pyrrolidine appended to the aromatic ring. As mentioned, this molecule displays potent activity at rat P2X$_7$ receptors and has additionally been found to possess activity in the rat CFA model of inflammatory hyperalgesia as assessed using a weight bearing measurement [42].

Further expanding on the theme of heterocyclic amides such as the indazole analog **8**, both substituted pyridines (**A**) and quinolines (**B**) (Table 1) were reported in two recent patent applications to possess potent P2X$_7$ activity [51, 52]. Most interestingly, these heterocycles are amenable to substitution with a variety of amine and alcohol groups that would be
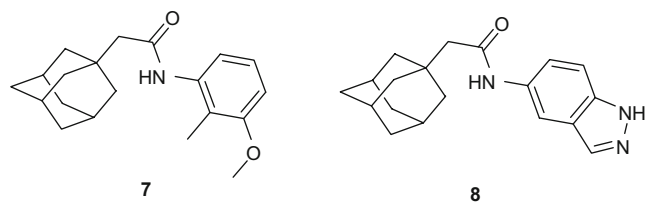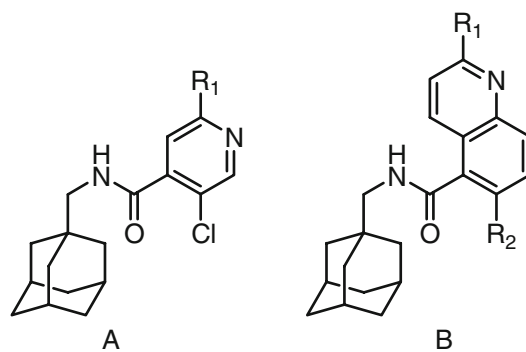
expected to significantly improve the solubility characteristics in this series. As can be seen in Table 1, both secondary and tertiary amines, as well as diamino (piperazine) and alcohol moieties are tolerated in the pendant chain. From the limited data, it is not possible to discern any significant differences between the pyridine- and quinoline-based analogs.
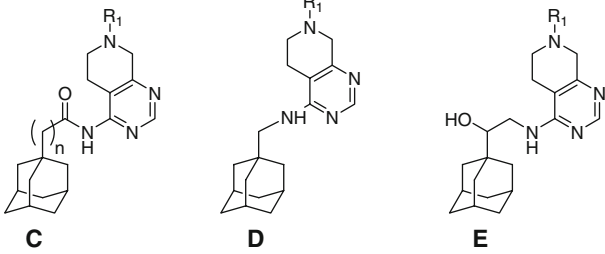
Additional adamantane-derived structural variations that have appeared in the patent literature are shown in Table 2. These analogs are based upon a 5,6,7,8-tetrahydropyrido [3,4-*d*]pyrimidine unit connected to the adamantane via an amide (**C**), aminomethyl (**D**), or aminoethanol (**E**) linker [53, 54]. The connectivity of the amides **18–22** is the same as for **7** and **8**, reversed from compounds **9–17** in Table 1. The amide may be directly attached to the adamantane as in **21** or connected through a methylene group (**18–20, 22**). Although only single concentration data are provided it is possible to gain a sense of some general SAR trends with these pyridopyrimidines. In all three variations **C-E**, there appears to be a significant degree of tolerance for structural variation at the R$_1$ position. Arylamides (**20, 26, 29**), alkylamides (**27, 28**), arylsulfonamides (**19, 25**), alkylsulfonamides (**22, 24**), and benzylamines (**18, 21, 23**) all show activity at less than 0.3 μM. The presence of an electron-withdrawing group attached to the nitrogen is not required as evidenced by the activity of the benzylamine examples. The primary amine example **30** showed appreciable activity but, interestingly, the analog that is epimeric at the alanine-derived carbon did not [54].

