Discovery of Potent and Selective Adamantane-Based Small-Molecule $P2X_7$ Receptor Antagonists/Interleukin-1β Inhibitors

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Abstract: A novel series of antagonists of the human $P2X_7$ receptor is described. Modification of substituents enabled identification of compounds selective for the rat $P2X_7$ receptor and provides useful pharmacological tools for evaluation of the role of $P2X_7$ in disease.

The cytokines interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α) are produced in response to inflammation, tissue injury, and microbial infection.[1] Overexpression of these cytokines has been implicated in the pathogenesis of a number of chronic inflammatory disorders, most notably, rheumatoid arthritis (RA). RA is a chronic disease characterized by joint inflammation accompanied by concurrent joint erosion and destruction, and extensive evidence from both in vitro and in vivo studies indicates that IL-1β, a proinflammatory cytokine, is involved in mechanisms leading to the progressive joint destruction observed in RA. Anakinra (Kineret), a recombinant human IL-1β receptor antagonist (IL-1Ra), is the first marketed treatment for RA that neutralizes IL-1β activity.[2] Anakinra is indicated as a monotherapeutic agent or can be used in combination with other disease-modifying antirheumatic drugs (DMARDs), such as methotrexate, and has shown significant improvement in patients with active RA, including a reduction in the rate of radiological disease progression.[2] While providing significant patient benefits, the possibility of providing a small-molecule IL-1β inhibitor that can be delivered by the oral route should provide the stimulus for further developments in this area; likely advantages would encompass patient compliance and health economics.

The $P2X_7$ receptor is a ligand-gated ion channel present on a variety of cell types involved in the inflammatory and immune process, specifically macrophages, mast cells, T lymphocytes, and B lymphocytes.[3] Activation of the $P2X_7$ receptor by extracellular adenosine triphosphate (ATP) leads to the processing and release of the proinflammatory cytokine IL-1β from monocytes and macrophages and to the degranulation of mast cells and the shedding of surface molecules L-selectin and CD23 from lymphocytes.[4] The $P2X_7$ receptor also regulates the release of interleukin-18 (IL-18), a proinflammatory cytokine which has also been implicated in the pathogenesis of RA.[5] Additional cell types on which $P2X_7$ receptors are located include antigen-presenting cells, microglial cells, salivary acinar cells, and human skin fibroblasts.[6] This role of $P2X_7$ in the processing and release of both IL-1β and IL-18 was viewed as an attractive approach to inhibiting levels of these cytokines with the target of providing a treatment for rheumatoid arthritis and other inflammatory conditions. Recently, evidence for the role of this receptor in disease processes has been demonstrated in mice in which the function of the $P2X_7$ receptor has been ablated, that is, in $P2X_7$ receptor knock-out (ko) mice. Sequential treatment of blood taken from wild-type mice, but not $P2X_7$-ko animals, with LPS and ATP yielded large amounts of cell-free cytokine IL-1β.[7] In addition, $P2X_7$-ko mice showed a reduced severity of arthritis in an anticollagen antibody arthritis model.[7]

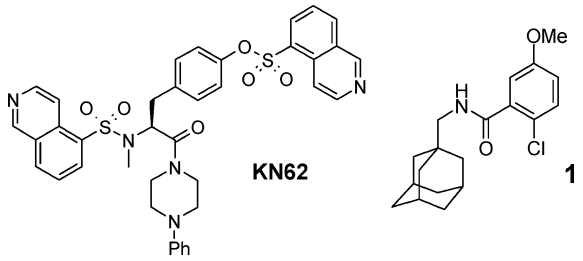

Figure 1. KN62[8] and an initial lead structure from high-throughput screening and a Hit-to-Lead exercise (human $P2X_7$ $pA_2 = 8.8$, rat $P2X_7$ $pA_2 < 5$)[11].

