

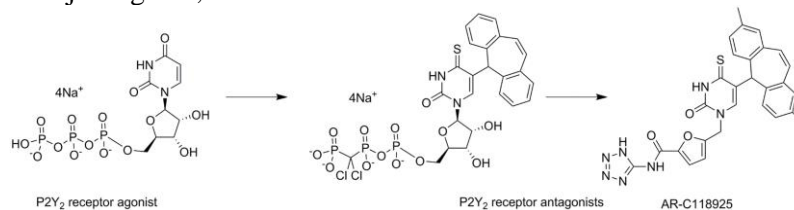
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From UTP to AR-C118925, the Discovery of a Potent non Nucleotide Antagonist of the P2Y₂ Receptor

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From UTP to AR-C118925, the Discovery of a Potent non Nucleotide Antagonist of the P2Y2 Receptor

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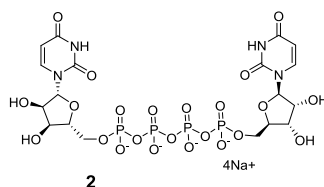
ABSTRACT

The G protein-coupled P2Y₂ receptor, activated by ATP and UTP has been reported as a potential drug target for a wide range of important clinical conditions, such as tumor metastasis, kidney disorders, and in the treatment of inflammatory conditions. However, pharmacological studies on this receptor have been impeded by the limited reported availability of stable, potent and selective P2Y₂R antagonists. This article describes the design and synthesis of AR-C118925, a potent and selective non-nucleotide antagonist of the P2Y₂ receptor discovered using the endogenous P2Y₂R agonist UTP as the chemical starting point.

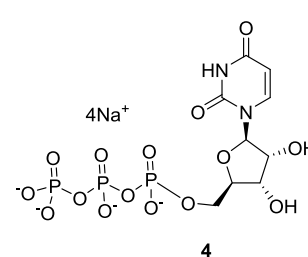
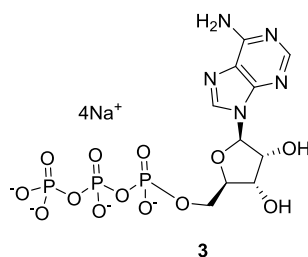
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Purinergic receptors are divided into P1 (adenosine) and P2 (ATP, ADP) receptors,¹ with P2 subdivided into P2X (trimeric ion channels) and P2Y (metabotropic G-coupled receptors).² The eight members of the P2Y family of receptors, so far characterized, have been further subdivided based on their primary signaling through specific coupled G-proteins. The first subgroup P2Y_{1,2,4,6,11} act through G_q and the second group, P2Y_{12,13,14}, through G_i.³ The P2Y₂ receptor (P2Y₂R) has been found in a variety of different tissues and cell types. Cell types include: epithelial cells, endothelial cells, smooth muscles cells and leukocytes. A study using P2Y₂ knockout mice revealed that the receptor mediates 85–95% of nucleotide-stimulated chloride secretion in the trachea. This suggests that P2Y₂R agonists have therapeutic potential as a treatment for cystic fibrosis (CF), as activation of this chloride secretion channel could compensate for the defective chloride secretion in the respiratory epithelium of CF patients.^{4, 5} Indeed, a P2Y₂ agonist, diquafosol **2**, mediating chloride secretion, has been approved in Japan for the topical treatment of dry eye disease.⁶

Agonism of the P2Y₂R can also lead to keratinocyte proliferation and neutrophil migration, indicating that P2Y₂ antagonists have therapeutic potential as a treatment for psoriasis.^{5, 7} It has also been reported that ATP released from tumor-cell activated platelets, acting through the P2Y₂R, induce opening of the endothelial barrier, leading to migration of tumor cells and hence cancer proliferation. P2Y₂R antagonists, therefore have therapeutic potential as anti-metastatic agents.⁸



Despite the appeal of the P2Y₂R as an important drug target, limited reports on P2Y₂R antagonists have appeared to date^{9–11} and indeed the only reported discovery of drug-like P2Y₂R antagonists¹² is from an industrial research group within AstraZeneca, disclosed within a series of chemical patents.^{13,14} One of the most potent and selective of these antagonists, AR-C118925, has been used as a tool for pharmacological studies on the P2Y₂ receptor.^{15,16} More recently, its selectivity profile against a range of P2 receptors has been published. AR-C118925 was at least 50-fold selective against P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₄, P2X₂, P2X₄ and P2X₇, whilst ~ 40-fold against P2X₁ and ~15-fold against P2X₃.¹⁷

