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Hit-to-Lead Studies: The Discovery of Potent Adamantane Amide $P2X_7$ Receptor Antagonists

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Abstract—A Hit-to-Lead optimisation programme was carried out on the adamantane high throughput screening hit **1** resulting in the discovery of a number of potent $P2X_7$ antagonists.
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The use of High Throughput Screening (HTS) is now widespread in the pharmaceutical industry. There was an expectation that once a screen was established for a particular target then potent lead compounds or candidate drugs would be found. The reality is often far from this. Bridging the gap between the end of a HTS and the start of a full Lead Optimisation (LO) project has been described as Hit-to-Lead (HtL).[1] Hits from HTS are profiled and compared to a generic target lead criteria. The lead target profile used is shown in Figure 1. Lead series then have a balance of properties — potency and SAR as well as an encouraging metabolic and selectivity profile — such that rapid (less than two years) further optimisation should provide Candidate Drugs (CDs) suitable for progression into clinical development.

Potency $pA_2 > 7.0$
Rat Hepatocytes clearance < 14μL/min/10^6 cells
Human liver Microsomes clearance < 23μL/min/mg
Rat iv Clearance < 35mL/min/kg, Vss > 0.5L/kg, $T_{1/2} > 0.5$hr
Molecular Weight < 450, clogP < 3.0, logD < 3.0

Figure 1. Hit-to-Lead generic lead target profile.

The $P2X_7$ receptor is a ligand-gated ion channel present on a variety of cell types involved in the inflammatory/immune process, specifically macrophages, mast cells and lymphocytes (T and B).[2–5] Activation of the $P2X_7$ receptor by the extracellular nucleotide adenosine triphosphate (ATP), leads to the processing and release of the proinflammatory cytokine interleukin-1β (IL-1β) from monocytes and macrophages,[6] degranulation of mast cells and the shedding of surface molecules, L-selectin and CD23 from lymphocytes.[7] $P2X_7$ receptors are also located on antigen-presenting cells, keratinocytes, salivary acinar cells and hepatocytes. It was desirable to make compounds effective as $P2X_7$ receptor antagonists for use in the treatment of chronic inflammatory diseases where the $P2X_7$ receptor may mediate the release of IL-1β. Recently, evidence for the role of this receptor in disease processes was published; $P2X_7$-KO mice showing a reduced severity in an anti-collagen antibody arthritis model.[8]

Few classes of $P2X_7$ antagonists are known in the literature. KN-62, a bis-isoquinolinesulphonyltyrosine derivative, was first described by Gargett and Wiley[9] and has an IC_{50} of 51 nM (see Fig. 2). Subsequently various groups have explored this area and have found more potent analogues such as MRS2306 (IC_{50} 40 nM)[10] and a phenylpiperazine derivative (IC_{50} 1 nM).[11] Whilst being interesting and useful tools to investigate the biology of $P2X_7$ antagonism, they do not represent good starting points for the discovery of oral drugs. These compounds have large molecular weights (> 700),

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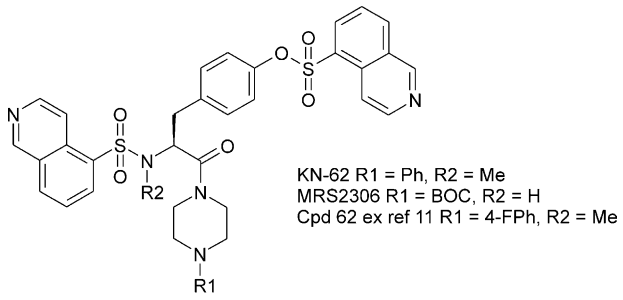


Figure 2. KN-62 and analogues.

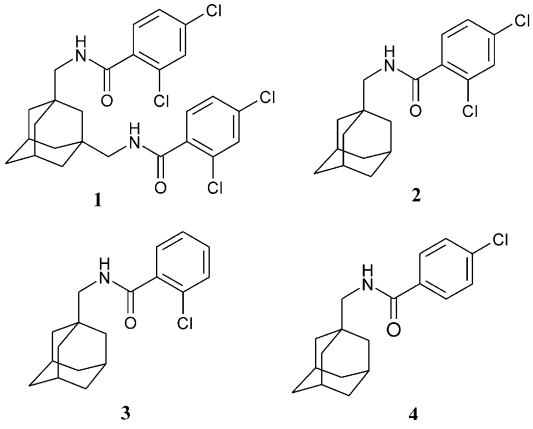
are very lipophilic (clogP >6) and would be expected to be metabolically labile by virtue of the essential sulphonate group. The published SAR[10] indicates that the amide, sulphonamide and sulphonate are all essential for potency and so the transformation of KN-62 analogues into compounds with drug-like properties[12] would be very challenging.

The biological screen for the assessment of $P2X_7$ receptor inhibition was the reduction of plasma membrane pore formation in a monocytic cell line stimulated with the synthetic ATP analogue, benzoylbenzoyl adenosine triphospate (BzATP). This characteristic of $P2X_7$ receptor activation was measured by the entry of the fluophore, ethidium bromide through the membrane pores with a consequent increase in total cellular fluorescence.[13] A HTS was undertaken using this assay in 96-well plate format and the adamantane **1** was identified.[14] Potency estimates for the compounds were initially made from pIC_{50} determinations and then more robustly with pA_2 estimates.