Few examples of $P2X_7$ antagonists are described. 4-[(2*S*)-2-[(5-Isoquinolinylsulfonyl)methylamino]-3-oxo-3-[4-phenyl-1-piperazinyl)propyl]phenylisoquinoline sulfonic acid ester (KN-62, IC_{50} 51nM) (Figure 1) and derivatives have been most studied[8] but do not represent an attractive starting point for a medicinal chemistry program. More recently, triazole[9] and aryl cyanamide[10] $P2X_7$ inhibitors have been reported. We have recently described the discovery of a series of small-molecule $P2X_7$ antagonists through a high-throughput screening approach.[11] The most potent compounds (e.g., **1**) have low nanomolar activity in an assay-measuring reduction of $P2X_7$-mediated plasma membrane pore formation in a monocytic cell line stimulated with the synthetic ATP analogue benzoylbenzoyl adenosine triphospate (BzATP).[12] With structural simplicity and high potency as starting point, efforts were directed toward a number of issues with these compounds. First, the contribution of individual structural features to receptor binding was unknown, with only limited exploration of structure−activity having been accomplished in the Hit-to-Lead program. Second, the properties of these compounds were known to be far from ideal, and the high lipophilicity, low associated solubility, and poor in vitro metabolic stability presented challenges which needed to be overcome. Finally, a lack of activity at the rodent $P2X_7$ receptor, due to poor species crossover, would make target validation in vivo more difficult, and it was deemed necessary to achieve this crossover within selected compounds. Each of these three areas was targeted for investigation.

The central adamantylmethylbenzamide unit was readily constructed by a simple amide coupling of adamantylmethylamine with an appropriate acid chloride. Compounds with a directly linked amine substituent **2** and **3** were prepared from the corresponding bromo compound either by a palladium-

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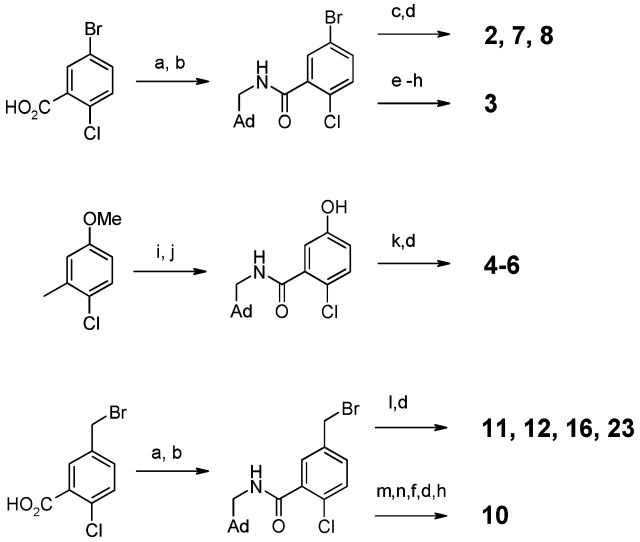
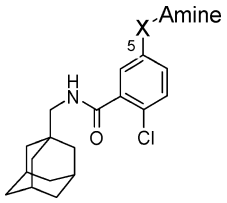
Scheme 1. General Method for the Synthesis of Adamantylmethylbenzamides[a]



[a] Reagents: (a) $(COCl)_2/CH_2Cl_2$/cat. DMF; (b) adamantylmethylamine/ iPr_2NEt/CH_2Cl_2; (c) mono-*N*-BOC-diamine/$Pd(OAc)_2$/ BINAP/Cs_2CO_3/ toluene; (d) HCl/MeOH; (e) MeLi /THF/−70 °C then *n*BuLi/−70 °C; (f) *N*-BOC-4-piperidone; (g) *c*HCl; (h) H_2/PtO_2; (i) $KMnO_4$ then (a), (b); (j) BBr_3/CH_2Cl_2; (k) *N*-BOC-aminoalcohol; (l) mono-*N*-BOC-diamine; (m) $P(OMe)_3$/toluene; (n) LDA 2.3 equiv/THF/−70 °C.

