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Perspective

Antagonists of the P2X$_7$ Receptor. From Lead Identification to Drug Development

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1. Introduction

The P2X$_7$ receptor is implicated in numerous diseases including pain, neurodegeneration, and inflammatory diseases such as rheumatoid arthritis and osteoarthritis.[1] Since the first patents[2−4] appeared in 1999, there has been a steady increase in publications describing the identification and optimization of new P2X$_7$ receptor antagonists from a growing list of pharmaceutical companies and academic groups. While no P2X$_7$ receptor antagonists have yet reached the market, two compounds have entered clinical trials (see section 4).

Numerous review articles have been published describing the biology, pharmacology, therapeutic potential, and medicinal chemistry of the P2 receptor family in general[5,6] and the P2X$_7$ receptor in particular.[1,7,8] This Perspective focuses on the study of P2X$_7$ receptor antagonists specifically aimed at the identification and development of drugs. The objective is to provide a comprehensive review of published information in this area from both the patent and journal literature to September 2008. An attempt has been made to group related series of compounds, highlight common issues, and describe the strategies being used to address these problems.

2. Biology

Purinoreceptors can be categorized into two broad families; P1 receptors are activated by nucleosides, while for P2 receptors nucleotides are the endogenous agonists. The P2 family can be further divided into P2Y receptors, which are seven-transmembrane G-protein-coupled receptors, and P2X receptors, having a two-transmembrane motif, which are ligand-gated ion channels.[9] For the P2X$_7$ receptor (formerly known as P$_{2Z}$) the endogenous ligand, ATP[a] **1**, is required at relatively high concentrations but the synthetic analogue benzoylbenzoylATP (BzATP) **2** is a useful, higher potency agonist that allows easier manipulation in in vitro systems, particularly for antagonist screening programs (Figure 1). Reports of an alternative ligand for P2X$_7$ in mouse, using nicotinamide adenine dinucleotide (NAD) and the ADP-ribosyltransferase-2 enzyme (ART-2), give a compelling complexity to P2X$_7$ biology, but at the moment this is confined to rodent species because humans lack the required ART-2 enzyme.[10,11]

The P2X$_7$ receptor, first cloned from rat brain and soon after from human monocytes, is the most disparate of the P2X subtypes, in terms of both structure and function.[12,13] Structurally, it differs from the other P2X subtypes in having a long intracellular C-terminal chain. Functionally, upon brief stimulation of the P2X$_7$ receptor, a nonselective cation channel is opened. However, following prolonged exposure to agonist, activation of the P2X$_7$ receptor leads to formation of a membrane pore that can be permeable to molecules up to 900 Da, depending upon the cell type.[14] It was not until the recent work in the Surprenant laboratory[15,16] that dissociation of the pore-forming protein from the P2X$_7$ receptor itself could be made; pannexin-1, a member of a hemichannel gap-junction-like family, is now thought to be the pore-forming protein with the P2X$_7$ receptor functioning as the ion channel.

The P2X$_7$ receptor is found on numerous cell types including macrophages, osteoclasts, and glial cells. The reported conse-

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[a] Abbreviations: ATP, adenosine triphosphate; BzATP, benzoylbenzoylATP; NAD, nicotinamide adenine dinucleotide; ART-2, ADP-ribosyltransferase-2; KO, knockout; LPS, lipopolysaccharide; FCA, Freund's complete adjuvant; SAR, structure−activity relationship; DIDS, 4,4′-diisothiocyano stilbene-2,2′-disulfonic acid; ip, intraperitoneal; iv, intravenous.

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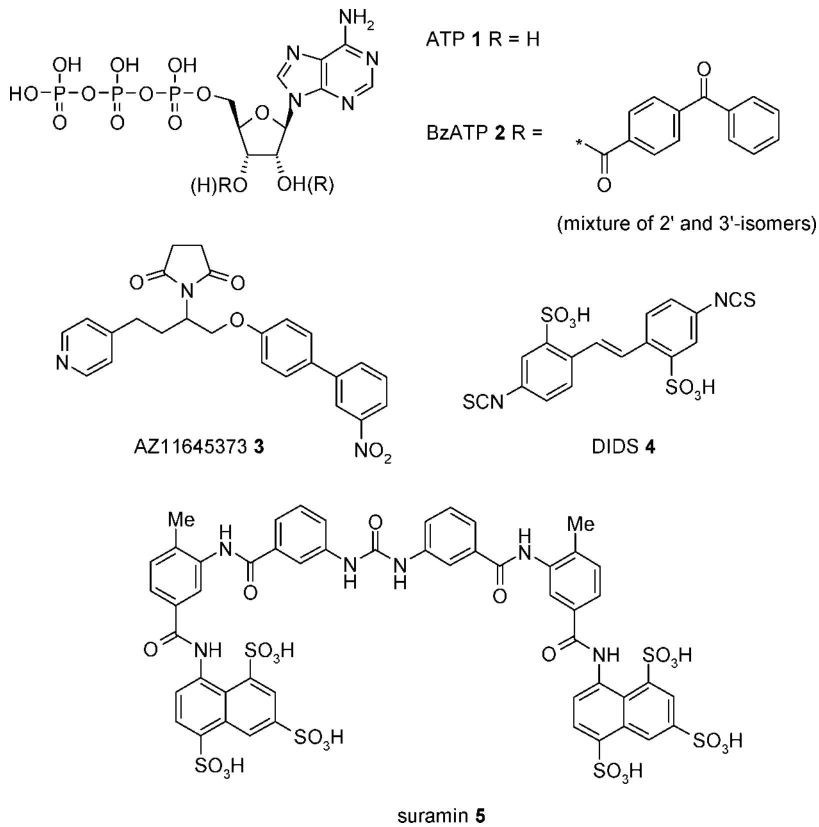


Figure 1. Structures of ATP **1**, BzATP **2**, cyclic imide **3**, DIDS **4**, and suramin **5**.

quences of $P2X_7$ receptor activation in vitro are manifested in a panacea of potential cellular response that are cell type and condition dependent. For example, dependent upon the extent of receptor activation, cell death, via apoptosis and necrosis, and cellular proliferation may occur and are reported within the same cell.[17] The $P2X_7$ receptor has a potential role in cell–cell fusion, initially shown in $P2X_7$ transfected HEK systems, first using antibodies to define colocalization with sites of cell fusion and then using blocking antibodies to inhibit this function.[18] These data have been extended using blocking antibodies to inhibit osteoclast precursor cell fusion in vitro, a bone-resorbing cell type that exists predominately in the multinucleated form.[19] It is noted that other mechanisms for fusion may exist, as osteoclast giant cells can be formed in vitro with cells from $P2X_7$ KO mice. By far the most accepted consequence of $P2X_7$ receptor activation, certainly in vitro, is the established link with the processing and externalization of the proinflammatory cytokine interleukin-1β (IL-1β)[20−22] and its related family member interleukin-18 (IL-18).[23] $P2X_7$ receptor ligation activates caspase-1 and stimulates the secretion of both caspase-1 and the processed bioactive 17 kDa form of IL-1[24] through a nonclassical secretory mechanism of plasma microvesicle loss from the cell surface.[25,26] This link was strengthened further with data from $P2X_7$ receptor KO mice that showed ablation of the IL-1β recovered from the peritoneum of animals pretreated with lipopolysacharide (LPS) and then subsequently with ATP.[27] Furthermore, $P2X_7$ receptor KO animals have been valuable for linking this receptor to potential disease states. $P2X_7$ KO mice have an ablated inflammatory response in a collagen antibody-induced arthritis model, including a reduction in cartilage degradation.[28] Controls using a tetanus toxin response demonstrated no effect of $P2X_7$ receptor deletion on normal immune responses. Also, $P2X_7$ KO mice were shown to have a reduced response to both chronic inflammatory pain (Freund's complete adjuvant (FCA) subplantar injection) and neuropathic pain (partial sciatic nerve ligation).[29]

These studies have, to some extent for joint inflammation and certainly for pain indications, been reinforced with the use of pharmacological intervention using the $P2X_7$ receptor antagonists described in more detail below. Aventis[30] have claimed activity in a collagen antibody-induced arthritis model, a model of inflammatory bowel disease, and a carrageen inflammatory model. Examples from the Astrazeneca adamantane amide series have been shown to successfully inhibit the histological damage associated with the streptococcal cell wall model of joint destruction in the rat, as well as reduce pain sensitivity in this model[31,32] and in a separate inflammatory pain model.[33] Abbott's series of adamantane acyl hydrazides,[34,35] triazoles,[36] and cyanoamidines[37] have reported activity in a peritonteal, zymosan-induced IL-1β release model (with no exogenously added agonist reported). Furthermore, activities in a variety of models of pain (inflammatory, neurogenic, and chemical-induced) have been reported, and these have been extended to triazoles,[38] tetrazoles,[39−41] and cyanoguanidines.[42,43] Lastly, GSK has reported activity of a 5-oxoproline-2-amide series in both inflammatory and neurogenic pain models.[44] It is encouraging to see such reports of activity in disease models emerging, as a number of chemical series have shown poor activity at rat and mouse $P2X_7$ receptor despite having good activity at the human homologue. Additionally, combining animal activity with suitable pharmacokinetic properties has been challenging. For example, the AstraZeneca cyclic imides, characterized pharmacologically in the form of AZ11645373 **3** (Figure 1),[45] have no equity with regard to testing in animal models, as they show human selectivity over both rat and mouse receptors. Sequence homology between human and rodents is 80%,[13,46] and differences in human, rat, and mouse $P2X_7$ function with regard to

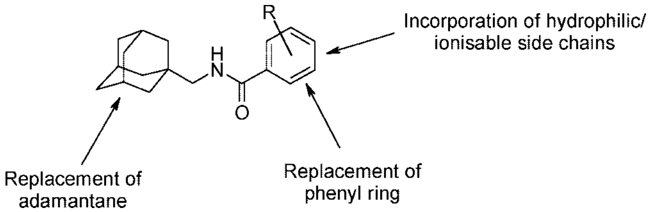
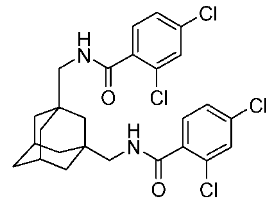


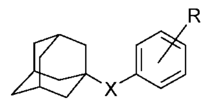
Figure 2. Generic structure of adamantane amides indicating various strategies employed to reduce lipophilicity.



6
hP2X$_7$ pA$_2$ 6.9

Figure 3. Structure of initial AstraZeneca hit compound **6**.

