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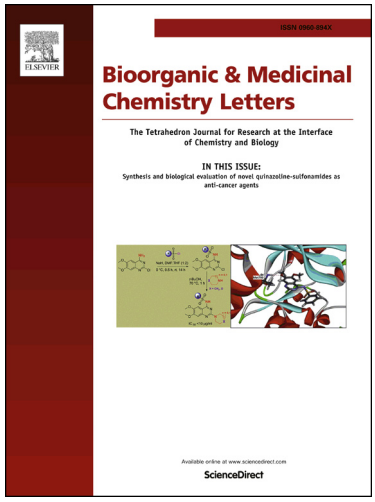

Pharmacological evaluation of a novel series of urea, thiourea and guanidine derivatives as $P2X_7$ receptor antagonists

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Pharmacological evaluation of a novel series of urea, thiourea and guanidine derivatives as $P2X_7$ receptor antagonists

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Abstract - We report on $P2X_7$ receptor antagonists based on a lead adamantly-cyanoguanidine-aryl moiety. We have investigated the importance of the central cyanoguanidine moiety by replacing it with urea, thiourea or guanidine moieties. We have also investigated the linker length between the central moiety and the aryl portion. All compounds were assessed for their inhibitory potency in a pore-formation dye uptake assay at the $P2X_7$ receptor. None of the compounds resulted in an improved potency illustrating the importance of the cyanoguanidine moiety in this chemotype.

Keywords: $P2X_7R$; inflammation; cyanoguanidine; urea; thiourea.

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Adenosine 5'-triphosphate (ATP) is the endogenous ligand and physiological agonist of the $P2X_7$ purinoreceptor ($P2X_7R$), a member of the P2X superfamily of trimeric ligand-gated cation channels (P2X1-7).[1-4] The $P2X_7R$ is predominantly and highly expressed on immune cells of hematopoietic origin such as peripheral monocytes and macrophages, as well as their centrally located counterparts, microglia.[2,5] Transient $P2X_7R$ activation by low concentrations of extracellular ATP permits the influx of calcium and sodium ions, and efflux of potassium ions within milliseconds.[1,4,6] However, psychological stress and insults to the CNS (including neurodegenerative and ischemic related) incite enhanced release of ATP into the extracellular environment.[7-9] The prolonged activation of the $P2X_7R$ results in the immediate rearrangement of the cell membrane and cytoskeleton,[10,11] the formation of a large >900 Da macro-pore, and eventual apoptosis.[2,7] Prior to cell death, the proinflammatory cytokines IL-1β and IL-18 are also processed and secreted,[8,10,12-15] Significantly higher serum levels of IL-1β, among other proinflammatory cytokines, have been reported in patients suffering from Alzheimer's disease (AD)[16] and major depressive disorder (MDD),[17] suggesting the dysregulation of the inflammatory response may underpin these, and many other, neurodegenerative (including Parkinson's disease), and neuropsychiatric (including bipolar disorder) conditions, as well as neuropathic pain.[7,18-21] Mice lacking the $P2X_7R$ generated macrophages that were incapable of releasing mature IL-1β in response to extracellular ATP, or to its more stable and potent analogue, 2'(3')-O-(4-benzoylbenzoyl)adenosine-5'-triphosphate tri(triethylammonium) salt (BzATP).[22,23] Additionally, in several animal models of depression, abolishing $P2X_7R$ expression offered an antidepressant effect.[23] When stimulated with the amyloid β protein (Aβ), a hallmark of AD pathology, lipopolysaccharide (LPS)-primed microglia from wild type mice released ATP and large amounts of IL-1β, whereas their $P2X_7R$-deficient counterparts did not.[24] Moreover the microglia derived from these $P2X_7R$-knockout mice were resistant to Aβ-induced plasma membrane permeabilisation, potentially through their inability to form the large $P2X_7R$ pore. Considering the significant influence the $P2X_7R$ has over the inflammatory response, and that microglial $P2X_7R$ expression is upregulated in conditions such as AD and multiple sclerosis (MS),[1,25] $P2X_7R$ antagonism appears as a potential treatment strategy.