Table 1. Human and Rat $P2X_7$ Activities for Amine-Substituted Adamantylmethylaminebenzamides

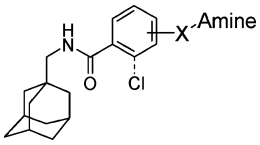


compound	X	amine	human[a] $P2X_7$ pA_2	rat[a] $P2X_7$ pIC_{50}
2	bond	1-piperazinyl	7.5	5.9
3	bond	4-piperidinyl	7.8	6.5
4	O	4-piperidinyl	7.8	6.2
5	O	3(*R*)-piperidinyl	8.2	6.5
6	O	3(*S*)-piperidinyl	8.0	7.0
7	NH	3-(±)-piperidinyl	8.3	7.5
8	NH	4-piperidinyl	7.6	6.3
9	CH_2	3-(±)-piperidinyl	8.2	7.5
10	CH_2	4-piperidinyl	7.8	7.2
11	CH_2	1-piperazinyl	8.0	6.4
12	CH_2	(1*S*,4*S*)-2,5-diaza-bicyclo[2.2.1]-heptane	8.3	6.6

[a] This screen relies on entry of the fluorescent compound ethidium bromide through $P2X_7$ receptor-activated pores. The increase in fluorescence of the intracellular DNA-bound ethidium bromide from cells is a measure of $P2X_7$ receptor activation, and this can be used to quantify the effect of test compounds on the $P2X_7$ receptor.[12]

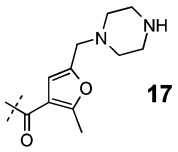
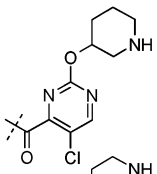
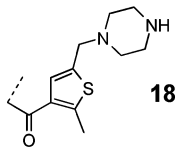
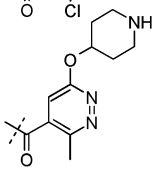
catalyzed coupling with the appropriate amine or, in the case of compound **3**, by a lithiation approach followed by addition to *N*-BOC 4-piperidone, acid-catalyzed dehydration, and selective hydrogenation of the resulting olefin. The same arylbromide was employed in palladium-catalyzed couplings to afford the NH-linked analogues **7** and **8**. Oxygen-linked compounds **4**−**6** were prepared using standard Mitsonobu chemistry and the methylene-linked analogues **9**−**12** by displacement of the corresponding benzylic bromide (Scheme 1). Displacement of this benzylic bromide with lithium tetrakis(*N*-dihydropyridine)-aluminate[13] followed by reduction of the resulting pyridine ring afforded the racemic 3-piperidinyl compound **9**. Analogous chemistry was used to prepare compounds described in Tables 2 and 3.

Table 2. Effect of Aromatic Substituent Regiochemistry (2, 3, 4, and 6 Positions) on $P2X_7$ Activity



compound	X	amine	human $P2X_7$ pA_2[12]	rat $P2X_7$ pIC_{50}[12]
13	O	2-(4-piperidinyl)	<5	<5
14	CH_2	3-(1-piperazinyl)	6.7	<5
15	CH_2	4-(1-piperazinyl)	6.9	6.1
16	O	6-(4-piperidinyl)	<5	<5

Table 3. Replacement of Phenyl with Heterocycles

	Human $P2X_7$ pA_2 (rat pIC_{50})[12]		Human $P2X_7$ pA_2 (rat pIC_{50})[12]
 **17**	<5 (<5)	 **19**	6.6 (6.5)
 **18**	6.5 (<5)	 **20**	5.8 (6.1)

SAR from the Hit-to-Lead exercise,[11] combined with the additional complexity and lack of a demonstrable advantage from functionalizing the adamantane ring itself, led us to explore substitution on the aromatic ring as the favored approach to introduce groups which could improve the physical properties of the compounds. In particular, we were interested in incorporation of polar and charged groups into this region of the molecule if this could be achieved without adversely affecting potency. Initial attempts to introduce a carboxylic acid functionality were not promising, and direct replacement of the *O*-methyl group in the 5 position of the aryl ring of compound **1** with a carboxylate group gave a large drop in potency (pA_2 6.2). Incorporation of amine substituents, however, was more beneficial (Table 1).