Aryl carbohydrazides

A series of hydrazide-containing P2X$_7$ antagonists (Fig. 4) has recently appeared in the literature which bear a resemblance to some adamantane amide analogs described above [55]. Starting with the high throughput screening lead **31** (hP2X$_7$ pIC$_{50}$=7.3), SAR studies led to the discovery of the highly potent antagonist A-847227 (**32**: hP2X$_7$ pIC$_{50}$=8.0). Adamantane substitution and variations thereof provided the most potent analogs in this series but other groups also gave rise to potent, if somewhat attenuated, activity. For example, cycloalkyl (**33**: hP2X$_7$ pIC$_{50}$=7.7), alkyl (not shown), and aryl spirocyclohexyl (**34**: hP2X$_7$ pIC$_{50}$=7.3) groups all showed potent activity as P2X$_7$ antagonists. On the left hand aryl group, an *ortho* substituent was essential for potency as has been observed in other series. The absence of an *ortho* substituent is not compensated for by substitution elsewhere on the aryl group. Tying the *ortho* substituent back in the form of a quinoline ring (**32**) retained the P2X$_7$ potency while significantly improving the physiochemical properties relative to similar substituted phenyl analogs. The isoquinoline moiety in this region also showed appreciable potency (not shown).

Fig. 3 Adamantane carboxamide P2X$_7$ antagonists

Table 1 Pyridine and quinoline adamantane amides

Compound	Heterocycle	R_1	R_2	P2X$_7$ pIC$_{50}$
9	A		-	7.1
10	A		-	7.8
11	B		H	8.1
12	B		H	8.0
13	B		Cl	8.3
14	B		Cl	7.6
15	B		Cl	7.7
16	B		CH$_3$	7.8
17	B		Cl	7.5

Table 2 Tetrahydropyridopyrimidine adamantane amides

C D E

Compound	Heterocycle	R₁	n	Activity
18	C	2,5-difluorobenzyl	1	≥75% IL-1β inhibition @ 0.3 µM
19	C	3-CF₃-phenyl sulfonyl	1	
20	C	3-CF₃-benzoyl	1	
21	C	3-CN-benzyl	0	
22	C	propyl sulfonyl	1	
23	D	benzyl	-	≥75% IL-1β inhibition @ 0.1 µM
24	D	ethyl sulfonyl	-	
25	D	2-F-phenyl sulfonyl	-	
26	D	benzoyl	-	
27	E	cyclopentyl propanoyl	-	≥75% IL-1β inhibition @ 0.3 µM
28	E	cyclohexyl carbonyl	-	
29	E	4-tBu-benzoyl	-	
30	E	2-amino propanoyl	-	

Several hydrazide analogs were assayed for the ability to inhibit IL-1β release in vitro and in a zymosan peritonitis model in vivo. As observed for other classes of P2X₇ antagonists, activity to inhibit Ca^{2+} flux translated into comparable potency to inhibit IL-1β release in vitro (A-847227 (**32**; IL-1β pIC_{50}=8.3). In addition, these molecules demonstrate significant activity to attenuate IL-1β release in the zymosan model in mice. For example, A-847227 (**32**) was found to reduce IL-1β release by 63% when dosed at 20 µmol/kg (i.p.). Consistent with the effects seen with compounds **5** and **6**, A-847227 (**32**) also displayed antiallodynic activity in the Chung model of neuropathic pain with an ED_{50} of 92 µmol/kg (i.p.).

Cyanoguanidines

A novel class of cyanoguanidines, represented by A-740003 (**5**), has recently been discovered to possess potent and selective P2X₇ antagonist properties. The relatively good pharmacokinetic (PK) properties and clean pharmacology of A-740003 made it an attractive tool compound with which to probe the potential therapeutic consequences of selective P2X₇ receptor antagonism. In contrast to some earlier classes of P2X₇ receptor antagonists reported in the literature, A-740003 was found to possess potent activity at the rat P2X₇ receptor, making it suitable for in vivo efficacy studies in rats. A-740003, a potent, competitive P2X₇ antagonist, was efficacious in models of neuropathic and inflammatory pain upon i.p. administration in rats. In vitro, A-740003 potently inhibited Ca^{2+} flux, Yo-Pro uptake, and IL-1β release (pIC_{50}=7.0–7.3).