The profile of the adamantane **1** is shown in Table 1 compared with key lead criteria — potency, molecular weight and lipophilicity. Whilst the potency was acceptable, the molecular weight and lipophilicity were appreciably outside the lead criteria limits. Reduction of these parameters therefore became an essential first step in assessing if adamantanes were to become lead compounds.

The preparation of compounds in this study was carried out using standard synthetic methods (Scheme 1).

Table 1. Profile of adamantanes **1–4**

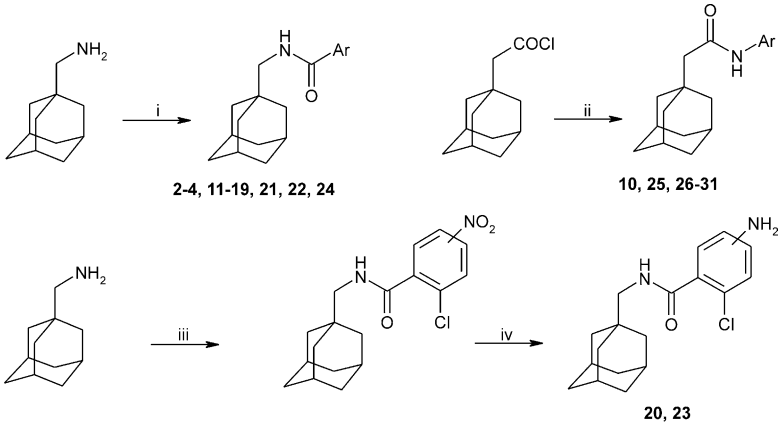


Generic lead criteria	**1**	**2**	**3**	**4**
$P2X_7$ $pA_2>7.0$	6.9	6.4	8.1	NA[a]
$M_r<450$	540	338	304	304
clogP <3.0	6.2	5.2	4.5	5.0

[a]NA = $<50\%$ inhibition at 10 μM.

Commercially available amines and acid chlorides were reacted to give the corresponding amides (**2–6**, **8–19**, **21–22**, **24–33**). Borane reduction of the amide **4** gave amine **7**. The anilines **20** and **23** were prepared by iron ammonium chloride reduction of the corresponding nitro compounds.

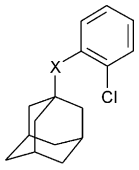
Removal of one of the amide side chains gave **2** which maintained most of the potency of the bisamide **1** but now had acceptable molecular weight. At this time the two mono-chloro amides **3** and **4** were also prepared. Surprisingly, the 2-chloroamide **3** was significantly more potent than amides **1** or **2** and the 4-chloro amide **4** was inactive. It is assumed that the presence of 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 *para* position is not allowed. At this point, variation of the adamantane was undertaken. Many 2-chlorobenzamides were tested from the corporate collection, compound purchase and from the synthesis of combinatorial libraries. No replacements for adamantane were found (data not shown).



Scheme 1. (i) ArCOCl, Et_3N, CH_3CN; (ii) $ArNH_2$, Et_3N, CH_3CN; (iii) ArCOOH, CDI, DMF; (iv) Fe, NH_4Cl.

Amide **3** then became the new starting point for further variation. The nature of the linking group between the adamantane and 2-chlorophenyl ring was investigated (Table 2). *C*- and *N*-methylation, carbonyl reduction and methylene removal (**5**–**8**) all caused a loss of potency. Chain extension (**9**) maintained potency whilst amide reversal (**10**) caused some potency loss. At this point combinatorial libraries were prepared using the reaction of adamantanemethanamine with a large variety of acid chlorides or via standard amide coupling with carboxylic acids. After testing for potency, key compounds were re-prepared and characterised chemically and biologically as solid samples (Table 3). General trends indicated that only aromatic amides had activity as $P2X_7$ antagonists (data not shown). A clear SAR around the benzamide ring was uncovered — a 2-substituent is required (**3**, **12**–**14** vs **11** and **16**) and 4-substitution is deleterious (**2** vs **3**). Amongst the dichlorobenzamides, 2,3 (**16**) and 2,5 (**17**) are better than 2,6 (**18**) with the dichloro compounds either lacking a 2-substituent — 3,5 (**19**)— or with a 4-substituent — 2,4 (**2**)— being much less potent confirming the conclusions drawn from the mono-chloroamides. Extension of the preferred 2,3- and 2,5-substitution pattern to other functional groups gave a range of sub-10 nM antagonists (compounds **16**, **17**, **20**–**24**).

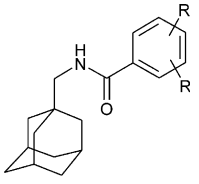
Table 2. $P2X_7$ antagonist potencies—linker variation



	X	$P2X_7$ pA_2[a]
3	CH_2NHCO	8.1
5	CHMeNHCO	6.4 (pIC_{50})
6	CH_2NMeCO	5.4 (pIC_{50})
7	CH_2NHCH_2	5.4
8	NHCO	NA
9	$(CH_2)_2NHCO$	7.8
10	CH_2CONH	6.3 (pIC_{50})

[a]NA, $<50\%$ inhibition at 10 μM.