Table 1. Variation of the Linking Group between the Adamantane and a Substituted Phenyl Ring



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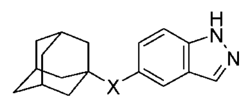


21

compd	X	R	hP2-X$_7$[a,b]	compd	X	R	hP2-X$_7$[a,b]
7	CH_2NHCO	2,4-diCl	6.4[a]	**15**	CH_2NHCO	2,3-diCl	8.8[a]
8	CH_2NHCO	2-Cl	8.1[a]	**16**	CH_2NHCO	2,5-diCl	8.3[a]
9	CH_2NHCO	4-Cl	<5[b]	**17**	CH_2NHCO	2-Cl,5-OMe	8.8[a]
10	CH_2NMeCO	2-Cl	5.4[b]	**18**	CH_2CONH	2-Me,3-OMe	8.3[a]
11	CH_2NHCH_2	2-Cl	5.4[a]	**19**	CH_2CONH	2-Me,5-OMe	8.0[a]
12	NHCO	2-Cl	<5[b]	**20**	CH_2CONH	2-Me	6.8[a]
13	$(CH_2)_2NHCO$	2-Cl	7.8[a]	**21**	CH_2CONH		7.4[a]
14	CH_2CONH	2-Cl	6.3[b]				

[a] pA_2. [b] pIC$_{50}$ in a BzATP induced ethidium uptake assay in a human monocyte cell line.

kinetics[47] and potency[48] are known, and this may underlie lack of species crossover in some series. Certainly, the commonality of the agonist, ATP, may have given some confidence for species crossover, but the indication that some series are allosteric in nature[49,50] may account for species differences. Other series with antagonist profiles consistent with a competitive nature[42] do demonstrate more robust species crossover, but the definitive mechanistic work using radiolabeled compounds has not been performed. It is still unknown which antagonist profile is best suited to demonstrate clinical efficacy, as all compounds are certainly reversible antagonists.

Different studies have used a variety of biological end points to monitor P2X$_7$ function in order to determine antagonist activity at the P2X$_7$ receptor. These include measurement of channel activity using cation flux (usually influx of calcium or barium but also efflux of potassium), exploitation of the pore forming properties by measuring uptake of fluorescent DNA binding dyes (e.g., ethidium bromide or Yo-Pro), and measurements of inflammatory readouts by monitoring IL-1β release. The standardization of affinity estimates of putative antagonists in biological systems, particularly functional assays, can be complex and is outside the scope of this article. Suffice it to say that despite the majority of reports using pIC$_{50}$ as the simplest measurement, the absence of knowing exact experimental conditions of a particular assay means caution should be applied when comparing values across assays. For this reason attempts to compare results between studies have been avoided wherever possible; rather, SAR has been derived from within a single study.

Historically, P2 receptor studies have relied on two broad classes of compounds that have been used to aid receptor classification and to understand pharmacology, although both can suffer from poor selectivity.[8] First, compounds derived from ATP **1** have been used extensively as tools, but this work is complicated by the instability of such compounds in assay systems and/or effects on metabolizing enzymes (e.g., ecto-nucleotidases). The second class consists of highly charged polysulphonated dyes such a DIDS **4** and suramin **5**. Neither class provides an attractive lead toward a drug, especially when oral bioavailability is desired,[51,52] and so will not be described further here.

3. Druglike Antagonists of the P2X$_7$ Receptor

3.1. Arylamides and Related Series. Several groups have disclosed P2X$_7$ receptor antagonists related to a series of adamantane amides first described by AstraZeneca. Early examples of these compounds, represented in Figure 2, have suffered from high lipophilicity, which is likely to give rise to issues such as poor metabolic stability and low aqueous solubility. A number of strategies have been employed to reduce lipophilicity within this pharmacophore including incorporation of hydrophilic and ionisable side chains, replacement of the phenyl ring with heterocyclic analogues, and replacement of the adamantane (or other lipophilic group) with less lipophilic derivatives.

AstraZeneca described the derivation of this amide series following the discovery and subsequent elaboration of an initial hit **6**, identified from high-throughput screening (Figure 3).[2,3,53] The compound had reasonable potency (determined in a BzATP-induced ethidium uptake assay in a human monocyte cell line) but suffered from a combination of high molecular weight (MW = 540) and high lipophilicity (cLogP = 6.2)[54] and therefore failed to meet lead criteria. Removal of one of the amide side chains afforded a monoamide **7**, which was shown to have acceptable molecular weight (MW = 338, cLogP = 5.2) and similar potency (Table 1). Analysis of a series of analogues quickly established that the 2-chloroamide **8** (MW = 304, cLogP = 4.5) was significantly more potent than amides **6** and **7** and that the 4-chloroamide **9** had poor activity. This result was rationalized by suggesting that the ortho-substituent causes a twist in the orientation of the benzamide that is necessary for potent P2X$_7$ antagonism and that substitution in the 4-position is disfavored. Baxter reported on an extensive search for adamantane replacements through screening of many 2-chlorobenzamides; however, at this point no replacements were found and so **8** became the new starting point for further optimization with a view to further reduce lipophilicity and increase potency.

Exploration of the linking group between the adamantane and the 2-chlorophenyl ring showed that N-methylation, **10**, carbonyl reduction, **11**, and removal of the methylene group, **12**, all resulted in reduced activity, whereas chain extension, **13**, and reversal of the amide, **14**, led to a more limited loss in potency. Exploration of the core structure via a parallel synthesis approach using an array of carboxylic acids and adamantylmethylamine rapidly demonstrated that only aromatic amides had potency against P2X$_7$ and SAR around the benzene ring was

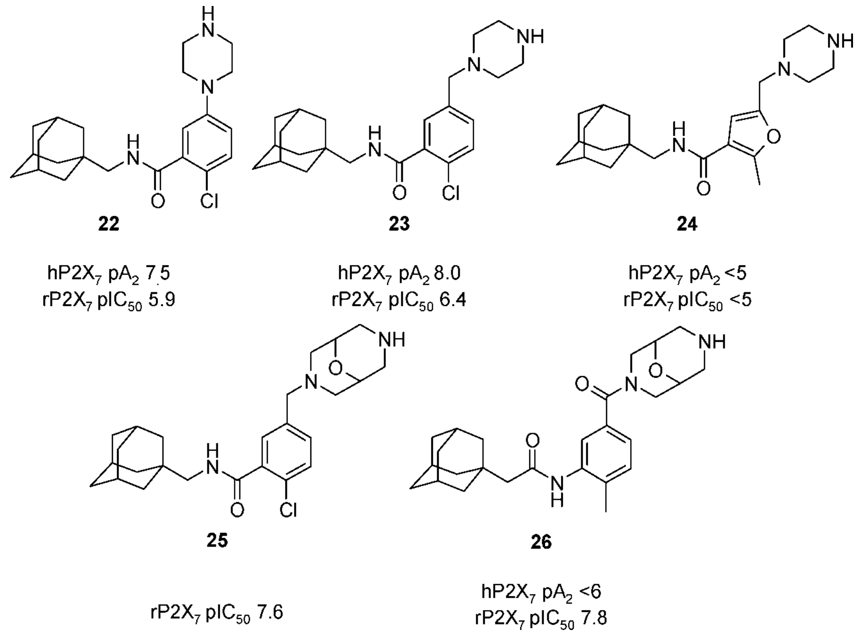


Figure 4. Structures of **22**−**26**.

rapidly uncovered. From this work it was confirmed that the 2-chloro substituent on the phenyl ring was required for potency and some of the most potent compounds obtained were the 2,3-dichlorophenyl **15** and 2,5-disubstituted **16** and **17** analogues. Even though the reverse, *N*-arylamide **14** was less potent than the *C*-arylbenzamide **8**, an examination of SAR similarities and differences between the two series was undertaken via parallel synthesis. It was shown that the 2,3-disubstituted **18** and 2,5-disubstituted **19** analogues were again the most potent. It was also shown that the 2-methylphenyl compound **20** was more potent than the corresponding 2-chlorophenyl compound **14**. Even though these compounds had reached sub-10 nM potencies, they appeared to suffer from high lipophilicity, which may well lead to high in vitro and in vivo metabolism. This was shown to be the case, as the majority of the compounds reported had very high rat intrinsic clearances (Cl_{int}) in vitro [**8** and **16** both had rat hepatocytes $Cl_{int} > 100$ (μL/min)/million cells]. Confirmation of the poor metabolic stability within this series was demonstrated in vivo through dosing of compound **16**, which exhibited very poor in vivo rat pharmacokinetics ($Cl_p >$ 100 (mL/min)/kg, $V_{ss} = 3$ L/kg, $T_{1/2} = 0.5$ h). Screening of compounds for improved metabolic stability revealed that the indazole amide **21** had reduced in vitro clearance (rat hepatocytes $Cl_{int} = 5$ (μL/min)/million cells). This was confirmed in vivo ($Cl_p = 47$ (mL/min)/kg, $V_{ss} = 2$ L/kg, $T_{1/2} = 1.0$ h) and indicated that further optimization could well be achieved within this series.

Recently, AstraZeneca[55−57] published on an extension of the adamantane amide series by producing a series of 2-chloro or 2-methyl substituted phenylamides containing a basic side chain attached to the 5-position of the phenyl ring. In this study they were looking for increases in potency against the human isoform of $P2X_7$ combined with an attempt to identify compounds that were active at the rat $P2X_7$ receptor in order to evaluate the role of $P2X_7$ in disease models. A BzATP-induced dye uptake assay was used in both cases. Addition of the basic side chain could also lead to an increase in metabolic stability of the initial hit-to-lead series through lowering of the overall lipophilicity of the compounds. Many examples were reported, and weak activity was eventually observed at the rat $P2X_7$ receptor for compound **22** (Figure 4).

During the study, changes to the aryl ring were also employed and two compounds, **23**, a potent phenyl-substituted compound, and **24**, an inactive furyl-substituted compound (to act as a negative control), were taken forward into in vitro and in vivo assays. In an assay measuring BzATP-induced IL-1β release from isolated human monocytes, **23** showed complete inhibition of cytokine release ($pIC_{50} = 7.1$), whereas **24** showed no activity in this assay. The compounds displayed a similar pattern of activity in an IL-18 release assay with **23** giving complete inhibition of cytokine release ($pIC_{50} = 7.4$), whereas **24** again showed no activity.

For target validation studies, a compound was required with a combination of good activity at the rat $P2X_7$ receptor as well as good exposure when dosed orally. It was remarked that although promising activity at the rat $P2X_7$ receptor was observed in some compounds, these were generally the compounds that had poor metabolic stability. Extensive screening of many analogues led to the discovery of **25**, which had the desired combination of high activity at the rat $P2X_7$ receptor coupled with good in vitro and in vivo stability but unfortunately did not have sufficient oral bioavailability. Further optimization led to **26** which had a very good profile, combining high rat (but not human) $P2X_7$ potency, low plasma protein binding (66% bound), and importantly, high exposure when dosed orally. Bisamide **26** was reported to be highly selective when screened against a range of other enzyme and receptor targets, including $P2X_{1-5}$ receptors. In a rat model of arthritis, at oral doses of 10−60 mg/kg twice daily, **26** showed a significant reduction in pain response and histological end points but not in ankle swelling.[32,58] Moreover, an additive effect on the same outcomes was shown when **26** was tested in combination with a range of COX-2 inhibitors.[31]

From these and related disclosures,[59−61] it is apparent that the physical properties of the adamantane amide series can be manipulated through wide variation of the group in the 5-position of the phenyl ring. Reports suggest that many basic and acidic groups are well tolerated and that this position is a good point of focus for modulating the physical properties to aid in increased solubility and metabolic stability. Examples of such compounds include **27**−**32** which are shown, along with associated potencies at the $P2X_7$ receptor (Figure 5). Substitution

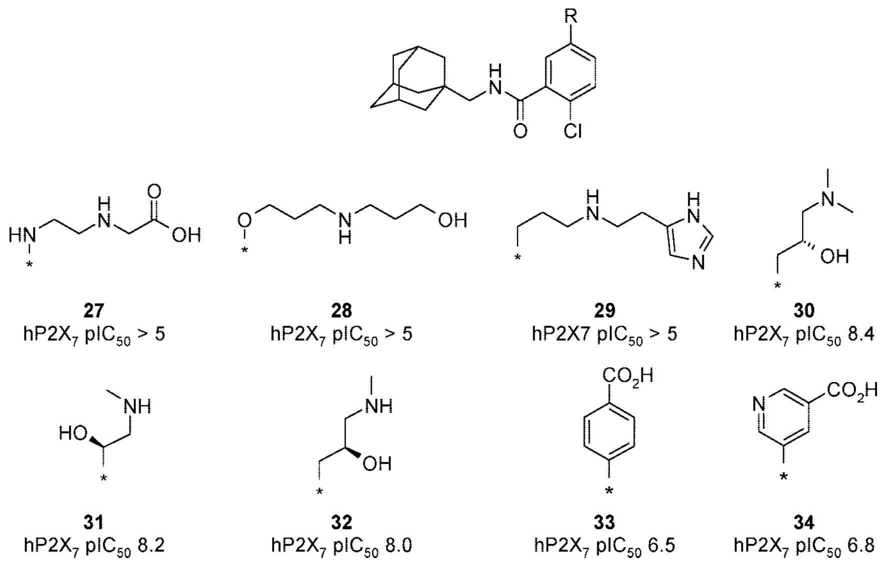


Figure 5. Structures of **27**−**34**.