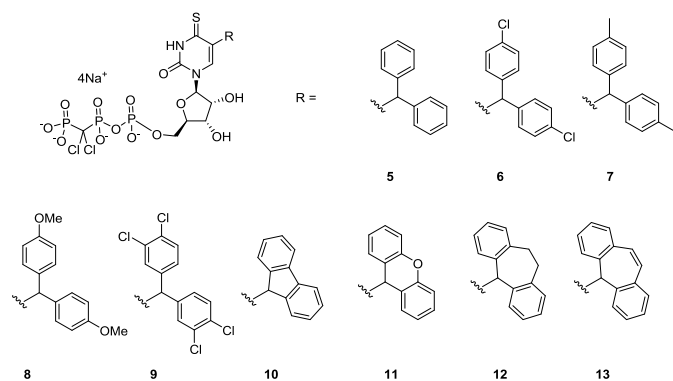


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Herein we report the design and synthesis of P2Y₂R antagonists, which led to the discovery of AR-C118925.

In the absence of a high throughput screening (HTS), the endogenous agonists, adenosine triphosphate (ATP) **3** and uridine triphosphate (UTP) **4** were considered as starting points for the research program. The greater selectivity of UTP for P2Y₂R over the other P2 purinoceptors led to its selection as the chemical starting point. Replacing the βγ-oxygen of the triphosphate moiety of UTP with a dichloromethylene unit and substitution of the oxygen in the 4-position of the uracil with sulfur, gave enhanced metabolic stability, whilst maintaining agonist activity.^{18–20} Interestingly, it was discovered that that P2Y₂R antagonism could be achieved through substitution of the 5-position of the uracil ring with lipophilic substituents (Table 1). The introduction of a benzhydryl group into this position (**5**) gave a compound with a pA₂ for the P2Y₂R of 6. P2Y₂R activity was substantially increased by either symmetrically adding substituents to the benzhydryl group, compounds **6**, **7**, **8**, and **9**, or by linking the two phenyl rings of the benzhydryl to form a tricycle, compounds **10**, **11**, **12**, and **13**, with the highest activity achieved with the dibenzosuberonyl group, compound **13** (pA₂ 8.5).

Table 1: Exploration of the 5-position of the uracil ring



Compound	P2Y ₂ pA ₂ ^{a, b}
5	6.0 ± 0.2
6	7.9 ± 0.2
7	7.8 ± 0.2
8	7.1 ± 0.2
9	7.7 ± 0.2
10	7.2 ± 0.2
11	7.3 ± 0.2
12	8.0 ± 0.2
13	8.5 ± 0.2

a) The assay used a human P2Y₂R clone which was isolated from HL60 cells cDNA and then stably transfected into a Jurkat cell line. The cloned receptor mediates an increase in intracellular calcium in the cell line, which possesses no endogenous nucleotide receptor of its own. Inhibition of UTP mediated calcium responses were measured using 17 ktM fluo-3AM dye on a SPEX Fluomax using 508 nm excitation and 525 nm emission wavelengths at room temperature.^b n ≥ 2 replicates

The P2Y₂R antagonist program ultimately required the development of an oral drug-like compound.²¹ This necessarily meant moving away from the high molecular weight, highly charged substituted nucleotides. Removing the triphosphate group gave the nucleoside analogue **14** (Table 2). Whilst this compound was substantially less potent than its parent triphosphate **13**, it did retain some antagonist activity (pA₂ 4.7). Furthermore, it was discovered that the ribose ring could be replaced with the structurally less complex 3-methylenebenzoic acid to give **15**, with a slight gain in activity. The rationale for having the carboxylic

acid was to mimic any binding interactions the alpha phosphate of UTP might have with the P2Y₂R.

Table 2: Replacement of the ribose-triphosphate group

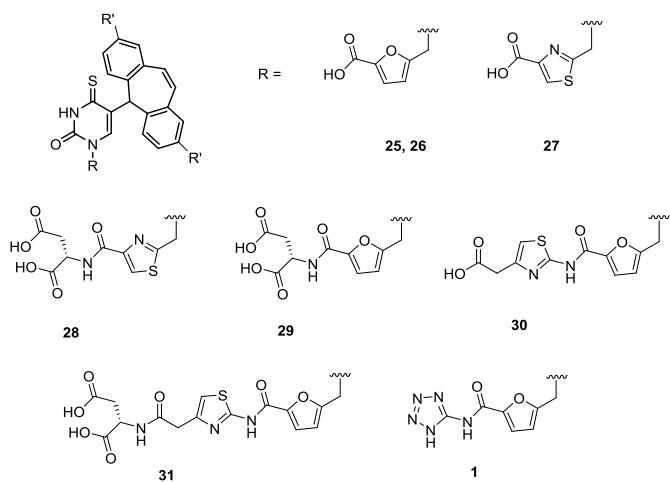
Compound	X	P2Y ₂ pA ₂ ^a
14	S	4.7 ± 0.2
15	S	5.4 ± 0.2
16	S	5.7 ± 0.2
17	S	5.6 ± 0.2
18	S	5.4 ± 0.2
19	S	5.9 ± 0.2
20	S	5.7 ± 0.2
21	O	4.4 ± 0.2
22	S	5.8 ± 0.2
23	S	5.2 ± 0.2
24	S	5.6 ± 0.2