While a drop in potency is seen compared to **1**, most of these cyclic amine substituted compounds retain a good level of activity and, importantly, have improved physical properties (e.g., solubility) and are appreciably more stable to metabolism. The most promising example from this series was judged to be compound **11**. The compound is stable in in vitro hepatocyte and microsomal preparations and displays good pharmacokinetics in vivo.[14] Further studies have also shown this compound to be selective for the $P2X_7$ receptor after screening widely against other receptor types, including related P2X receptors. An X-ray structure (Figure 2) shows the amide carbonyl to be twisted 44° out of the plane of the phenyl group by the *ortho*-

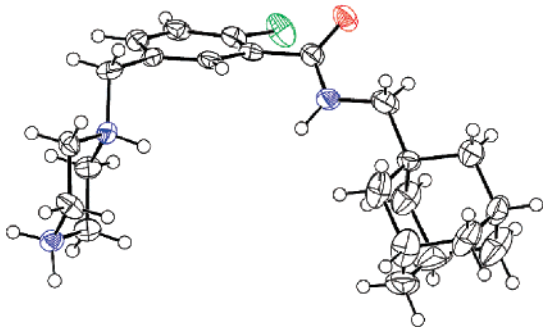


Figure 2. The molecular conformation of compound **11** dihydrochloride monohydrate. The two chloride ions and the water molecule are excluded for clarity. The angle between the carboxamide plane [O=C−NH−C] and the aromatic Cl−phenyl plane is 44.4°.

chlorine atom, a common feature within this series of compounds and one associated with high activity at the $P2X_7$ receptor.

It is apparent from this small series of compounds that introduction of an amine substituent had introduced activity at the rat $P2X_7$ receptor which was not present in the starting compound **1**. Although this activity varied considerably between the compounds and compound **11** had relatively weak activity, at least two members of the series (**7** and **9**) had activity approaching $IC_{50} = 30$ nM. Further investigation led us to move the amine substituent into the other available positions on the aromatic ring in compound **1** and to examine a greater variety of amine groups. The results of the first exercise can be illustrated with just four examples (Table 2). Depending on the chemistry used to construct the compounds, the linking group X was either oxygen or a methylene unit. Attachment of the amine group through the 2 (replacing the Cl group) or 6 positions led to inactive compounds. Attachment to the 3 or 4 position retained a degree of activity, but this was substantially reduced compared to that of the 5 position.

Having established the 5 position as optimal for attaching a charged group, we investigated replacement of the phenyl ring itself with a limited selection of heterocyclic groups (Table 3). While this work did not provide an advance in terms of properties, it did reveal the furan ring compound **17** as an inactive structural analogue which could be used as a standard for in vitro and in vivo testing. In an assay-measuring release of IL-1β from monocytes, compound **11** showed complete inhibition of cytokine release (pIC_{50} 7.1 ± 0.1), whereas compound **17** showed no activity in this assay (Figure 3). Compounds **11** and **17** displayed a similar pattern of activity in an IL-18 release assay (Figure 4), compound **11** giving complete inhibition of cytokine release (pIC_{50} 7.4 ± 0.1).

Target validation studies required a compound with activity at the rat $P2X_7$ receptor combined with the ability to achieve

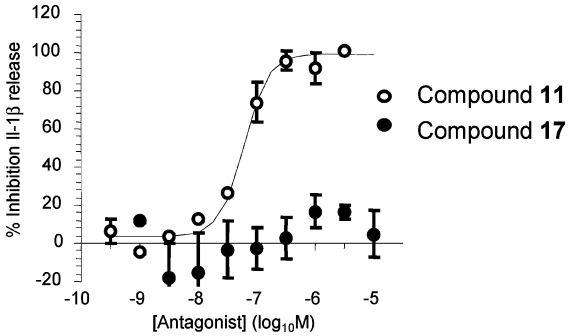


Figure 3. BzATP-induced IL-1β release from isolated human monocytes—effect of compounds **11** and **17** ($n = 3$)[15].