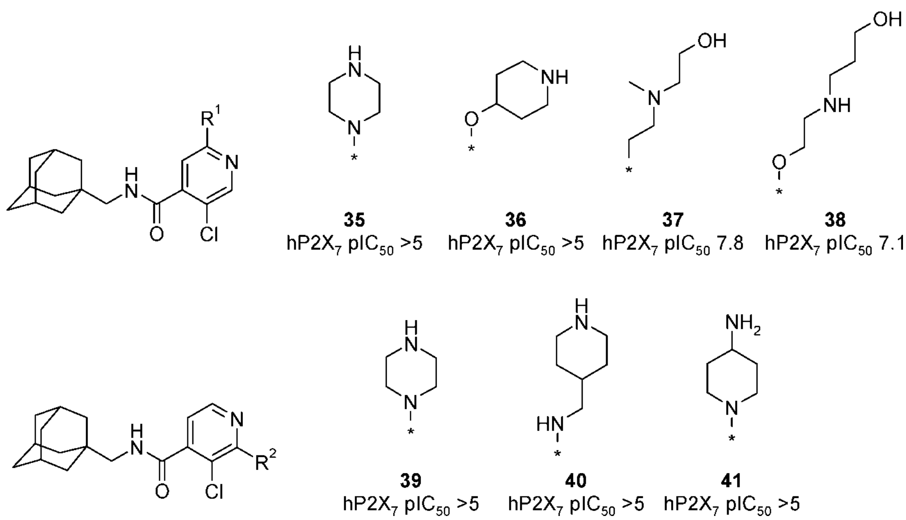


Figure 6. Structures of **35**−**41**.

with a second aryl ring containing a carboxylic acid at the 5-position of the adamantane 2-chlorobenzamides has produced a series of interesting biaryl compounds (e.g., **33** and **34**), although these compounds have high plasma protein binding due to their high lipophilicity (cLogP of 5.4 and 3.9, respectively).[62]

AstraZeneca has disclosed that the phenyl ring from the adamantane amide series can be replaced with a pyridine,[56,63] quinoline,[64] or indole[65] ring, thus maintaining potency in less lipophilic series of compounds. In the pyridine series a range of amine substituents was investigated, including **35**−**41** (Figure 6). It appears that $P2X_7$ receptor antagonism can be achieved in this series through substitution with cyclic amines at both the 2- and 6-positions of the pyridine ring (e.g., **35** vs **39**).

More diverse replacements were disclosed within the quinoline series of adamantane amides. Examples of some of the reported compounds **42**−**45** are shown in Figure 7, along with associated potencies at the $P2X_7$ receptor. From this disclosure, some interesting SAR was highlighted. First, the majority of compounds belong to the reverse (*N*-aryl) amide class, and it is interesting to note that, in this case, high potency can be achieved without an *o*-chloro substituent.

Quinoline derivatives with a *C*-arylamide orientation were the focus of a more recent report.[66] As in the phenyl series, it is apparent that the ortho-substituent is important for potency. A range of carboxylic acids and bioisosteres at varying distances to the core (**46**−**49**) appear to be tolerated (Figure 8).

AstraZeneca has also disclosed work in the amide series through the exploration of adamantane replacements (Figure 9).[67] In their first disclosure, it was shown that the lipophilic adamantane could be replaced by simple cycloalkane groups (e.g., **50** and **51**) while retaining potency at the $P2X_7$ receptor. They also demonstrated that this holds for *N*-aryl as well as *C*-aryl linked quinoline amides such as **52**, but again, in the latter case, the ortho-substituent appears important for potency. Substitution of the quinoline suggests that the ionizable group is not contributing positively to the potency of the compounds within the series (e.g., **53** vs **52**) but is instead acting to moderate physical properties. A more recent disclosure has described the replacement of the adamantane and cyclohexane groups with cycloheptanes (e.g., **54** and **55**) in a biarylamide series.[68]

AstraZeneca has revealed further series of arylamides attached through various linkers to a second (substituted) aromatic ring (Figure 10).[69−71] These series contain a pharmacophore similar to the adamantane amides, and it may be speculated that they

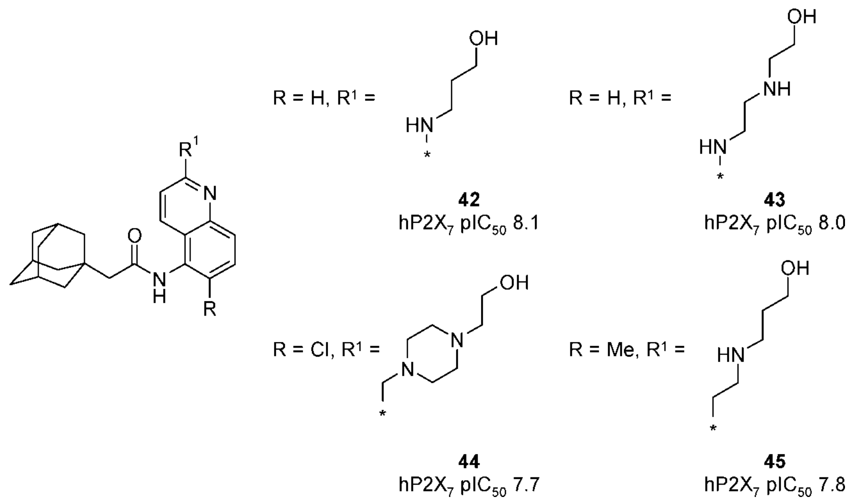


Figure 7. Structures of **42**−**45**.

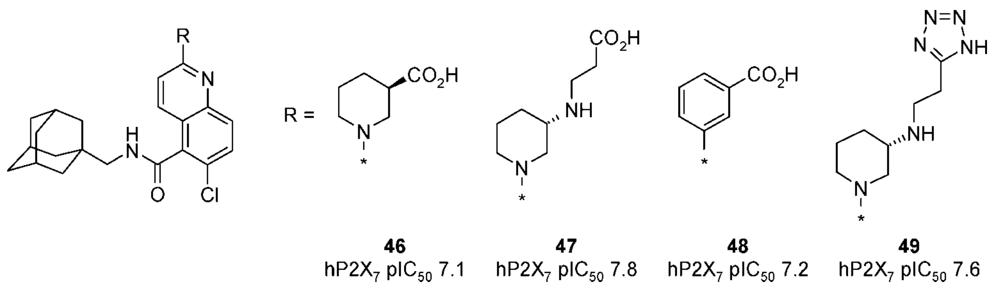


Figure 8. Structures of **46**−**49**.

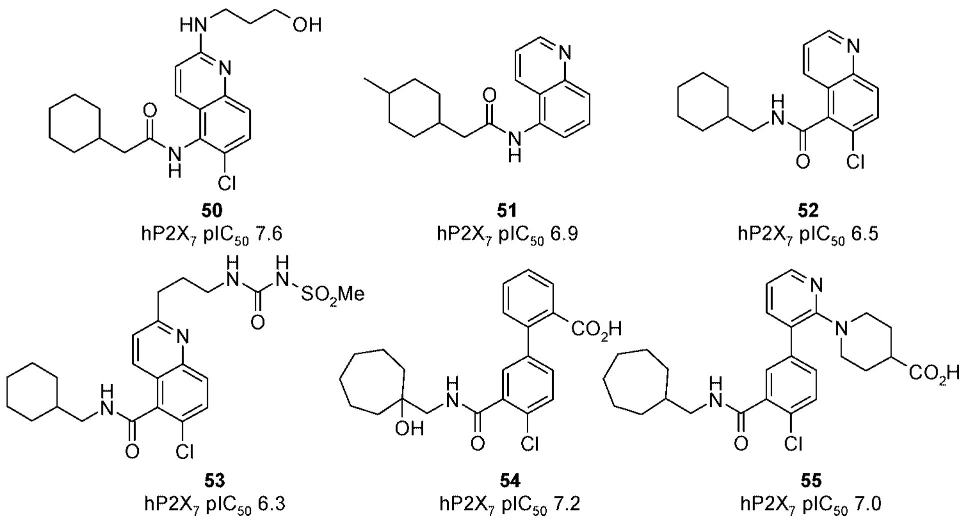


Figure 9. Structures of **50**−**55**.

might bind at a common site in the $P2X_7$ receptor. In both the quinoline amide compounds **56**−**59** and the phenyl amide **60**, the heteroatom or carbon linked aromatics might be thought of as adamantane replacements. The similarity is strengthened by the use of hydrophilic groups to moderate the physical properties of the compounds.