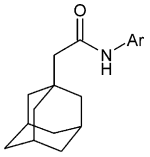
Table 3. $P2X_7$ antagonist potencies—benzamides

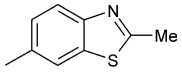
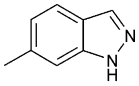
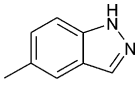


	R	$P2X_7$ pA_2		R	$P2X_7$ pA_2
3	2-Cl	8.1	**17**	2,5-DiCl	8.3
11	H	7.1	**18**	2,6-DiCl	7.4
12	2-Br	8.0	**19**	3,5-DiCl	5.4 (pIC_{50})
13	2-OMe	7.1	**20**	2-Cl,3-NH_2	8.2
14	2-Me	7.9	**21**	2-Cl,5-OMe	8.8
15	3-Cl	7.2	**22**	2-Cl,5-OH	8.0
16	2,3-DiCl	8.8	**23**	2-Cl,5-NH_2	8.4
2	2,4-DiCl	6.4	**24**	2-Cl,6-NH_2	8.3

Even though the reverse amide **10** was less potent than the benzamide **3**, an examination of SAR similarities and differences between the two series was undertaken. Again combinatorial libraries were prepared using standard amide coupling methods between adamantaneacetic acid and a variety of amines. After potency testing, chemical and biological characterisation was undertaken on re-prepared solid samples (Table 4). 2,3- and 2,5-disubstitution was again found to be the most potent as was found previously with the benzamides (**10** and **25** vs **26**–**28**). Larger potency increases were seen though so that again sub-10 nM antagonists were found (compounds **27** and **28**). In addition a separate sub-set of anilides was found based on 6,5-ring systems, the indazole **31** has good antagonist potency.

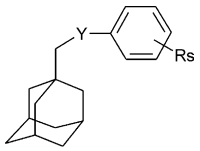
Table 4. $P2X_7$ antagonist potencies—anilides



	Ar	$P2X_7$ pA_2
10	2-ClPh	6.3 (pIC_{50})
25	2-MePh	6.8 (pIC_{50})
26	2-Cl,5-OMePh	7.2
27	2-Me,3-OMePh	8.3
28	2-Me,5-OMePh	8.0
29		6.5
30		5.9
31		7.4

A key part of Hit-to-Lead strategy is to assess and improve the Drug Metabolism and PharmacoKinetic (DMPK) properties at an early stage. With a variety of antagonists with good potency in hand, measurement of intristic clearance (Clint) in vitro using rat hepatocytes and human liver microsomes were carried out. This data should be compared with the lead criteria shown in Figure 1. Results of some of these experiments are presented in Table 5. Not surprisingly, the simple lipophilic benzamides were all rapidly cleared by rat hepatocytes (**3**, **16**, **17**). Confirmation in vivo was carried out for the dichlorobenzamide **16**. Interestingly some of the other 2,3- and 2,5-disubstituted benzamides had better in vitro rat hepatocyte clearance (**20**, **22**, **23**) but, in the case of the best compound (**23** with rat heps Clint 24), this did not translate into any improvement in vivo. A similar picture was observed for the simple anilides **26**–**28**, all having high clearances in vitro. Unexpectedly the indazole amide **31** has reduced in vitro clearance and was the first compound to fulfil the in vitro clearance generic lead criteria. Clearance in vivo for **31** is close to criteria with volume of distribution and half-life estimate inside limits.

Table 5. $P2X_7$ antagonist potencies and DMPK parameters



	Y	Ar subst. Rs	$P2X_7$ pA_2	Rat heps Clint	Hu mics Clint	Rat pharmacokinetics iv		
						Cl	Vss	$T_{1/2}$
3	NHCO	2-Cl	8.1	>100				
16	NHCO	2,3-DiCl	8.8	>100	33	>100	6.2	1.0
17	NHCO	2,5-DiCl	8.3	>100				
20	NHCO	2-Cl,3-NH_2	8.2	56				
22	NHCO	2-Cl,5-OH	8.0	45	88			
23	NHCO	2-Cl,5-NH_2	8.4	24	19	>100	3	0.5
24	NHCO	2-Cl,6-NH2	8.3	>100	>100			
26	CONH	2-Cl,5-OMe	7.2	45	79			
27	CONH	2-Me,3-OMe	8.3	>100	>100			
28	CONH	2-Me,5-OMe	8.0	46	>100			
31	CONH	5-Indazolyl	7.4	5	21	47	2	1.0

From this study the adamantine indazole amide **31** was identified as a lead compound fulfilling most of the target lead criteria. Excellent sub-10 nM potency was also achieved in a number of compounds (e.g., **16**). Poor pharmacokinetic data was a feature of the adamantanes but this could be ameliorated by structural variation as in indazole **31**. The encouragement obtained from this clearance data, in vitro and in vivo, suggested that further improvements could be made and so this study was approved as a full LO project.

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