The cyanoguanidine-containing P2X₇ antagonist A-740003 bears a remarkable similarity to compound **35** (Fig. 5), a structure that was previously discovered to possess activity as an ATP-sensitive potassium channel (K_{ATP}) opener [56, 57]. This is an interesting finding, given that the K_{ATP} channel and P2X₇ are each a ligand-gated ion channel wherein the endogenous ligand is ATP. Another parallel between compounds **5** and **35** is that they both were derived from thiourea screening hits, which themselves were not pursued due to toxicological concerns associated with this functional group. A number of cyanoguanidine K_{ATP} openers were assayed at P2X₇ and generally found to possess very weak activity (Donnelly-Roberts, unpublished results), providing some indication that the SAR trends for K_{ATP} and P2X₇ within this pharmacophore do not substantially overlap. The human P2X₇ pIC_{50} for the K_{ATP} opener **35** was approximately 5.7, considerably weaker than **5**. In general, direct attachment of the aryl group to the amide on the right hand side (RHS) of the structure was a requirement for potent activity at K_{ATP}, whereas the presence of an additional 1 carbon spacer conferred more potent P2X₇ activity. On the left hand side (LHS), a substituent in the
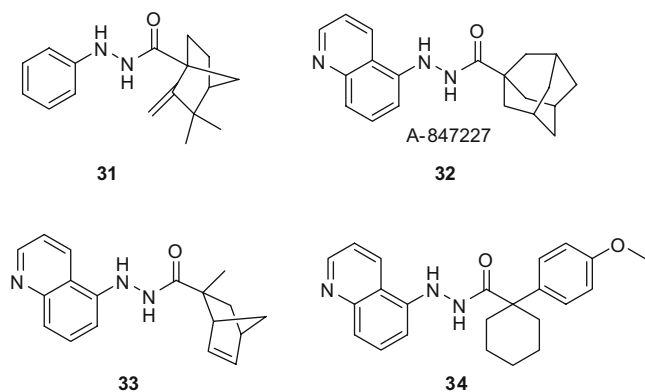
Fig. 4 Arylhydrazide P2X$_7$ antagonists

ortho position increased activity at P2X$_7$ (Donnelly-Roberts, unpublished observations) but decreased K$_{ATP}$ activity [62, 63]. Although substitution patterns that were preferred for K$_{ATP}$ tended to erode activity at P2X$_7$, there were some exceptions. Compound **36**, for example, a hybrid of a preferred LHS for P2X$_7$ and RHS for K$_{ATP}$ possesses potent activity at hP2X$_7$ (pIC$_{50}$=7.2) but weak activity at K$_{ATP}$ (pIC$_{50}$ ~ 5) [57].

The preceding discussion concerns observations that are confined to the aromatic rings on either end of the molecule. Additional SAR studies around A-740003 led to a novel modification wherein the central *t*-butyl-substituted carbon linker has been replaced with a substituted piperazine unit (Fig. 6) [58]. In the case of **37** and **38**, the piperazine is substituted with a phenyl group in either of the two available positions. Both of these compounds retain good potency as P2X$_7$ antagonists (**37**: hP2X$_7$ pIC$_{50}$=7.2; **38**: hP2X$_7$ pIC$_{50}$=7.3), seemingly indifferent to the location of the phenyl substituent. Replacement of the 3,4-dimethoxyphenyl on the RHS was tolerated with various heterocycles such as pyridine, isoxazole, and thiophene. The carbon chain connecting the piperazine amide with the aromatic group on the RHS could be varied from zero to three carbons with little effect on hP2X$_7$ potency. On the left hand side, the *ortho*-tolyl group could be replaced with the 5-quinolinyl present in A-740003 (Fig. 6; compound **39**). Although most compounds from this series showed three- to fivefold greater potency at hP2X$_7$ than rP2X$_7$, **39** was nearly equipotent at rat (pIC$_{50}$=7.5) and human (pIC$_{50}$=7.2) P2X$_7$.
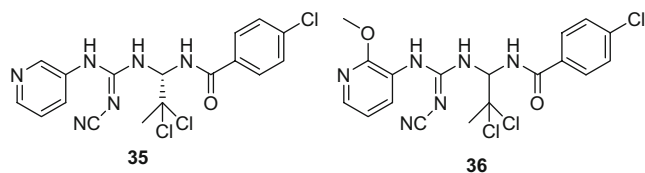
**Fig. 5** Cyanoguanidine K$_{ATP}$ opener **35** and P2X$_7$ antagonist **36**
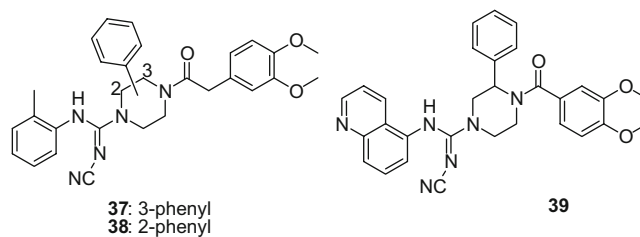
Fig. 6 Cyanoguanidine-piperazine P2X$_7$ antagonists