Initial $P2X_7R$-focussed medicinal chemistry was directed at generating $P2X_7R$ antagonists for the treatment of peripheral inflammatory diseases including rheumatoid arthritis and Crohn's disease, with a number of compounds having progressed to clinical trials.[2,26] Adamantyl amide **1** was developed by AstraZeneca and showed promising pharmacokinetic

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properties from *in vivo* studies in rats (Figure 1). Abbott Laboratories developed compounds focused on the cyanoguanidine moiety. These compounds, with **2** and **3** being examples of the more potent in the series, showed nanomolar potency, but a poorer pharmacokinetic profile. We have recently reported an adamantyl-cyanoguanidine hybrid that combined pharmacologically beneficial features from the Abbott cyanoguanidine derivatives with the inclusion of an adamantane moiety for improved pharmacokinetics and enhanced blood-brain barrier (BBB) penetration.[27] The small cyanoguanidine hybrid **4a** was reported to have nanomolar potency at the $P2X_7R$ (IC_{50} = 100 nM). Potency could be improved by lengthening the chain and incorporating a nitrogen atom into the aryl portion, particularly at the 3-position to give **4b** (IC_{50} = 69 nM). Further conclusions from this work showed that a methylene linker between the cyanoguanidine and adamantyl moieties was essential for potency, while either no linker (n = 0) or a methylene linker (n = 1) was acceptable for the aryl portion.

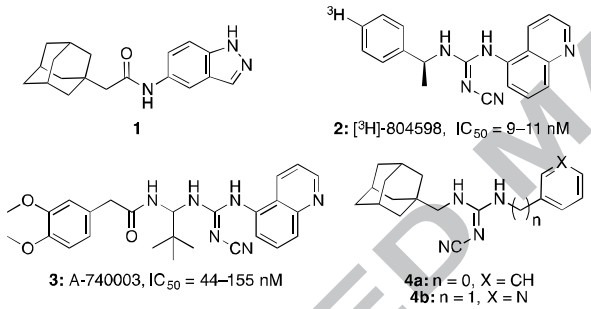

Figure 1. P2X7R antagonists.

While there have been advancements in potency optimization and BBB permeability, many currently disclosed compounds lack desirable characteristics for targeting central $P2X_7R$, being unable to permeate the BBB or offer insufficient target engagement for potential therapeutic value.[1] Therefore to increase the potential for translational clinical utility, modifications were made to the cyanoguanidine moiety in an effort to optimize lipophilicity. However, it is unknown whether the cyanoguanidine moiety was essential for potency or if substitutions could be tolerated. In this article, we report urea, thiourea and guanidine derivatives of this cyanoguanidine hybrid which explore the significance of the cyanoguanidine linker moiety and the positioning of the phenyl group on $P2X_7R$ antagonist potency.

The (adamantan-1-yl)methanamine hydrochloride (**5**) was obtained using previously reported procedures[28] and then reacted with the commercially available phenyl isocyanate (**6**)

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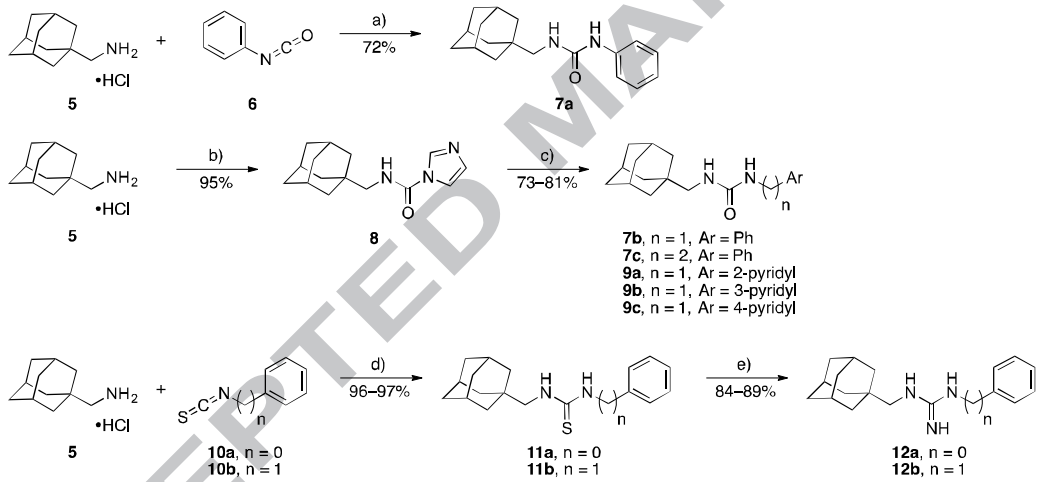
to afford **7a** in 72% yield (Scheme 1). Considering the range of analogues that we wanted to develop, we sought adamantyl isocyanate as a key building block. However, we were interested in a previous report of a methyl isocyanate equivalent derived from 1,1'-carbonyldiimidazole (CDI) that was crystalline and stable.[29] By using CDI and **5**, compound **8** could be obtained as a white solid in 95% yield. This compound was stored for a year under ambient conditions without any observed decomposition, making it a suitable isocyanate equivalent. Reacting **8** with benzylamine or phenethylamine afforded **7b** and **7c**, respectively. The same isocyanate equivalent **8** could be converted to the pyridyl containing ureas **9a**-**c**, using 2-, 3-, or 4-picolylamine respectively. The high yields obtained (73-81%) illustrate the utility of **8** as a precursor for forming (adamantan-1-yl)methyl ureas.