Pfizer has reported arriving at a similar series of 2-chlorobenzamides through high-throughput screening (Figure 11).[72−74] The hydrocarbon chain of the lead compound **61** was modified in a sequence of steps, targeting reduced lipophilicity and increased potency in an assay measuring inhibition of ATP-induced IL-1β release in human monocytes. This was achieved in 2-chlorophenethyl derivative **62**, which also exhibited moderate pharmacokinetics in rat (Cl_p = 9 (mL/min)/kg, V_{ss} = 0.3 L/kg, $T_{1/2}$ = 3 h, F = 44%). This compound was also active in vivo in a mouse model measuring ATP-induced IL-1β production with an ED_{50} of 20 mg/kg. Potency could be further improved by introduction of an adamantane group **63**, as in the AstraZeneca compounds, but the lipophilicity of this compound moved in the wrong direction and the in vitro clearance was correspondingly higher. A marked reduction of lipophilicity was achieved through replacement with a hydroxylated cyclohexane group, affording **64**, and homologation to the cycloheptane ring gave **65** which was both potent and efficacious in vivo in the mouse model (ED_{50} = 2 mg/kg). The in vivo results with these compounds are noteworthy because of the generally poor species

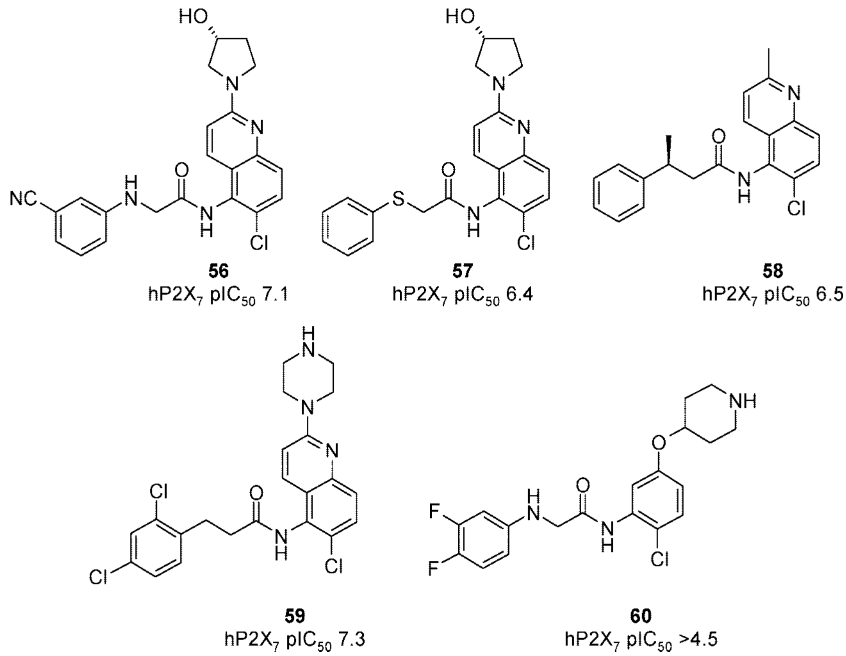


Figure 10. Structures of **56**−**60**.

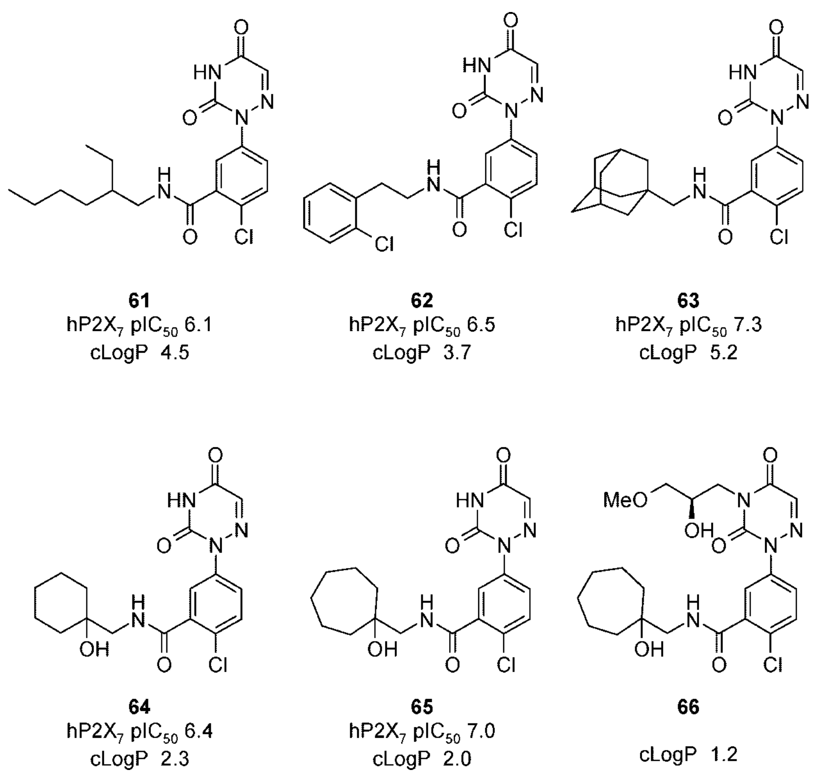


Figure 11. Structures of **61**−**66**.

crossover to rodents seen within the related AstraZeneca series of compounds, although Pfizer provided no in vitro data for activity against the mouse $P2X_7$ receptor. Further reduction in lipophilicity was achieved by addition of a polar side chain to the heterocyclic ring giving **66**, which was specifically identified by Pfizer in both compound[72,75] and combination patents[76] for use in the treatment of rheumatoid arthritis.

Pfizer has separately reported on compounds in which the azauracil ring was replaced by other heterocycles (Figure 12).[77,78] The 2-chlorophenethyl and hydroxycycloheptylmethylamide substituents were preferred in the cited examples with activities spanning a range from $pIC_{50} < 6$ to $pIC_{50} = 8.6$ in an assay measuring inhibition of BzATP-induced Yo-Pro influx in human THP-1 cells. The pyridine compound **67** had modest potency in the pore formation assay, whereas the dimethylcyclohexanol analogue **68** was significantly more potent. This does not appear general, since in an analogous pyrazole series, which represented a focus for the Pfizer group, a similar jump in potency was not observed (**69** vs **70**). In this series a methyl group adjacent to the pyrazole nitrogen was found to produce a profound effect on potency (**69** vs **71**). This subseries of compounds was generally quite lipophilic and does not appear to have progressed as far as the corresponding 6-azauracil series. Compound **72** was one of the more potent examples described

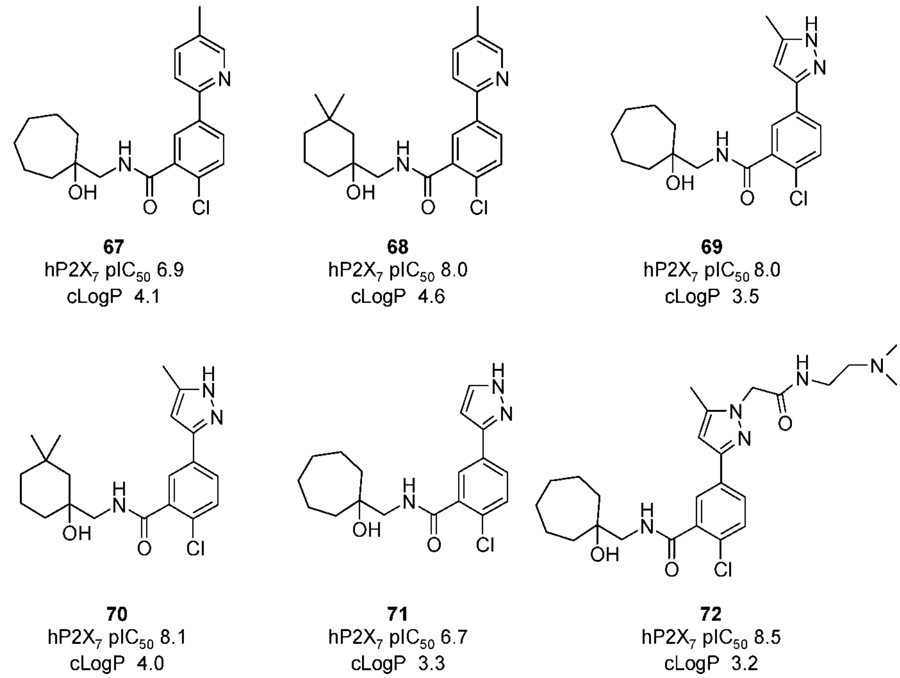


Figure 12. Structures of **67**−**72**.

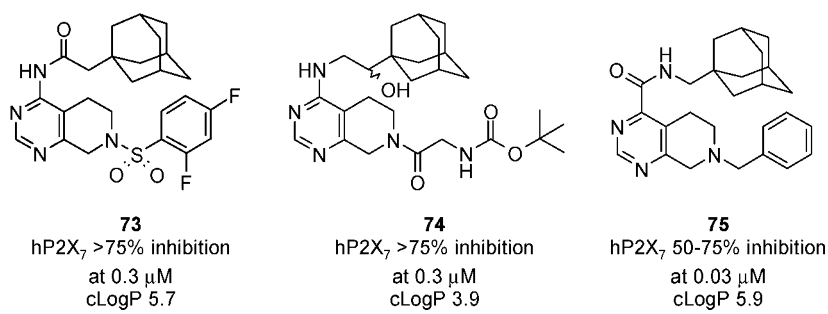


Figure 13. Structures of **73**−**75**.

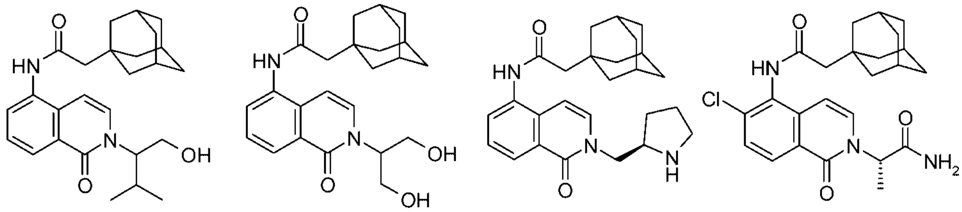
and because of the presence of a basic amine side chain would have a significantly lower log *D* and might be anticipated to possess improved properties.

Meanwhile, Renovis (now Evotec) has published several patents claiming potent $P2X_7$ receptor antagonist activity for partially saturated pyridopyrimidine and isoquinoline derived compounds for the treatment of diseases such as pain, inflammation (including rheumatoid arthritis and osteoarthritis), trauma, and Parkinson's disease.[79−87] Both series of compounds bear a close structural similarity to the quinolines claimed by AstraZeneca, having a lipophilic group attached via an amide link to an aromatic heterocycle bearing another, often polar or H-bonding, group.

In the pyridopyrimidine series (**73**−**75**) (Figure 13) a large number of compounds was prepared having an amide or sulfonamide at N-7, with either an *N*-aryl or *C*-aryl linked amide or an amine at C-4. The majority of the compounds contained an adamantane group and consequently had high lipophilicity. Potencies were determined in an assay measuring inhibition of BzATP-stimulated IL-1β production and quoted as a range, with the most potent compounds showing 50−75% inhibition at 0.03 μM or >75% inhibition at 0.3 μM.

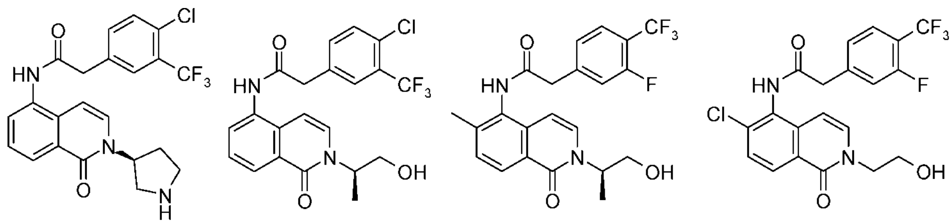
In the isoquinoline series, an initial patent outlined a number of adamantane-substituted isoquinolinones and dihydroisoquinolinones.[85] The majority of the compounds described gave >75% inhibition of IL-1β production at 0.3 μM, and a wide variety of neutral and basic side chains was tolerated. Subsequent patents have disclosed further work in this area, with more detailed biological activity and basic pharmacokinetic data disclosed for a number of the compounds.[79,81,82] Most of the compounds fall into the general area represented by structures **76**−**79** shown in Figure 14. In Renovis's IL-1β screen, more than 20 compounds are reported with $pIC_{50} > 9$ including the very potent alcohol **76**; however, all but a few are adamantane-containing compounds with correspondingly high lipophilicities. With the exception of a few basic compounds, this series suffered from poor in vitro metabolic stability and, where reported, oral bioavalabilities were mostly <25%.