Aryltetrazoles/aryltriazoles

The growing diversity of small molecule P2X$_7$ antagonists is exemplified by the recent reports detailing SAR studies on aryltetrazoles [40] and aryltriazoles [50] represented respectively by A-438079 (**6**) (hP2X$_7$ pIC$_{50}$=6.3; Fig. 2) and **44** (hP2X$_7$ pIC$_{50}$=7.1; Fig. 7) (see also Table 3). The nature of the heterocyclic core in this pharmacophore was found to be critical for activity as potency decreased in the following order: tetrazole>triazole>pyrazole>imidazole [50]. The connectivity of the aryl and benzyl groups to the core via either nitrogen or carbon appears to be of little consequence as potency for the *N*-aryl compounds **41** and **46** is similar to the *C*-aryl connected counterparts **6** and **43** (Table 3). In the case of the triazole core, three additional regioisomers were evaluated beyond those shown in Fig. 7, for which the potencies were comparable to **43** and **46**. As will be described below in greater detail, the aryltetrazoles and aryltriazoles display the same tendency observed with other chemotypes in requiring the presence of an *ortho*-substituted aromatic group in the molecule for potency to be maintained.

SAR exploration of the RHS of the tetrazole pharmacophore began with an evaluation of preferred substitutions on the phenyl ring. Although highly potent compounds such as **40** were discovered, the poor physiochemical properties of these molecules led to an investigation of heterocyclic attachments that might confer improved properties in this regard. The 3-pyridyl group was found to be an acceptable moiety on the RHS, balancing potency with improved solubility characteristics (e.g., A-438079). With a 3-pyridyl group in place, it was possible to explore variations on the LHS of A-438079. On the left hand aromatic ring of the structure, substitution in both the *ortho* and *meta* positions with either halogen or CF$_3$ groups was a definite requirement for potency. In the *meta* position, CF$_3$ proved superior to chloro in terms of P2X$_7$ potency (**42** vs **41**). Additional placement of fluorine or chlorine in the *para* position was tolerated although typically no further improvement in potency was realized. Other arrangements of halogens on the left side aromatic were substantially less active as were modifications that replaced the halogens with alkyl or alkoxy groups. Replacement of the *ortho*-substituted phenyl with a 5-quinolinyl group, present in A-740003, on the LHS of the tetrazole resulted in a completely inactive analog [40].