The commercially available isothiocyanates **10a**-**b** could be reacted with **5** to form the thiourea derivatives **11a**-**b** and further converted to the guanidine derivatives **12a**-**b**.



Scheme 1. Synthesis of ureas, thioureas and guanidines. *Reagents and conditions:* a) Et_3N (1.0 equiv), CH_2Cl_2, RT, 18 h; b) 1,1'-carbonyldiimidazole (1.0 equiv), MeCN/DMF (3:1), RT, 2 h; c) requisite amine (1.0 equiv), Et_3N (1.0 equiv), CH_2Cl_2, RT, 18 h; d) Et_3N (1.0 equiv), CH_2Cl_2, RT, 18 h; e) 1) MeI (10 equiv), MeCN, 40 ºC, 1 h; 2) NH_4OH, RT, 3h.

The adamantyl urea, thiourea and guanidine compounds **7a**-**c**, **9a**-**c**, **11a**-**b** and **12a**-**b**, were assayed for their ability to inhibit BzATP-induced $P2X_7R$ activity in human THP-1 cells. BzATP is a stable and potent $P2X_7R$ agonist with the $P2X_7R$ being endogenously expressed by THP-1 cells. The cellular uptake of the fluorescent dye, YO-PRO-1, was measured as an indicator of pore formation, and therefore $P2X_7R$ activity. Experimental protocols are detailed in the supplementary information. In addition to the newly synthesized compounds, the previously reported cyanoguanidine **4a** was also subjected to the same experiments.

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The significance of the cyanoguanidine moiety to the nanomolar potency yielded by **4a** in previous reports[27] was explored by replacing this with other functional groups, whilst retaining the adamantyl and aryl portion of the molecule (Table 1). With the exception of compound **11b**, replacing the cyanoguanidine moiety with all three functional groups (urea, thiourea, guanidine) decreased the lipophilicity (cLogP) value compared to **4a** (Table 1). Comparing variations in central functionality showed that substituting the cyanoguanidine moeity (**4a**) with: (i) a guanidine (**12a**) proved detrimental to the molecule's solubility, (ii) a thiourea (**11a**) was poorly tolerated with an almost 24-fold decrease in potency and yielded only low micromolar potency (2.4 μM), and (iii) a urea (**7a**) revealed an even greater 50-fold reduction in potency (4.9 μM).

Our previous studies on the adamantyl cyanoguanidine compounds showed that altering the linker length between the central moiety and aryl group impacted potency.[27] Therefore, the linker length between the phenyl group and the central functional group was investigated in all the urea, thiourea, and guanidine derivatives to determine if this might result in improved compound potency. Although extending the phenyl portion by one carbon from the guanidine group **12b** restored the molecule's solubility, potency remained low (**12b** > 10 μM). Extending the phenyl group away from the urea group of **7a** by one carbon only slightly improved compound potency (**7b** = 4.54 μM), and by two carbons led to a further decrease in potency (**7c** = 6.41 μM). These results mirror the results of the adamantyl cyanoguanidine derivatives where a linker length of n = 0 or 1 was most tolerated and that extending the linker further was detrimental. The thiourea derivatives showed a decrease in potency when the linker was increased from n = 0 to 1 (**11a** = 2.42 μM, **11b** = 4.24 μM, respectively) and so further extensions were not investigated. Overall, increasing the distance between the central functional group and the phenyl group did not produce higher potency compounds, and may reflect a smaller binding pocket on the $P2X_7R$ where binding residues may favor shorter molecules.[30]