A range of compounds having adamantyl replacements, including substituted aryl and cycloalkyl compounds **80**−**87**, were prepared resulting in lower lipophilicity (Figure 15).[82−84] This gave a concomitant improvement in metabolic stability in human microsomes but at the price of reduced potency, mostly greater than 10-fold. However, a number of interesting compounds did exhibit good overall properties. For example, compounds **81** and **82** exhibit high potency (pIC_{50} of 8.9 and 9.4, respectively), good metabolic stability in human microsomes (half-lives of 1.0 and 1.5 h, respectively), and good oral bioavailability in rat (25−75%). The nonchiral derivative **83** has a slightly lower potency (pIC_{50} of 8.2) but improved metabolic stability in human microsomes (half-life of 3.7 h) and high oral bioavailability in rat (>75%).

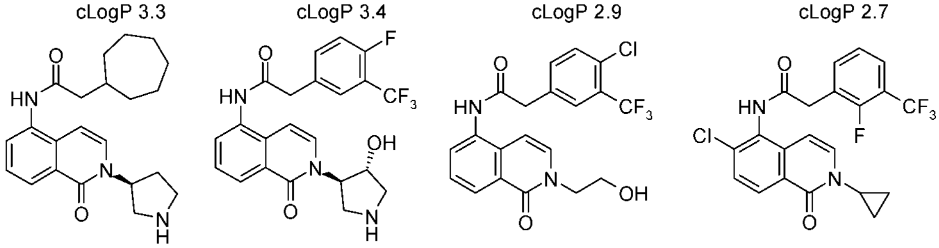


76 hP2X7 pIC50 10.3 cLogP 5.7 — **77** hP2X7 pIC50 9.7 cLogP 4.4 — **78** hP2X7 pIC50 9.0 cLogP 5.3 — **79** hP2X7 pIC50 10.1 cLogP 4.3

Figure 14. Structures of **76**−**79**.

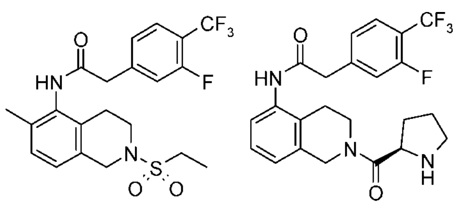


80 hP2X7 pIC50 7.8 cLogP 3.3 — **81** hP2X7 pIC50 8.9 cLogP 3.4 — **82** hP2X7 pIC50 9.4 cLogP 2.9 — **83** hP2X7 pIC50 8.2 cLogP 2.7



84 hP2X7 pIC50 8.5 cLogP 3.6 — **85** hP2X7 pIC50 8.6 cLogP 2.9 — **86** hP2X7 pIC50 8.7 cLogP 3.1 — **87** hP2X7 pIC50 9.5 cLogP 3.8

Figure 15. Structures of **80**−**87**.



88 hP2X7 pIC50 7.6 cLogP 3.1 — **89** hP2X7 pIC50 7.1 cLogP 3.0

Figure 16. Structures of **88** and **89**.

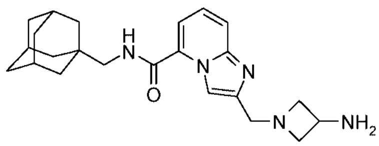
A series of non-adamantane tetrahydroisoquinolines (lacking the 1-carbonyl) was prepared (e.g., **88** and **89**), but these were less interesting than the isoquinolones having $pIC_{50} \leq 7.6$ and no reported pharmacokinetic data (Figure 16).[80]

Neurogen has also described a series of adamantane amides bearing a substituted imidazo[1,2-*a*]pyridine ring as exemplified by compound **90** (Figure 17).[88] A large number of substituents were reported at the 5-position of the imidazopyridine ring, but no biological data were provided. Neurogen also examined replacements for the adamantyl ring, and *cis*-myrtanyl, as exemplified by **91**, has most exemplification within the patent. This particular adamantane replacement has also been employed by AstraZeneca[68] and by Pfizer.[73]

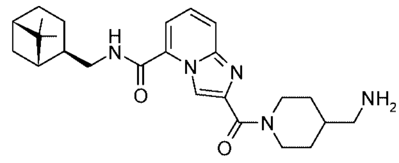
In a recent publication, scientists from Sydney University have reported the replacement of the adamantyl group found in AstraZeneca's $P2X_7$ receptor antagonist **17** with a cubyl moiety **92** that has the advantage of conferring lower lipophilicity.[89,90] These compounds were assessed in rat spinal cord microglia cells stimulated with BzATP measuring inhibition of dye uptake. As expected from previously published rat $P2X_7$ data on compounds related to **17** ($pA_2 < 5$),[55] their activity at the rat receptor was relatively weak, which will probably limit their utility as tools in rodent models.

Researchers from Abbott have reported a series of the acyl hydrazides that were derived from a high-throughput screening hit **93** (Figure 17).[35] The SAR in this series, looking at BzATP-induced calcium influx in human and rat $P2X_7$-transfected cells, appears to mirror that of the amide series described above, but lipophilicity is predicted to be lower. Adamantane **94** demonstrated activity in models of neuropathic pain and inflammation.[34,35] It is noteworthy that potency against the rat $P2X_7$ receptor is much greater here than in the phenylamide series.

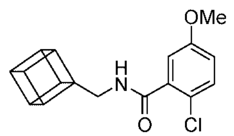
3.2. Miscellaneous Amide Series. In 2005, Bayer described a series of $P2X_7$ antagonists based around a pyrazole template.[91] Thirty examples were prepared by solid phase synthesis and tested in a calcium influx assay in human $P2X_7$-tranfected HEK293 cells. Compound **95** had a reported activity of $pIC_{50} \geq 6.7$ in this assay, and compounds of structures **96**−**99** were also claimed to be of particular interest (Figure 18). Although no data have been provided, the compounds were claimed to be selective and potent in a range of in vitro and in vivo ATP or BzATP induced IL-1β release screens. Although these compounds have a reasonable molecular weight, they are



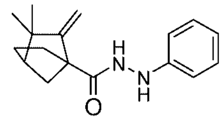
90
cLogP 4.8



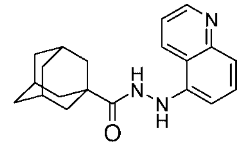
91
cLogP 3.5



92
cLogP 2.7

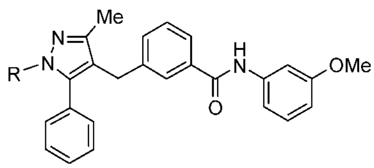


93
$hP2X_7$ pIC_{50} 7.3
$rP2X_7$ pIC_{50} 7.3
MWt 270, cLogP 3.3

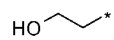


94
$hP2X_7$ pIC_{50} 8.0
$rP2X_7$ pIC_{50} 7.4
MWt 321, cLogP 3.6

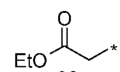
Figure 17. Structures of **90**−**94**.



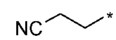
R =



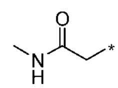
95
MWt 441
cLogP 4.2



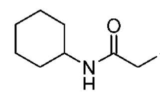
96



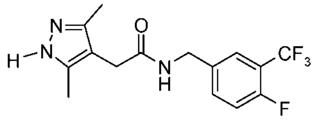
97



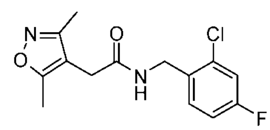
98



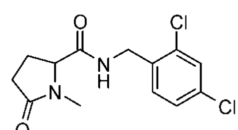
99



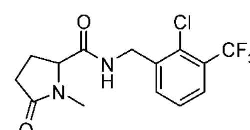
100
MWt 329
cLogP 2.3



101
MWt 297
cLogP 2.0



102
MWt 301
cLogP 2.3



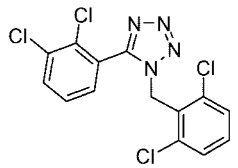
103
MWt 334
cLogP 2.3

Figure 18. Structures of **95**−**103**.

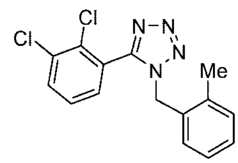
relatively lipophilic, and this may limit their solubility, absorption, and metabolic stability.

In 2007, GSK also reported a series of $P2X_7$ antagonists that are structurally similar to that of Bayer's series but in which the central phenyl ring was removed.[92] The compounds were profiled in an ATP-stimulated ethidium accumulation assay and/or a calcium influx assay, both assays using a HEK293 cell line expressing human recombinant $P2X_7$ receptors. Typical compounds reported in this application are illustrated by compound **100** and are claimed to have $pIC_{50} > 4.7$ in the calcium influx assay and/or $pIC_{50} > 5.5$ in the ethidium accumulation assay. In another application, GSK has also claimed isoxazoles as a replacement for the pyrazole core in the series of compounds described above.[93] The range of analogues reported in this application is typified by compound **101**. They have been profiled in the same assays as the pyrazoles and have similar levels of activity. Although these antagonists are smaller and more polar than the series described above and should have a more favorable pharmacokinetic profile, no in vivo evaluation procedure was described in either of these two applications, which might suggest that none of these compounds had a level of activity and/or crossover to rodent that was suitable for further evaluation.

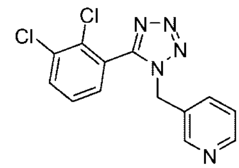
More recently, a GSK patent application claiming a series of 5-oxoproline-2-amides was published.[44] Although there were no specific in vitro figures reported for any of the compounds, two analogues **102** and **103** have been evaluated in a range of pain models in the rat. In the neuropathic pain model, the two compounds were administrated twice daily orally for 8 days and significant reversal of CCI-induced mechanical allodynia compared to vehicle response was observed. In the rat model of joint pain, the compounds administrated twice daily orally for 5 days significantly reversed FCA (intra-articular) induced differences in weight bearing capacity compared to vehicle with an $ED_{50} < 20$ mg/kg. Finally, in a rat model of acute inflammatory pain, **102** and **103** dosed orally significantly reversed FCA (intraplantar) induced differences in weight bearing compared to vehicle with an $ED_{50} < 20$ mg/kg. Although not specified in the application, one may speculate that the relatively high doses of compounds required to achieve efficacy in the different models could be a reflection of weak activity of these antagonists at the rodent receptors. However,



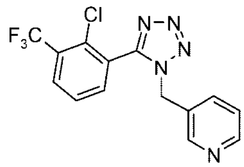
104
hP2X7 pIC50 6.9
MWt 374, cLogP 6.2



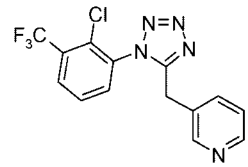
105
hP2X7 pIC50 7.8



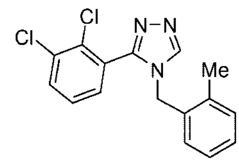
106
hP2X7 pIC50 6.9
rP2X7 pIC50 6.5
MWt 306, cLogP 3.2



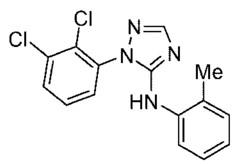
107
hP2X7 pIC50 7.9
MWt 340, cLogP 3.3



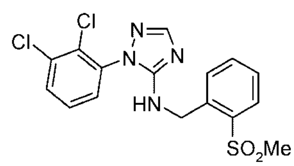
108
hP2X7 pIC50 7.9
MWt 340, cLogP 2.1



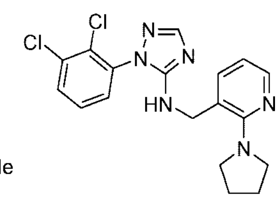
109
hP2X7 pIC50 7.1
rP2X7 pIC50 6.7
MWt 318, cLogP 4.0



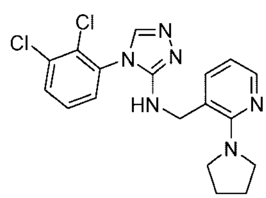
110
hP2X7 pIC50 7.7
rP2X7 pIC50 6.6
MWt 320, cLogP 5.5



111
hP2X7 pIC50 7.7
rP2X7 pIC50 7.4
MWt 397, cLogP 2.8



112
hP2X7 pIC50 7.9
rP2X7 pIC50 7.6
MWt 389, cLogP 3.8



113
hP2X7 pIC50 7.8
rP2X7 pIC50 7.9
MWt 389, cLogP 4.0

Figure 19. Structures of **104**−**113**.

if the activity of these antagonists at the human receptor were of a significant level, their low molecular weight and moderate lipophilicity would constitute attractive druglike features. **102** and **103** were both synthesized on a large scale as single L-isomers, suggesting a significant level of interest in these compounds.

