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Cubyl amides: Novel $P2X_7$ receptor antagonists

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ABSTRACT

Polycyclic amides **2** and **5**–**9** were successfully synthesised and their lipophilicity profiles were evaluated using reverse-phase HPLC. All synthesised compounds possessed $P2X_7R$ antagonistic properties when tested on rat spinal cord microglia cells. Extensive screening for binding to other neuroreceptor subtypes demonstrated their $P2X_7$ selectivity.

The $P2X_7$ receptor ($P2X_7R$) is an unusual, non-desensitising cation-selective ion channel, directly gated by extracellular ATP. It is ubiquitously found in a variety of cell types, most notably those of haematopoietic origin, such as mast cells, macrophages and lymphocytes, as well as brain glial cells, including microglia and astrocytes.[1] It possesses a 240-amino acid residue, cytoplasmic, carboxy-terminal tail that is necessary for its bifunctionality as a selective ion channel assembled from $P2X_7$ subunits, or as a non-selective pore. There are two hypotheses explaining $P2X_7$-associated pore formation; in both scenarios brief activation of the receptor with ATP leads to opening of the ion channel by conformational changes within the $P2X_7$ structure. In order to form the membrane pore, the receptor complex recruits further subunits to the original receptor complex. Alternatively, prolonged activation of the $P2X_7$ receptor complex leads to activation of intracellular machineries that signal the opening of $P2X_7$-independent pore forming proteins such as pannexin 1 allowing entry of large (up to molecular weights of 900 Da) cationic species.[2]

Since the $P2X_7R$-encoding cDNAs were isolated and characterised from three different mammalian sources (rats,[2] mice[3] and human[4]) about a decade ago, there has been increasing interest in delineating different aspects of the $P2X_7R$ function, particularly its role in microglia activation and neurodegenerative diseases,[5,6] chronic pain modulation[7] and the exact mechanism of the channel-to-pore transition.[8,9] $P2X_7$-knockout mice have been reported to show reduced incidence and severity of arthritis in an anti-collagen antibody arthritis model.[10] More strikingly, chronic inflammatory and neuropathic hypersensitivity are totally abolished in $P2X_7$-knockout mice despite the remaining normal nociceptive processing.[11] Furthermore, antagonism of the $P2X_7R$ on oligodendrocytes leads to failure of multiple sclerotic conditions to develop in mouse EAE models of multiple sclerosis.[12] In addition, linkage studies show that one of the best logarithm of odds (LOD) scores for bipolar disorder, clinical depression and anxiety disorders is the $P2X_7$ receptor.[13,14] It could therefore be suggested that potent, selective $P2X_7$ antagonists may serve as novel all-purpose analgesics.

To date, structure–activity relationship (SAR) studies of several small, drug-like $P2X_7$-active molecules have been reported and recently reviewed (Fig. 1).[15] Cyclic imide **1** and adamantyl benzamide **2** were discovered by AstraZeneca through a high-throughput

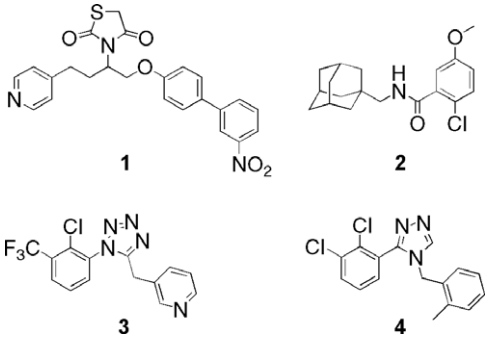

Figure 1. Drug-like $P2X_7$-active molecules.