Fig. 7 Aryltetrazole and aryltriazole P2X$_7$ antagonists
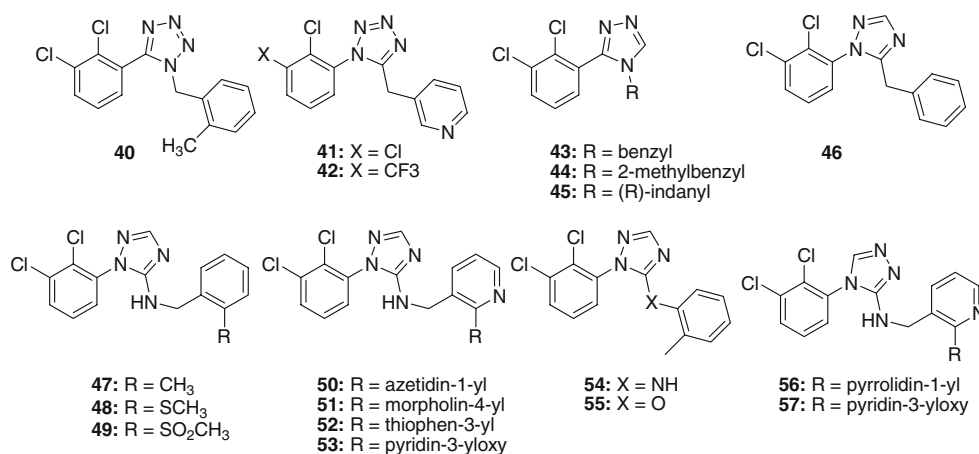
In the case of the aryltriazoles, even the weak basicity of the core was sufficient to allow extensive study of the preferred substitution patterns on the RHS without the solubility limitations associated with the tetrazole. Thus, complementary SAR studies were conducted on the left and right hand sides of the tetrazoles and triazoles, respectively. A more extensive study on the right hand aromatic of the triazoles around **43** revealed a fair degree of tolerance for the interchange of groups in the *ortho* position. Substitution of relatively small electron-donating (OCH$_3$) and electron-withdrawing groups (CF$_3$, CHF$_2$) similarly increased potency in comparison to the simple phenyl [50]. The best potencies, however, were achieved with the more weakly electron-donating methyl (compound **44**) (same trend observed for the tetrazole **40**) and methylthio groups (not shown). Both 2,3 and 2,5 disubstitution was found to retain

Table 3 Aryltetrazole and aryltriazole P2X$_7$ antagonists

	Compound	hP2X$_7$ pIC$_{50}$	rP2X$_7$ pIC$_{50}$
Tetrazoles	**6**	6.9	6.5
	40	7.8	7.2
	41	7.0	6.6
	42	7.9	7.3
Triazoles	**43**	6.3	6.4
	44	7.1	6.7
	45	7.7	7.1
	46	6.7	6.4
Aminotriazoles	**47**	7.8	6.7
	48	7.3	6.5
	49	7.7	7.4
	50	8.0	7.6
	51	7.6	6.7
	52	7.8	6.9
	53	7.9	6.9
	54	7.8	6.6
	55	5.8	5.6
	56	7.8	7.9
	57	7.5	7.5

the potency of the simple *ortho*-substituted analogs; however, the presence of a lone substituent in the *meta* or *para* position markedly decreased potency regardless of the particular moiety present. Connecting the *ortho* substituent to the benzylic position by forming an indane ring provided the most potent triazole reported (**45**), with the (R) enantiomer being several times more potent than the (S) enantiomer. Interestingly, this same modification on the tetrazole core did not result in a similar increase in potency (Donnelly-Roberts, unpublished observations). In both the tetrazoles and triazoles, increasing the length of the spacer between the core and the right hand side aromatic group to two carbons significantly decreased P2X$_7$ potency.

In animal pain models, A-438079 and **44** were found to inhibit mechanical allodynia in the Chung model of neuropathic pain upon i.p. dosing with ED$_{50}$s of 76 and 125 μmol/kg, respectively. Additionally, A-438079 displayed activity against vincristine-induced neuropathy and was effective in the formalin model of persistent pain [59]. Up to a dose of 300 μmol/kg (i.p.), A-438079 did not attenuate rotarod performance. A-438079 has been further shown from in vivo electrophysiological experiments to inhibit both evoked and spontaneous firing of different classes of spinal cord neurons following intravenous administration [59].

A noteworthy extension of the scope of the SAR in this class of P2X$_7$ antagonist has recently appeared showing the potential for nitrogen incorporation into the linker between the core and the RHS aryl group [60] (Fig. 7, compounds **47**–**57**). Although an oxygen linker led to weak activity (**55**), amino (**54**) and aminomethyl (**47**) linkers were well tolerated with the latter variation giving rise to a number of highly potent antagonists, particularly at hP2X$_7$. The activity seen with the two atom aminomethyl linker is especially surprising in light of the poor activity reported with a two carbon linker [40, 50].

Some important similarities and differences are observed between the SAR trends in the methylene-linked (**43**–**46**) and amino/aminomethyl-linked (**47**–**54**, **56**–**57**) analogs.