3.3. Tetrazoles and Related Series. In 2005, Abbott disclosed a series of tetrazoles as novel $P2X_7$ receptor antagonists identified through high-throughput screening of their corporate compound collection (Figure 19).[39,41,94] 2-Substituted benzyl groups (e.g., **104** and **105**) exhibited good potency but possessed limiting solubility. Heterocycle replacement of the benzyl motif afforded pyridylmethyl derivative **106**, a potent $P2X_7$ antagonist with selectivity against other P2 receptors ($P2X_3$, $P2X_4$, and $P2Y_2$). Investigation of substitution on the directly attached phenyl ring confirmed that 2,3-substitution was optimal, while replacement of the 3-chloro substituent with trifluoromethyl gave **107**, a more potent antagonist. Reversal of the tetrazole connectivity was investigated with little influence on potency (**107** vs **108**). Interestingly, this series exhibited potency in recombinant rat cell lines, typically 3-fold to 20-fold lower than in human (both screened in an assay measuring BzATP induced calcium flux at recombinant $P2X_7$ receptor in stably transfected human 1321N1 astrocytoma cells devoid of endogenous P2X receptor function). Pyridine **106** (A-438079),[40] with more leadlike physicochemical properties, demonstrated a dose dependent reversal of mechanical allodynia in the Chung model of neuropathic pain (10−300 μmol/kg, dosed ip), and in a separate ip pharmacokinetic study **106** showed 19% bioavailability and 1 h half-life.

Further work centered on replacement of the tetrazole core heterocycle and subsequent reoptimization of the pendent substituent.[36,38,95] A progressive loss of potency was observed as the core ring became less electron deficient (tetrazole > triazole > imidazole > pyrrazole), while it was determined that the absolute positioning of the ring nitrogens was of less importance.[38] During this optimization, triazole **109** was shown to demonstrate antinociceptive activity in the Chung model of neuropathic pain when dosed intraperitoneally (ip) with an ED_{50}

of 125 μmol/kg and a maximum efficacy of 68% along with acceptable ip pharmacokinetics ($T_{1/2}$ = 1.7 h, F = 62%).

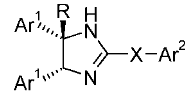
Further SAR studies around regioisomeric triazoles have been reported.[96] Aniline substituents in the 1,2,4-triazole series **110** were found to be active but a small ortho-substituent proved critical for high potency. The need for an ortho-substituent is reminiscent of the adamantane amide series described above and may suggest an overlay between these series. However, various other SAR features appear to differ. Larger ortho-substituents that could attenuate high lipophilicity (e.g., **111**) were more readily tolerated in the homologated *N*-benzyl series than in the anilines (e.g., **110**), while potency could also be retained in the absence of any substitution. Conversely, the SAR of *N*-pyridylmethyl analogues **112** stipulated an ortho-substituent, which in the case of azetidinyl and pyrrolidinyl afforded good species crossover. This species crossover was most broadly realized in the isomeric 1,2,4-triazole series (e.g., **113**) where several analogues differing only by their ortho-substituents were shown to be equipotent in human and rat recombinant cell lines, making them good compounds for further target validation studies.[42,43,97] The degree of crossover differs from the earlier amide series, further challenging the possibility of these series sharing a common binding mode.

3.4. Dihydroimidazoles. Aventis has reported a series of *cis*-4,5-diarylimidazolines as potent $P2X_7$ receptor inhibitors.[30,98] Lead compound **114** was identified from a high-throughput screening campaign and was followed by rapid parallel synthesis using a combination of solid phase and microwave techniques. Activity was assessed using a fluorescence-based Yo-Pro dye uptake assay in $P2X_7$ transfected U373 cells. The chain length and effect of substitution on the portion linking the 4,5-diphenylimidazoline core and the phenyl ring were studied. It was found that direct attachment **115** gave poor activity and that activity also decreased once the linker's length was greater than two carbon atoms, **116** and **117**. It was also shown that substitution on the ethylene linkers **118** and **119** and ring fusion of the pendent aryl ring back onto the linker **120** were both well tolerated (Table 2).

The introduction of substituents onto the terminal phenyl ring was then investigated in order to improve metabolic stability. A range of substituted analogues was synthesized, but no dramatic improvement in potency was observed and no effect on metabolic stability was reported, implying that this strategy was not successful. Substitution with electron-donating substituents (e.g., **121**) led to a marked drop in potency, while introduction of halogens and small alkyl groups (**122**−**129**) generally retained activity. In the phenethyl analogues, ortho- and meta-substitution was generally preferred to para (**124**−**126**), whereas in the benzyl series, meta- and para-substitution tended to be preferred over ortho (**127**−**129**). Amino (**130**−**134**), thio (**135**), and alkoxy (**136**) linkers, as well as substitution on the groups at the 4- and 5-positions of the imidazoline core (**131**−**134**), have all been exemplified, while *trans*-4,5-diarylimidazoline analogues are claimed to be inactive at inhibiting the $P2X_7$ receptor.

The ability of some of these compounds to inhibit IL-1β release from human macrophages has been evaluated. For example, compound **131** was found to have a pIC_{50} of 5.9 in this assay. Compounds of this invention have been claimed to display significant activity in a range of in vivo anti-inflammatory models at a once-daily dose of 50 mg/kg given orally or 30 mg/kg injected intraperitoneally. It is worth noting that there was no indication given to the level of crossover between the human receptor and that of the species used in the in vivo models (mouse and rat). Again, the dosing regimen described in these experiments could be indicative of weak activity at the corresponding rodent receptor and/or poor pharmacokinetic properties expected for these highly lipophilic analogues (e.g., **131** cLogP = 5.3).

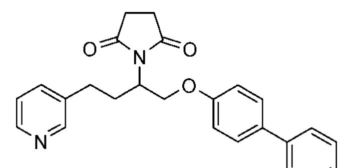
Table 2. SAR of Dihydroimidazoles



114-136

compd	Ar^1	R	X	Ar^2	$hP2X_7$[a]
114	C_6H_5	H	$-(CH_2)_2-$	C_6H_5	7.1
115	C_6H_5	H		C_6H_5	5.4
116	C_6H_5	H	$-(CH_2)_3-$	C_6H_5	6.3
117	C_6H_5	H	$-(CH_2)_4-$	C_6H_5	6.2
118	C_6H_5	H	$-CH_2CHMe-$	C_6H_5	7.5
119	C_6H_5	H	CH_2CMe_2	C_6H_5	8.0
120	C_6H_5	H		2-indanyl	7.5
121	C_6H_5	H	$-(CH_2)_2-$	4-MeOC_6H_4	<5.5
122	C_6H_5	H	$-(CH_2)_2-$	2-MeC_6H_4	7.2
123	C_6H_5	H	$-CH_2-$	2-MeC_6H_4	6.5
124	C_6H_5	H	$-(CH_2)_2-$	2-ClC_6H_4	7.2
125	C_6H_5	H	$-(CH_2)_2-$	3-ClC_6H_4	6.9
126	C_6H_5	H	$-(CH_2)_2-$	4-ClC_6H_4	<6
127	C_6H_5	H	$-CH_2-$	2-FC_6H_4	6.2
128	C_6H_5	H	$-CH_2-$	3-FC_6H_4	7.1
129	C_6H_5	H	$-CH_2-$	4-FC_6H_4	7.1
130	C_6H_5	H	$-NHCH_2-$	3-FC_6H_4	7.0
131	3-FC_6H_4	H	$-NHCH_2-$	3-FC_6H_4	7.2
132	3-MeC_6H_4	H	$-NHCH_2-$	3-FC_6H_4	6.9
133	3-FC_6H_4	Me	$-NHCH_2-$	3-FC_6H_4	7.4
134	2-ClC_6H_4	H	$-NHCH_2-$	3-FC_6H_4	6.4
135	C_6H_5	H	$-SCH_2-$	3-FC_6H_4	6.7
136	C_6H_5	H	$-OCH_2-$	3-FC_6H_4	6.7

[a] pIC_{50} in a BzATP-induced Yo-Pro uptake assay in human $P2X_7$ transfected U373 cells.



137

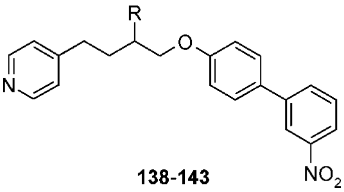
Figure 20. Structure of **137**.

3.5. Cyclic Imides and Related Series. AstraZeneca has reported[4,99] on a further series once more derived from high-throughput screening. Even though the initial hit **137** (Figure 20) was weak, a clear SAR was observed and it was shown that 4-pyridyl was the preferred heterocycle and that a 3′-susbstituent on the biphenyl portion led to beneficial activity (**3**, pA_2 = 6.9) (Figure 1).

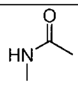
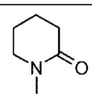
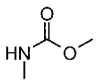
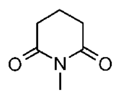
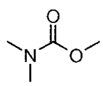
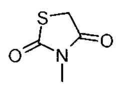
Replacement of the imide ring with an acyclic amide **138**, carbamate **139**, *N*-methyl carbamate **140**, as well as δ-lactam **141**, afforded compounds with reduced activity. However, replacement of the imide ring with other heterocycles such as the six-membered imide **142** and thiazolidine-2,4-dione **143** showed increased potency (Table 3). The thiazolidine-2,4-dione **143** was subsequently disclosed to be a highly selective and potent antagonist at the human (but not rat) $P2X_7$ receptor and was used as a tool compound to study the effects of inhibition of the $P2X_7$ receptor in human cell lines.[45]

3.6. Benzoxazinones. A series of benzoxazinones having potency of $pIC_{50} \geq 4.5$ in a human $P2X_7$ receptor screen (BzATP-stimulated ethidium bromide uptake in THP-1 cells) has also been described by AstraZeneca.[100] The piperidine can be linked to either a phenyl ring containing a strong electron

Table 3. SAR of Cyclic Imides and Related Compounds



138-143

cmpd	R	$hP2X_7$	cmpd	R	$hP2X_7$
138		<5[a]	**141**		5.2[a]
139		<5[a]	**142**		7.5[b]
140		<5[a]	**143**		7.7[b]

[a] pIC_{50}. [b] pA_2 in an assay measuring BzATP induced ethidium uptake in a human monocyte cell line.

withdrawing group **144** or a pyridine ring **145**. The majority of the compounds possess either a chloro- or nitro-substituent in the 2-position of the aryl ring although some examples where the heteroaromatic ring is pyrimidine **146** do not require this group (Figure 21).