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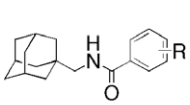
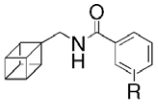
Table 1
Cubyl amides and adamantyl amides



2: R = 2-Cl-5-OMe
5: R = 2-F
6: R = 4-F



7: R = 2-Cl-5-OMe
8: R = 2-F
9: R = 4-F

Compound	∠OCCC[a] (°)	*C(O)–H*(poly) distance[b] (Å)	Clog*P*[c]
2	−49.8	7.392	5.40
5	−12.6	7.376	5.11
6	−24.4	7.377	5.52
7	−50.4	6.797	2.67
8	−12.3	6.772	2.39
9	−23.5	6.757	2.80

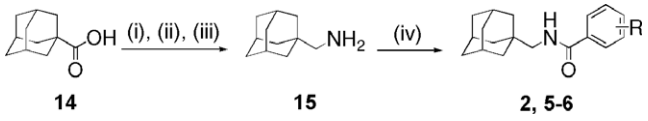
[a] The dihedral angle about the amide C=O and aromatic ring obtained from Macromodel™.
[b] The distance between the carbonyl carbon atom to the furthest hydrogen atom in the polycyclic moiety obtained from Macromodel™.
[c] The Clog*P* values were obtained from ChemDraw 9™. Surface areas of adamantane = 156 Å^2, cubane = 122 Å^2 (Macromodel™).

screening process of their compound library.[16,17] More recently, tetrazole **3** and triazole **4** have also been found to inhibit rat and human P2X$_7$R activity.[18–21] These compounds are highly amenable to structural modifications, indicating their potential as lead molecules for further improving the potency and selectivity at P2X$_7$R.

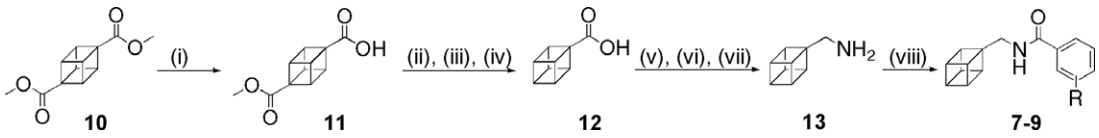
Of these the adamantyl benzamide **2** represents an intriguing class of compound for development of P2X$_7$ antagonists since the adamantane could be replaced with other polycyclic moieties and their influence on P2X$_7$ receptor activity assessed. These molecules could potentially be used not only as therapeutics but also as diagnostic probes when radiolabelled for use with in vivo molecular imaging. We hypothesised that by replacing the adamantane with a cubane moiety, these compounds could be rendered suitable as in vivo radiotracers for positron emission tomography (PET) whilst maintaining activity (Table 1). This would allow for the first time to image P2X$_7$R expression in disease and disease progression. In addition they could also be used to assess the efficacy of P2X$_7$R therapy.

Although the original report by AstraZeneca mentioned that no replacements were found for adamantane, we believed cubyl amides **7**–**9** could offer unique possibilities for development of P2X$_7$R-active molecules due to their rigid molecular structure and ability to orient substituents in three dimensions. As shown in Table 1, the distance between the carbonyl carbon atom to the furthest hydrogen atom in the adamantane series is ca 0.6 Å longer than the cubane series. The dihedral angle about the amide C=O and aromatic ring is very similar in both series, suggesting that each of the cubyl amides adopts a very similar lowest energy conformation to the adamantane analogues. Moreover, systematic alteration of their lipophilicity would allow easier access across membranes or the blood–brain barrier. To the best of our knowledge, there have only been a few studies that reported radiolabelling of P2X$_7$-active compounds.[22,23] Thus far, no attempts have been undertaken to radiolabel-specific P2X$_7$R ligands for use in PET imaging of the living brain. Herein, we report an efficient synthesis of cubyl amides and their preliminary biological evaluation at the P2X$_7$R. Variations in the aromatic segment of the target compounds are based primarily on maintaining strong binding to the receptor whilst investigating potential sites for incorporation of a fluorine-18 radiolabel (**2** vs **5**, **6** and **7** vs **8** and **9**). Their preliminary P2X$_7$ functionality was tested based on the inhibition of dye uptake using rat spinal cord microglia as P2X$_7$ source. The binding affinities towards other common neuroreceptors were also investigated.