Previous findings indicate the inclusion of a nitrogen atom in the aromatic ring of adamantyl cyanoguanidines improved activity at the $P2X_7R$.[27] Investigating the role of incorporating a nitrogen atom in the aromatic system was made on the urea moiety (n = 1) giving the isomeric pyridyl derivatives **9a**-**c**. Compound **9b** displayed a slight increase in potency (3.38 μM), while the analogues **9a** and **9c** were less potent than the phenyl analogue **7b** (**9a** and **9c**; 5.95 and 6.75 μM respectively). The improved potency of **9b** is in agreement with the observations of the cyanoguanidine derivative **4b** that also showed improved

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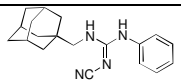
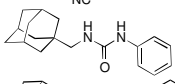
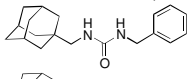
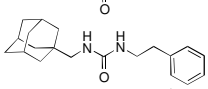
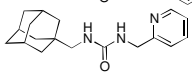
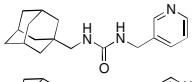
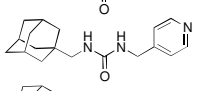
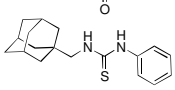
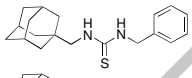
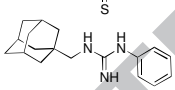
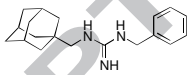
potency over its phenyl and pyridyl isomer analogues. The 3-pyridyl derivative **9b** (n = 1) positions the nitrogen atom in a similar location to that observed in the quinoline compounds **2** and **3**. These combined results suggest that, as a hydrogen bond acceptor, the nitrogen atom improves hydrogen bonding interactions with residues in the receptor's ligand-binding site.

Table 1. IC_{50} values of compounds **4a**, **7a-c**, **9a-c**, **11a-b** and **12a-b** derivatives against $hP2X_7R$ using the YO-PRO-1 dye uptake functional assay in human THP-1 cells

Compound	Structure	cLogP [a]	IC_{50} mean ± SEM (μM) [b]
4a		4.6	0.10 ± 0.01
7a		3.36	4.96 ± 0.24
7b		3.43	4.54 ± 0.28
7c		3.71	6.41 ± 0.26
9a		2.51	5.95 ± 0.04
9b		2.09	3.38 ± 0.14
9c		2.09	6.75 ± 0.17
11a		4.5	2.42 ± 0.12
11b		4.71	4.24 ± 0.14
12a		4.18	– [b]
12b		4.39	>10,000

[a] Calculated using ChemDraw Professional 15.

[b] Values are the mean of three to five experiments and uncertainty was determined by the standard error of the mean (SEM)

[c] IC_{50} could not be determined due to compound insolubility

We have reported the synthesis of 10 adamantyl-containing compounds as well as the adamantyl isocyanate analogue **8**. However, the poor potency resulting from the chemical modifications to the cyanoguanidine moiety, as revealed by the dye uptake functional assays, render the compounds synthesized of unlikely therapeutic use. These results have clearly shown the importance of the cyanoguanidine moiety for potent $P2X_7R$ inhibition in this class of compounds, but also confirmed that the incorporation of a nitrogen atom in the aromatic system, namely at the 3-position, can be used to improve potency. Based on these results, our

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current work in this area will be to return the cyanoguanidine moiety and develop functionality around that to improve potency.

Acknowledgments

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Supplementary Material

Full synthetic details, ^{1}H and ^{13}C NMR spectra and experimental protocols for biological assays are listed in the supplementary material.

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Graphical Abstract

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Pharmacological evaluation of a novel series of urea, thiourea and guanidine derivatives as $P2X_7$ receptor antagonists

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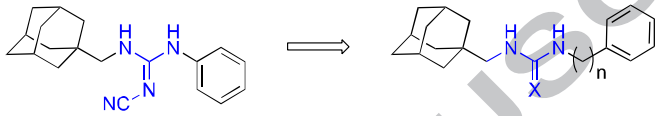

$P2X_7$ receptor IC_{50} = 100 nM

X = O, S, NH, n = 0–2
μM potency at $P2X_7$ receptor

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