3.7. Piperazines. A further patent from AstraZeneca described compounds that fit into two broad categories containing a piperazine either directly attached **147** or linked through a sulfonamide **148** to an aryl ring.[101] Examples are shown in Figure 22; however, there was no biological data given in the patent apart from the comment that all examples had a human $P2X_7$ potency of $pIC_{50} \geq 5.0$.

3.8. Amidines and Guanidines. In 2005, Abbott disclosed a structurally unusual series of cyanoguanidines[43] (e.g., **149** (A-740003) and **150** (A-759020)) derived from a series of ATP-sensitive potassium channel openers (K_{ATPs}) (Figure 23).[97] Compound **149** proved to be a useful tool compound, having demonstrated selective $P2X_7$ activity, a moderate pharmacokinetic profile (Cl_p = 62 (mL/min)/kg, V_β = 21 L/kg, $T_{1/2}$ = 4.0 h, and F = 20%), and significant antinociception in animal models of neuropathic and inflammatory pain.[42]

Replacement of an anilinic nitrogen with a carbon linker provided a more lipophilic series of cyanoamidines (e.g., **151**) capable of inhibiting IL-1β release from differentiated THP-1 cells at <10 μM. The patent claimed these cyanoamidines for the treatment of pain, inflammation, and neurodegeneration.[37] An undisclosed cyanoamidine was reported to inhibit IL-1β release with an ED_{50} of 90 μmol/kg (dosed sc) in the Zymosan induced peritonitus model of inflammation.

After replacement of the unusual aminal functionality, a series of cyanoguanidine piperazines, amenable to synthesis by multiple parallel synthesis, was recently described (Figure 24).[102,103] Reports highlighted progress toward the generation of tool compounds suitable for target validation studies in rat, possessing both cellular activity and good oral exposure. In the amide subseries (e.g., **152**), good species crossover was observed with potency and selectivity comparable to that of **149**.[103] This was also the case for a urea subseries.[104] It was remarked that the lead compound **153** demonstrated high rates of oxidative metabolism in rat and human microsomes and moderate rat iv pharmacokinetics (Cl_p = 68 (mL/min)/kg, V_{ss} = 5.3 L/kg, $T_{1/2}$ = 0.9 h). Two observations led to the discovery of **154** from lead **153**. First, 3-piperazinyl substituents suffered a surprising drop in potency in a whole blood assay relative to 2-piperazinyl substituents. Second, the most potent compounds in human whole blood (hWB) contained a basic replacement for the *o*-tolyl residue. Ultimately, the 2-methyl-5-quinolinyl group retained potency and sufficient microsomal stability (through blockade of quinoline N-oxidation) to merit in vivo testing. Unfortunately, while **154** had an acceptable iv pharmacokinetic profile in rat (Cl_p = 23 (mL/min)/kg, V_{ss} = 11.8 L/kg, $T_{1/2}$ = 4.4 h), it suffered from poor oral bioavailability of 5%, which the authors tentatively attributed to solubility-limited absorption. One might speculate that this series would again suffer from high lipophilicity and poor solubility, a feature common to many $P2X_7$ antagonist research programs.

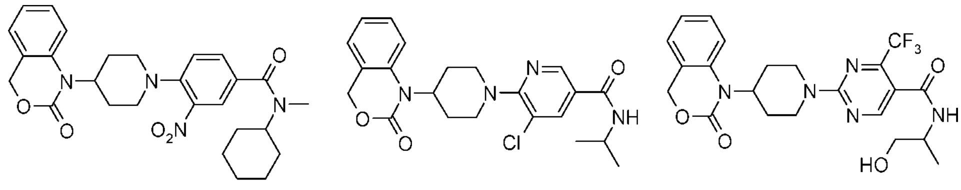
3.9. KN-62 Derivatives. KN-62 (**155**) and KN-04 (**156**) were serendipitously found in 1997[105] to be potent antagonists of the $P2X_7$ receptor. For example, they inhibit ATP-mediated Ba^{2+} influx in to human lymphocytes with a pIC_{50} of 7.9 and 7.8, respectively. These closely related analogues provide a rich scaffold allowing changes to be made at a variety of positions in a relatively facile manner. Although these compounds may be considered rather poor leads, especially for targeting oral drug administration, several SAR investigations have been reported, including an early effort to ring-constrain **155** leading to compounds such as **157** which were inactive (Figure 25).[106]

In the most extensive SAR study reported to date, Jacobson and co-workers have sequentially varied each of the pendent groups of **155**, aided by the fact that some synthetic protecting groups maintain biological activity.[107−109] In order to investigate substitution at the tyrosine nitrogen, a derivative of **155** having a BOC-protected piperazine **158** was employed as the starting point (Table 4). The isomeric quinoline **159** was slightly more active than **158** and comparable in activity to **155**. Moving to a phenylsulfonamide **160** resulted in an inactive compound; however, activity was regained in changing the linking group to a benzylcarbamate **161** or, more subtly, in substituting off the phenyl ring **162**. An amide linker **163** was less active while smaller groups such as ethyl carbamate **164**, methylsulfone **165**, and the parent amine **166** were inactive.

At the para-position of the tyrosine phenyl ring a range of aryl sulfonates **167**−**170** and esters **171** were tolerated while aliphatic sulfonates **172** and esters **173** as well as carbonates **174** and **175** and parent hydroxyl **176** were not.

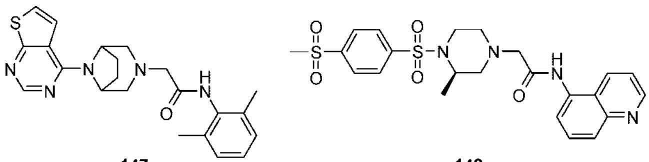
Following the same strategy, a Cbz-protected **155** derivative allowed investigation of the phenylpiperazine group. While benzyl carbamate **177** retained moderate activity, a sulfonamide linker **178** was not tolerated. Aliphatic amides **179** were also inactive but the corresponding phenyl amide **180** showed good activity. Attempts to improve potency through phenyl substitution were not successful.

In a related study (Table 5), Baradli and co-workers[102,110,111] examined phenylpiperazine variation while maintaining the rest of the molecule as in **155**. Insertion of a methylene linker **181** gave a small increase in potency, while addition of a second methylene **182** lost activity. Moving to a 4-benzylpiperidine group **183** reduced potency approximately 3-fold, suggesting that the distal piperazine nitrogen is making a specific interaction but is not critical for activity. Exploration of a large variety of substituted phenyl groups gave a wide range of potencies, again indicating a specific interaction with the receptor. In particular, the *p*-fluorophenyl compound **184** was much more active than



144 145 146

Figure 21. Structures of **144**−**146**.



147 148

Figure 22. Structures of **147** and **148**.

the parent and is one of the most potent **155** derivatives reported. Other para-substituents (e.g., **185**) gave potencies ranging over 100-fold. Interestingly, of the monochloro substituents, the para was significantly less active than either meta, **186**, or ortho, **187**. The *o*-methyl derivative **188** has been tritiated for use in binding studies.[112] Finally, replacement of the phenyl ring with pyrimidine **189** gave a small drop in activity, indicating an opportunity for reduced lipophilicity in this region. Another modification suggests that the N-methylated amino acid is slightly more active that the NH equivalent (e.g., **190** vs **184**).

Compound **155** has a number of features that makes it unattractive as an oral drug lead, including high molecular weight (MW = 722), high lipophilicity (cLogP = 5.4), and a metabolically labile sulfonate group. Recently, Baraldi's group has made an important advance by demonstrating that good activity can be achieved in significantly simplified structures.[113] In these compounds the tyrosine core was replaced with glycine, removing the sulfonate group and leading to less lipophilic compounds with a lower molecular weight while maintaining good levels of potency at the $P2X_7$ receptor (Table 6). For example, the *p*-fluoro derivative **191** had potency comparable with that of **155** with reduced molecular weight (MW = 429) and lipophilicity (cLogP = 3.4). Moreover, the related *o*-fluoro **192** and *p*-nitro **193** derivatives were more potent than **155**. However, SAR at this position was steep with many other substituted phenyl derivatives (e.g., **194** and **195**) being inactive. Unsubstituted phenyl **196** was also inactive, whereas the benzyl **197** and phenethyl **198** derivatives showed good potency, a SAR that differs from that of **155** (cf. **155**, **181**, and **182**, above). Replacement of the isoquinolin-5-yl with its isomers **199** and **200** was also not tolerated, again differing from previous SAR (cf. **158** vs **159**). Similarly, N-methylation, **201**, here destroyed activity, whereas previously this change was preferred (cf. **184** vs **190**).

The steep SAR at several positions raises a question about whether there is sufficient scope within this series to allow full optimization to achieve druglike compounds.

In contrast to the numerous reports describing **155** derivatives, very little work has been published on the closely related **156** where differences in SAR may be expected. Further work with **155** and **156** derivatives will continue to increase understanding of SAR in this interesting series. Notwithstanding the SAR limitations seen so far with glycine derivatives (**191**−**201**), these compounds provide hope that other simplified and druglike derivatives of **155** and **156** may be found in the future.

4. Clinical Trials

AstraZeneca were the first to enter clinical trials with a small molecule $P2X_7$ receptor antagonist. Compound **202** (AZD9056, structure not disclosed) has completed a phase I study where it was well tolerated, with no serious effects on major organ function at doses of ≤3000 mg. The maximum tolerated dose was 1500 mg, and the half-life and pharmacokinetic characteristics were consistent with once-daily dosing.[114] **202** has also completed a 1-month study for the treatment of rheumatoid arthritis giving positive clinical signals, supportive of further studies. **202** is now being investigated in a 6-month phase IIb study for the treatment of rheumatoid arthritis.[115] **202** has also entered clinical trials for osteoarthritis, chronic obstructive pulmonary disease, and inflammatory bowel disease.[116]

Pfizer is also reported to be in phase II studies for the treatment of rheumatoid arthritis with CE-224535 (structure not disclosed) at a dose of 500 mg twice daily.[115,117] A further study, investigating osteoarthritic knee pain, was terminated because of lack of efficacy.[115]

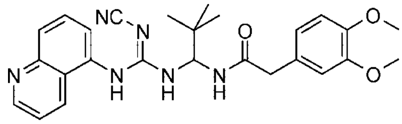
On the company Web site, Evotec (Renovis) describes their $P2X_7$ receptor antagonist program for the treatment of rheumatoid arthritis, irritable bowel disease, chronic obstructive pulmonary disease, and pain. They have reported that a candidate compound has been selected to begin clinical trials during 2008, but no structure or code number has yet been disclosed.[118]

5. Conclusions

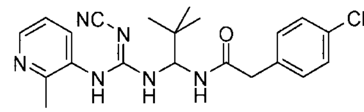
Over the past few years there have been many interesting developments in the study of $P2X_7$ receptor antagonists, including the identification of diverse new chemical series, increased understanding of receptor pharmacology, and reports of in vivo studies both in preclinical animal models of disease and, critically, in early clinical trials.