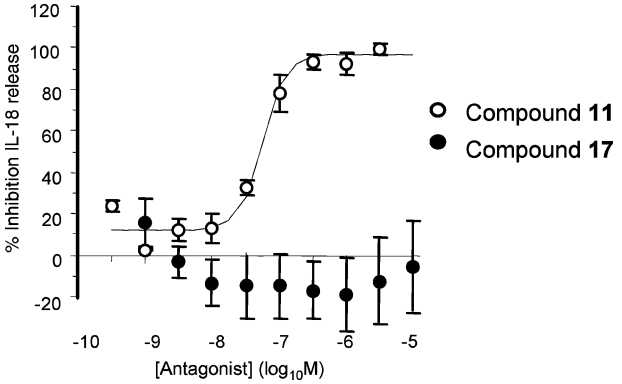


Figure 4. BzATP-induced IL-18 release from isolated human monocytes—effect of compounds **11** and **17** ($n = 3$)[15].

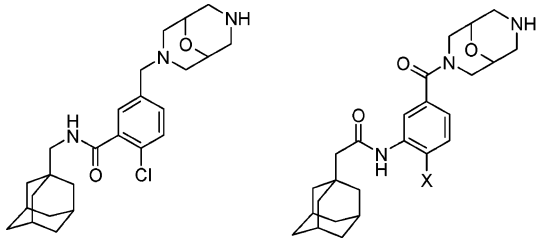


Figure 5. Compounds used for in vivo evaluation.

good exposure when dosed orally. Although promising activity at the rat $P2X_7$ receptor was observed in some of the compounds illustrated in Table 1, the more metabolically stable members, **4**, **11**, and **12**, were those with lower activity. Extensive screening of analogues led to the discovery of compound **21**, which has the desired properties of high activity at the rat $P2X_7$ receptor (pA_2 7.6) combined with good in vitro and in vivo stability but poor oral bioavailability. Further modification of this compound yielded compound **22**, which combined rat $P2X_7$ activity (pA_2 7.8), low rat plasma protein binding (66%), and high exposure when dosed orally, and compound **23**, with very similar properties. Interestingly, both compounds were selective for the rat versus the human $P2X_7$ receptor (pIC_{50} <6.0) and were highly selective when screened against a wide variety of receptor and enzyme systems (MDS Pharma),[16] including $P2X_{1-5}$ receptors.[17] $P2X_7$ antagonists, through their ability to inhibit release of IL-1β and IL-18, should have utility in the treatment of a range of inflammatory diseases where these cytokines play a role. The discovery of highly selective and metabolically stable small-molecule antagonists of the human $P2X_7$ receptor such as compound **11**, alongside the discovery of close structural analogues possessing activity at the rat $P2X_7$ receptor (**21**−**23**), will enable an evaluation of $P2X_7$ antagonists in both disease models and in human disease.

Supporting Information Available: Experimental details, ^1H NMR chemical shifts, HPLC and MS data are provided, including X-ray crystallographic data for compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Dayer, J. Interleukin 1 or tumor necrosis factor-α: which is the real target in rheumatoid arthritis? *J. Rheumatol.* **2002**, *29*, 10−15, Supplement 65. (b) Dinarello, C. A.; Wolff, S. M. The Role of interleukin-1 in disease. *N. Engl. J. Med.* **1993**, *328*, 106−113. (c).

van den Berg, W. B. Arguments for interleukin-1 as a target in chronic arthritis. *Ann. Rheum. Dis.* **2000**, *59*, i81−i84, Supplement 1.