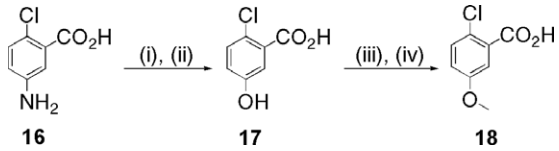
Synthesis of novel *N*-cuban-1-ylmethyl-benzamides is illustrated in Scheme 1 (see Supplementary data for details). Commercially available dimethyl 1,4-cubanedicarboxylate **10** was converted to cubane-1-carboxylic acid **12** in 70% yield overall following a literature procedure utilising Barton decarboxylation as the key step.[24] Subsequent transformation of the acid to amine **13** followed by amide couplings afforded the desired amides **7**–**9**.[25] To obtain more information on the optimal size of the 'hydrophobic pocket' at the P2X$_7$R binding site, the adamantyl amides **2**, **5**, and **6** were also prepared in a similar fashion (Scheme 2). 2-Chloro-5-methoxybenzoic acid **18** was readily prepared by diazotisation of commercially available 5-amino-2-chlorobenzoic acid **16**, followed by treatment with excess MeI and K_2CO_3 and subsequent



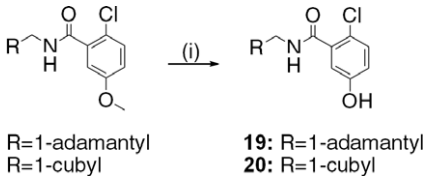
Scheme 2. Reagents and conditions: (i) $SOCl_2$, reflux, 3 h; (ii) aq NH_3, THF, 0 °C–rt, 1 h; (iii) $LiAlH_4$, THF, 0 °C to reflux, 16 h, 60% over three steps; (iv) aroyl chloride, NEt_3, MeCN, 0 °C–rt, 16 h.



Scheme 1. Reagents and conditions: (i) NaOH (1 equiv), MeOH, THF, rt, 16 h, 88%; (ii) $(COCl)_2$, CH_2Cl_2, 30 min; (iii) sodium salt of *N*-hydroxypyridine-2-thione, *hν*, DMAP, *t*-BuSH, benzene, reflux, 90 min; (iv) NaOH (1 equiv), MeOH, reflux, 1 h, 80% over three steps; (v) $(COCl)_2$, CH_2Cl_2, rt, 45 min; (vi) NH_3 (l), CH_2Cl_2, −78 °C, 30 min, 83% over two steps; (vii) $LiAlH_4$, THF, 0 °C to reflux, 16 h, 86%; (viii) aroyl chloride, NEt_3, MeCN, 0 °C–rt, 16 h.



Scheme 3. Reagents and conditions: (i) $NaNO_2$, H_2SO_4, 0–5 °C; (ii) hot H_2O, reflux, 30 min; 70% over two steps; (iii) K_2CO_3, MeI, DMF, 40 °C, 20 h, 94%; (iv) LiOH, THF/H_2O, reflux, 6 h, 87%.



Scheme 4. Reagents and conditions: (i) BBr_3, CH_2Cl_2, 0 °C–rt, 2 days, 84–100%.

hydrolysis of the resultant ester (Scheme 3). The 2-chloro-5-methoxybenzamides **2** and **7** were also successfully demethylated using boron tribromide to afford **19** and **20**, potential compounds for ^{11}C labelling (Scheme 4).

Since lipophilicity of the synthesised amides is central for their use as brain imaging probes, we conducted log*D* (log*P* at $pH_{7.4}$) measurements of these compounds. It is believed that the optimum log*P* value for therapeutic CNS-active compounds is between 2 and 3.5.[26] In this series, the log*D* values were evaluated using reverse-phase C-18 HPLC study by comparison of their average retention times from three injections to that of reference compounds with known log*D* values.[27] All measurements were performed on the same day to minimise day-to-day variations and acetone was used to determine the void volume of the column (1.72 min). The average retention time from three injections was recorded for each of the selected six reference compounds and the results are summarised in Table 2.