In the quest for better small molecule antagonists, many new and improved series have been disclosed. The amide series, first disclosed by AstraZeneca, still appears to be the most prevalent where numerous reports of structure−activity studies have resulted in molecules with significantly improved druglike properties relative to the early leads. It is evident that high-throughput screening has provided a rich source of leads for this target. However, high lipophilicity is a common feature in lead $P2X_7$ receptor antagonists and many medicinal chemistry programs are driven by the need to improve druglike properties. In the amide series this appears to have met with considerable success, while in some other series more work is still required. As further structurally diverse series are discovered, opportunities increase to transfer SAR understanding between series. However, at present, this approach is hampered by the lack of a good $P2X_7$ receptor model.[119]

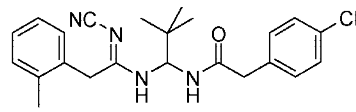
During early work, target validation was limited by poor species crossover. More recently, there have been numerous reports of activity in animal models, typically rat or mouse,



A-740003 **149**
$hP2X_7$ pIC_{50} 7.4
$rP2X_7$ pIC_{50} 7.7
MWt 474, cLogP 3.2

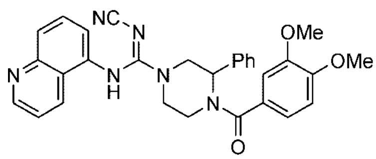


A-759020 150
MWt 413
cLogP 3.3

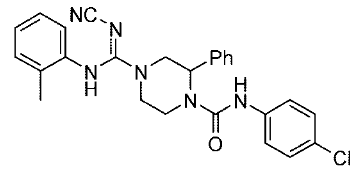


151
MWt 411
cLogP 5.4

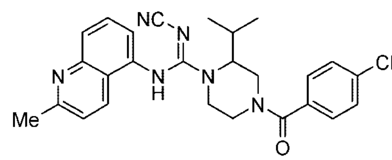
Figure 23. Structures of **149**−**151**.



152
$hP2X_7$ pIC_{50} 7.2
$rP2X_7$ pIC_{50} 7.5
MWt 520, cLogP 4.9

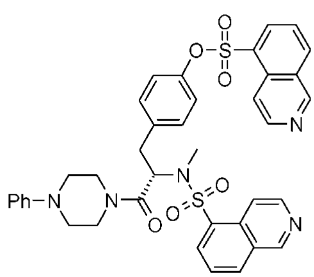


153
$hP2X_7$ pIC_{50} 6.9
$rP2X_7$ pIC_{50} 7.0
hWB pIC_{50} 5.9
MWt 473, cLogP 6.3

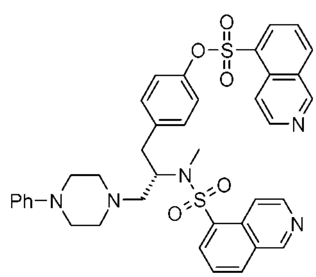


154
$hP2X_7$ pIC_{50} 7.0
$rP2X_7$ pIC_{50} 6.8
hWB pIC_{50} 6.9
MWt 475, cLogP 5.8

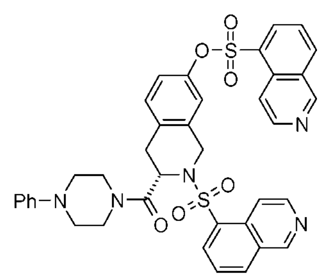
Figure 24. Structures of **152**−**154**.



KN-62 **155**



KN-04 **156**



157

Figure 25. Structures of **155**−**157**.

although relatively high doses indicate that further improvements in potency and/or pharmacokinetic properties would still be desirable. For some series, demonstrable in vivo efficacy is a result of good species crossover, while in other cases good rodent activity was specifically targeted. It is interesting to note that while rheumatoid arthritis appears to be the current front-running indication in the clinic, supported by preclinical in vivo data, many recent preclinical studies focus on in vivo models of pain.

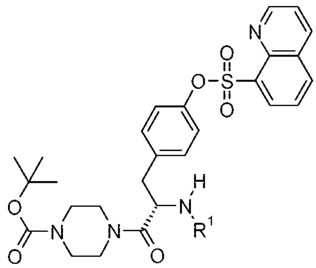
With an increasing number of publications from pharmaceutical companies and academic groups, new understanding around the biology of $P2X_7$ receptor activation, and the approaching disclosure of clinical trial data, it is an interesting time for $P2X_7$ receptor research.

Acknowledgment. The authors thank Suzanne Pears and Sarah-Jane Lynch for their assistance in the preparation of this Perspective.

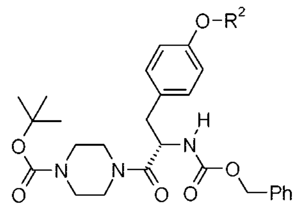
Biographies

Simon D. Guile graduated from The University of Leeds in 1988 and remained there to completed his Ph.D. studies in 1991 under

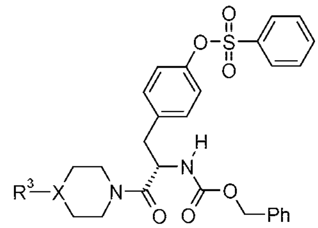
Table 4. SAR of **155** Analogues



158-166



167-176

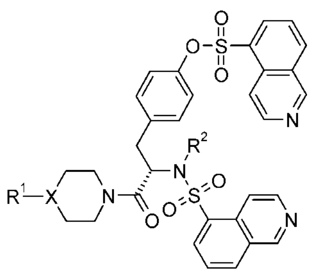


177-180

compd	R^1	%[a]	compd	R^2	%[a]	compd	R^3−X	%[a]
155		85	**167**	4-$MeC_6H_4SO_2$	71	**177**	$PhCH_2OCON$	48
158	5-isoquin-SO_2	37	**168**	4-$MeOC_6H_4SO_2$	62	**178**	$PhSO_2N$	ia
159	8-quin-SO_2	61	**169**	$PhSO_2$	59	**179**	EtCON	ia
160	$PhSO_2$	ia	**170**	5-isoquin-SO_2	43	**180**	PhCON	78
161	$PhCH_2OCO$	53	**171**	PhCO	47			
162	4-$MeC_6H_4SO_2$	32	**172**	$MeSO_2$	ia			
163	PhCO	13	**173**	EtCO	ia			
164	EtOCO	ia	**174**	EtOCO	ia			
165	$MeSO_2$	ia	**175**	$PhCH_2OCO$	ia			
166	H	ia	**176**	H	ia			

[a] % inhibition of ATP-induced potassium release in HEK293 cells stably transfected with $hP2X_7$ using 3 μM antagonist. ia: ≤10% inhibition at 3 μM.

Table 5. Variation of the Phenylpiperazine Group of **155**

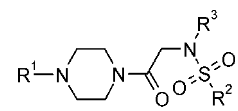


181-190

compd	R^1−X	R^2	pIC_{50}[a]
155	PhN	Me	7.3
181	$PhCH_2N$	Me	7.7
182	$PhCH_2CH_2N$	Me	6.2
183	$PhCH_2CH_2$	Me	7.2
184	4-FC_6H_4N	Me	8.9
185	4-ClC_6H_4N	Me	7.0
186	3-ClC_6H_4N	Me	7.9
187	2-ClC_6H_4N	Me	7.8
188	2-$CH_3C_6H_4N$	Me	7.8
189	Pyrimidin-2-yl-N	Me	7.1
190	4-FC_6H_4N	H	8.2

[a] Inhibition of ATP-stimulated calcium flux in HEK293 cells stably transfected with $hP2X_7$.

Table 6. SAR of Glycine Derivatives of **155**



191-201

compd	R^1	R^2	R^3	pIC_{50}[a]
155				7.3
191	4-FC_6H_4	isoquinolin-5-yl	H	7.2
192	2-FC_6H_4	isoquinolin-5-yl	H	7.9
193	4-$NO_2C_6H_4$	isoquinolin-5-yl	H	7.7
194	3-FC_6H_4	isoquinolin-5-yl	H	<6
195	4-CNC_6H_4	isoquinolin-5-yl	H	<6
196	Ph	isoquinolin-5-yl	H	<6
197	$PhCH_2$	isoquinolin-5-yl	H	7.5
198	$PhCH_2CH_2$	isoquinolin-5-yl	H	7.4
199	2-FC_6H_4	quinolin-5-yl	H	<6
200	2-FC_6H_4	quinolin-8-yl	H	<6
201	4-FC_6H_4	isoquinolin-5-yl	Me	<6

[a] Inhibition of ATP-stimulated calcium flux in HEK293 cells stably transfected with $hP2X_7$.

the supervision of Dr. J. Edwin Saxton. He then carried out 2 years of postdoctoral research at Stanford, CA, with Prof. Barry Trost. He joined Fisons Pharmaceuticals (which became Astra Pharmaceuticals and then AstraZeneca) in Loughborough in September 1993 as a Medicinal Chemist. Simon has experience as a Project Leader and Medicinal Chemistry Team Leader and has worked on a number of projects in cardiovascular, respiratory, and inflammation disease areas.

Lilian Alcaraz received a Ph.D. in Organic Synthesis on the synthesis of paclitaxel at the University of Strasbourg, France, in 1995 under the supervision of Dr. Charles Mioskowski and then carried out 2 years of postdoctoral research in York, U.K., with Prof. Richard Taylor. He joined Astra Pharmaceuticals (which became AstraZeneca) in January 1998 as a Medicinal Chemist and worked on different projects in the respiratory and inflammation therapeutic area. As a team leader in both Lead Generation and Lead Optimization, Lilian has led a wide range of activities from library design and synthesis to late stage drug discovery programs. Lilian is author or coauthor of 40 publications and patents and recently coauthored a book on medicinal chemistry.

Tim N. Birkinshaw graduated from the University of Oxford, U.K., in 1981 and after a brief spell at Glaxo completed his Ph.D. at the University of Cambridge, U.K., in 1987, under the guidance of Andrew Holmes. After postdoctoral studies in Geneva with Prof W. Oppolzer, he joined Fisons Pharmaceutical (which became Astra Pharmaceuticals and then AstraZeneca) in 1989 as a Medicinal Chemist, where he has worked on a number of lead optimization projects.

Keith C. Bowers has a degree in Pharmacology and received a Ph.D. in Cell Biology at the University of Liverpool, U.K., in 1992 under the supervision of Prof. Peter Cobbold. After completing postdoctoral studies in the same laboratory he joined Fisons Pharmaceuticals (which became Astra Pharmaceuticals and then AstraZeneca). He has experience as a lead optimization Project Leader and BioScientist Team Leader and has worked on a number of projects in the respiratory and inflammation disease areas.

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