(2) (a) Cohen, S. B.; Woolley, J. M.; Chan, W. W. Interleukin 1 receptor antagonist anakinra improves functional status in patients with rheumatoid arthritis. *J. Rheumatol.* **2003**, *30*, 225−231. (b) Bresnihan, B. Effects of anakinra on clinical and radiological outcomes in rheumatoid arthritis. *Ann. Rheum. Dis.* **2002**, *61*, ii74−ii77, Supplement 2.

(3) (a) North, R. A. Molecular physiology of P2X receptors. *Physiol. Rev.* **2002**, *82*, 1013−1067. (b) Burnstock, G. Overview of P2 receptors: possible functions in immune cells. *Drug Dev. Res.* **2001**, *53*, 53−59. (c) Di Virgilio, F.; Vishwanath, V.; Ferrari, D. On the role of the P2X7 receptor in the immune system. *Handb. Exp. Pharmacol.* **2001**, *151/II*, 355−374. (d) Guile, S. D.; Ince, F.; Ingall, A. H.; Kindon, N. D.; Meghani, P.; Mortimore, M. P. The medicinal chemistry of the P2 receptor family. *Prog. Med. Chem.* **2001**, *38*, 115−187.

(4) (a) MacKenzie, A.; Wilson, H. L.; Kiss-Toth, E.; Dower, S. K.; North, R. A.; Surprenant, A. Rapid secretion of interleukin-1β by microvesicle shedding. *Immunity* **2001**, *15*, 825−835. (b) Perregaux, D.; Gabel, C. A. Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. *J. Biol. Chem.* **1994**, *269*, 15195−15203. (c) Gu, B.; Bendall, L. J.; Wiley, J. S. Adenosine triphosphate-induced shedding of CD23 and L-selectin (CD62L) from lymphocytes is mediated by the same receptor but different metalloproteases. *Blood* **1998**, *92*, 946−951.

(5) (a) Mehta, V. B.; Hart, J.; Wewers, M. D. ATP-stimulated release of interleukin (IL)-1β and IL-18 requires priming by lipopolysaccharide and is independent of caspase-1 cleavage. *J. Biol. Chem.* **2001**, *276*, 3820−3826. (b) Banda, N. K.; Vondracek, A.; Kraus, D.; Dinarello, C. A.; Kim, S.; Bendele, A.; Senaldi, G.; Arend, W. P. Mechanisms of inhibition of collagen-induced arthritis by murine IL-18 binding protein. *J. Immunol.* **2003**, *170*, 2100−2105.

(6) (a) Mutini, C.; Falzoni, S.; Ferrari, D.; Chiozzi, P.; Morelli, A.; Baricordi, R.; Collo, G.; Ricciardi-Castagnoli, P.; Di Virgilio, F. Mouse dendritic cells express the P2X7 purinergic receptor: characterization and possible participation in antigen presentation. *J. Immunol.* **1999**, *163*, 1958−1965. (b) Collo, G.; Neidhart, S.; Kawashima, E.; Kosco-Vilbois, M.; North, R. A.; Buell, G. Tissue distribution of the P2X7 receptor. *Neuropharmacology* **1997**, *36*, 1277−1283. (c) Gibbons, S. J.; Washburn, K. B.; Talamo, B. R. P2X7 receptors in rat parotid acinar cells: formation of large pores. *J. Auton. Pharmacol.* **2001**, *21*, 181−190. (d) Solini, A.; Chiozzi, P.; Morelli, A.; Fellin, R.; Di Virgilio, F. Human primary fibroblasts in vitro express a purinergic P2X7 receptor coupled to ion fluxes, microvesicle formation and IL-6 release. *J. Cell Sci.* **1999**, *112*, 297−305.