Like **43–46**, compounds **50–53** and **54** showed ~tenfold greater potency relative to their counterparts lacking a substituent in the *ortho* position. The requirement of an *ortho* substituent was less pronounced, however, where the right hand aromatic was phenyl as in **47–49**. Another illustration of the differences encountered with the amino-methyl-linked triazoles can be seen by comparing the 2-SO_2Me and 2-SMe substitutions with their counterparts in the carbon-linked class. Whereas the 2-SO_2Me group lost potency relative to 2-SMe in the carbon-linked series [50], it instead shows relatively enhanced potency with the aminomethyl linker (**49** vs **48**), particularly at the rat receptor. Reduced potency with monosubstitution in the *meta* or *para* position is also observed in the aminomethyl-linked series.

The diversity of substitution at the *ortho* position has been greatly expanded in the aminomethyl series, with heterocyclic (**50–52, 56**) and heteroaryloxy (**53, 57**) groups all exhibiting potent activity. This tolerance for structural variation was confined to the aminomethyl-linked analogs, however, as replacement of the *ortho*-methyl on compound **54** with *ortho*-morpholinyl (structure not shown) resulted in a 100-fold reduction in activity compared with compound **51** [60].

Regarding regioisomers, similar to observations made in the methylene-linked triazoles, the regioisomeric amino-triazole **57** possessed essentially the same potency as **53** at the human P2X$_7$ receptor. Although no data have been published, it is worth noting that aminopyrazole and amino-tetrazole variations on this series have appeared in the patent literature [61, 62].

Reflecting a common theme in the P2X$_7$ field, different potencies at the rat and human P2X$_7$ receptors were often noted for both the tetrazoles and triazoles (Table 3). Generally, these structural types were three- to tenfold more potent at human P2X$_7$ compared to rat, although a few analogs possessed essentially equivalent potency across species such as **56** and **57**. Although the SAR trends summarized above for the tetrazole and triazole analogs are based upon data measuring the inhibition of Ca^{2+} flux at the recombinant human and rat P2X$_7$ receptors, very similar results were obtained using native THP-1 cells measuring Yo-Pro uptake [40]. Likewise, the rank order potencies for inhibition of IL-1β release paralleled those from the Ca^{2+} flux experiments, with the caveat that potencies were somewhat attenuated for cytokine release [40].

Conclusion

The discovery of potent and receptor-selective P2X$_7$ antagonists has significantly expanded the field of P2X receptor pharmacology. The plethora of emerging new chemotypes have greatly enhanced our appreciation for the important structural features that impart P2X$_7$ receptor activity while also serving to illustrate that this target remains fertile ground for future exploration. A noteworthy commonality that has been observed across the diversity of P2X$_7$ receptor antagonist structural types is the preference or even requirement for the presence of an *ortho*-substituted aromatic group in the molecule. Additionally, substantial progress has been made toward the discovery of potent antagonists at both rodent and human P2X$_7$ receptors that possess improved drug-like properties (e.g., solubility/pharmacokinetics). In this regard, the P2X$_7$ receptor appears to be both more drugable, as well as susceptible to pharmacological modulation in comparison to other P2X receptors like P2X$_3$ and P2X$_4$ [2].

While it is clear that P2X$_7$ receptors play an important mechanistic role in chronic ongoing inflammation, multiple P2 receptor-based mechanisms likely contribute to the ability of ATP to alter nociceptive sensitivity following tissue injury [64]. Activation of P2X$_3$, P2X$_{2/3}$, P2X$_4$, P2X$_7$, and P2Y (e.g. P2Y$_2$) receptors can modulate pain via direct (neuronal excitability) and non-direct (neural-glial cell interactions) mechanisms [63]. The recent report of functional heteromeric P2X$_{4/7}$ receptors [29] may also help bring some mechanistic clarity to the similar functional roles of these receptors in chronic inflammation and pain. While both P2X$_4$ and P2X$_7$ receptors have been implicated in glial-based modulatory mechanisms involved in sensory neurotransmission [9, 65], the discovery of selective P2X$_4$ antagonists are needed to differentiate the respective roles of these receptors in pain signaling. The identification of novel P2X$_7$ receptor-selective antagonists has provided new research tools for both in vitro and in vivo studies of P2X$_7$ receptor pharmacology and led to the generation of new data that indicate an expanded role for this receptor in pain signaling associated with nerve injury and inflammation.

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