An interesting observation in Table 2 is that whilst the *C*log*P* prediction for cubane derivatives **7**–**9** was quite accurate, it was not the case for the adamantane derivatives **2** and **5**–**6**. In contrast, the log*P* prediction was excellent for adamantane derivatives but not for the cubanes. Nevertheless, both *C*log*P* prediction and the measured log*D* from reverse-phase HPLC gave values greater than 4 for the adamantane derivatives, indicating that these compounds are probably less suitable for crossing the blood–brain barrier. On the other hand, the measured log*D* values of cubane derivatives **7**–**9** were between 2 and 3. These values are considered to be within the optimum range for potential PET radioligands ($2 < \log D < 3.5$) and therefore are suitable for brain imaging probes.[26]

Table 2
Lipophilicity measurements for amides **2** and **5**–**9**

Compound	Experimental log*D*	ChemDraw® log*P*	ChemDraw® *C*log*P*
2	4.07 ± 0.01	4.12	5.40
5	4.03 ± 0.01	3.84	5.11
6	3.81 ± 0.01	3.84	5.52
7	2.90 ± 0.01	1.94	2.67
8	2.72 ± 0.01	1.66	2.39
9	2.61 ± 0.01	1.66	2.80

The $P2X_7$ antagonistic properties of the polycyclic amides **2** and **5**–**9** were subsequently tested by measuring the decrease in the fluorescent propidium iodide dye uptake in rat spinal cord microglial cells stimulated by 2′(3′)-*O*-(4-benzoylbenzoyl)adenosine 5′-triphosphate (BzATP). To the best of our knowledge, this demonstrates the first $P2X_7$ functional studies performed on a native, activated rat microglia system with all other purinergic receptors intact. The cell types used for examining $P2X_7$ functionality in the literature so far include: human THP-1 cells, HEK293 cells expressing either human or rat $P2X_7R$, U373 cells expressing human $P2X_7R$ and human 1321N1 astrocytoma cells devoid of endogenous P2X function expressing either human or rat $P2X_7R$.[4,16–19,28–30] As shown in Figure 2, all the six polycyclic amides **2** and **5**–**9** exhibit a degree of antagonism on the rat $P2X_7R$ at 1 μM concentration. Whilst IC_{50} values are yet to be determined, it is noteworthy that the $P2X_7R$ antagonistic properties are retained upon alteration of both the aromatic and polycyclic moieties in a wild-type $P2X_7R$ source.

Extensive screening for binding to other common neuroreceptors was undertaken to ensure the specificity and selectivity of amides **2** and **5**–**9** towards the $P2X_7R$. Receptor-binding profiles were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program.[31] The receptor profiling studies indicated that none of the compounds **2** and **5**–**9** exhibited appreciable binding to the many neuroreceptor subtypes assayed (see Supplementary information for details). In addition, functional assays on $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptors expressed in HEK293 cells using ATP as the agonist at a concentration of 4 mM and drug concentrations of 10 μM, 3 μM, 1 μM, 300 nM, 100 nM, 10 nM and 1 nM indicated that none of the compounds **2** and **5**–**9** had activity at the assayed metabotropic purinergic receptors. The target structures and related compounds have been judiciously chosen, taking into consideration the desired lipophilicity range and ability to radiolabel, and can be readily accessed by concise synthetic routes from commercially available starting materials, employing well-precedented and reliable synthetic methods. The chemical synthesis and functional assays of related compounds will be conducted in parallel as part of a continuous and convergent assessment process to obtain derivatives with optimum $P2X_7$ activity (work in progress).

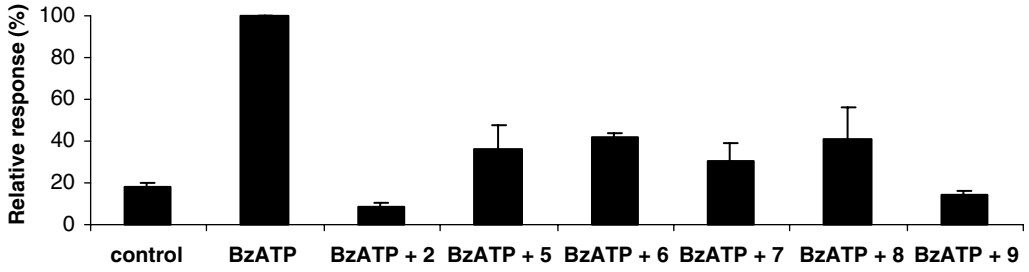


Figure 2. $P2X_7$ functional assay of amides **2** and **5**–**9**. All experiments were performed using 150 μM BzATP and 1 μM of the antagonists. Average reading from four replicates and standard errors are shown.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.062.

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