(7) (a). Labasi, J. M.; Petrushova, N.; Donovan, C.; McCurdy, S.; Lira, P.; Payette, M. M.; Brissette, W.; Wicks, J. R.; Audoly, L.; Gabel, C. Absence of the P2X7 receptor alters leukocyte function and attenuates an inflammatory response. *J. Immunol.* **2002**, *168*, 6436−6445. (b). Solle, M.; Labasi, J.; Perregaux, D. G.; Stam, E.; Petrushova, N.; Koller, B. H.; Griffiths, R. J.; Gabel, C. A. Altered cytokine production in mice lacking P2X7 receptors. *J. Biol. Chem.* **2001**, *276*, 125−132.

(8) (a) Gargett, C. E.; Wiley, J. S. The isoquinoline derivative KN-62 a potent antagonist of the P2Z-receptor of human lymphocytes. *Br. J. Pharmacol.* **1997**, *120*, 1483−1490. (b) Ravi, R. G.; Kertesy, S. B.; Dubyak, G. R.; Jacobson, K. A. Potent P2X7 receptor antagonists: tyrosyl derivatives synthesized using a sequential parallel synthetic approach. *Drug Dev. Res.* **2001**, *54*, 75−87. (c) Baraldi, P. G.; Nunez, M. C.; Morelli, A.; Falzoni, S.; Di Virgilio, F.; Romagnoli, R. Synthesis, biological activity and molecular modeling studies of 1,2,3,4-tetrahydroisoquinoline derivatives as conformationally constrained analogues of KN62, a potent antagonist of the P2X7-receptor containing a tyrosine moiety. *Arzneim. Forsch.* **2002**, *52*, 273−285.

(9) Carroll, W.; Florjancic, A.; Perez-Medrano, A.; Peddi, S. Triazole derivatives as $P2X_7$ receptor antagonists and their preparation, pharmaceutical compositions and use in treatment of disease. US2007105842, 2007.

(10) Carroll, W.; Florjancic, A.; Perez-Medrano, A.; Peddi, S. Preparation of aryl cyanoamidines as $P2X_7$ antagonists for the treatment of pain, inflammation and neurodegeneration. US2006025614, 2006.

(11) Baxter, A.; Bent, J.; Bowers, K.; Brough, S.; Fagura, M.; Lawson, M.; McInally, T.; Mortimore, M.; Robertson, M.; Weaver, R.; Webborn, P. Hit-to-Lead studies: the discovery of potent adamantane amide $P2X_7$ receptor antagonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4047−4050.

(12) Full details of the method for assessing benzyl ATP-induced changes in plasma pore formation (in human and rat cells) and for pA_2/IC_{50} determinations quoted in Tables 1−3 are provided in the Supporting Information. The pA_2/pIC_{50} values are the mean of a minimum on $n = 2$ determinations performed on separate days with individual values no more than 0.5 log units apart.

(13) Giam, C.; Abbott, S. D. Novel synthesis of 3-substituted pyridines from pyridine. *J. Am. Chem. Soc.* **1971**, *93*, 1294−1296.

(14) In vitro pharmacokinetic data for compound **11**: human hepatocytes $Cl_{int} < 3$ μl/min/1E6, rat hepatocytes $Cl_{int} < 5$ μl/min/1E6. In vivo pharmacokinetic data for compound **11** in Sprague Dawley rat: Cl 13 ml/min/kg, Vss 5.3l /kg, $T_{1/2}$ 5.2 h, bioavailability 79%.

(15) Full details of the method for assessing inhibition of the release of IL-1β and IL-18 from LPS-primed human monocytes is provided in the Supporting Information.

(16) The only significant off-target activities when tested against a panel of 153 enzyme and receptor assays at MDS Pharma were vasopressin V_{1A}, IC_{50} 7 μM, and nonselective sigma receptor activity, IC_{50} 4 μM.

(17) Inactive when tested at 10 μM against human $P2X_1$, $P2X_3$, $P2X_4$, and $P2X_5$ and rat $P2X_2$ and $P2X_{2/3}$ (heteromeric) receptors.

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