^a n ≥ 2 replicates

Changing the linker between the carboxylic acid and phenyl ring from a direct bond to a one or two atom linker, potentially placing the carboxylic acid group further into the triphosphate binding region, gave a small increase in activity (**16** and **17**). In addition, the P2Y₂R appeared tolerant of a range of hetero aromatic replacements for the phenyl ring. For example, the furanyl analogue **19** had a further slight gain in activity (pA₂ 5.9). The continuing importance of the 4-thiouracil relative to uracil was demonstrated with thiazole replacement, where the uracil analogue was > 10-fold less potent (compare compound **20** with **21**).

Having been able to replace the ribose triphosphate with a structurally less complicated and synthetically less challenging group, P2Y₂R antagonism could be increased through symmetrical substitution on the 5-suberonyl substituent **25**, **26** and **27** (Table 3). This combines the two features of change to the 5-benzhydryl group in the ribose triphosphate series, which independently gave an increase in receptor antagonism (Table 1).

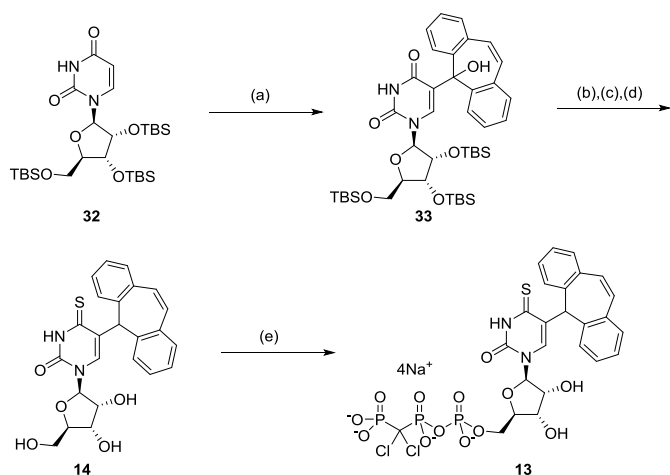
Table 3: Further changes to the substituent on the 1-position of the uracil ring



Compound	R'	P2Y ₂ pA ₂ ^a
25	Me	7.0 ± 0.2
26	Cl	6.7 ± 0.2
27	Me	6.6 ± 0.2
28	Me	7.4 ± 0.2
29	Me	7.6 ± 0.2
30	Me	7.5 ± 0.2
31	Me	7.2 ± 0.2
1	Me	7.8 ± 0.2

^a n=2 replicates

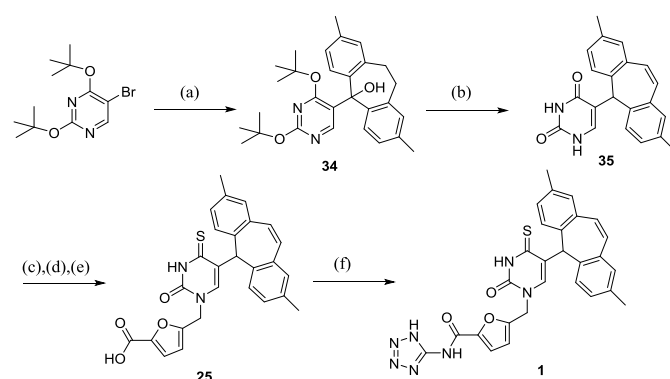
In a similar manner to compounds discussed in Table 2, moving the acidic group further into the triphosphate binding region gave an increase in receptor antagonism. Although the dicarboxylic acids **28** and **29** had similar activity to the mono carboxylic acid **30**, indicating perhaps that only one of the carboxylic acids is involved in binding to the receptor. Finally, bioisosteric replacement of the carboxylic acid was employed giving the most potent compound, AR-C118925 (**1**).



Scheme 1: Representative synthesis of nucleotide analogues (**5-13**). (a) (i) TMEDA (2.2 eq), *sec*BuLi (2.1 eq), THF, -78°C (ii) 5-dibenzosuberone (1.5 eq), THF, 55% (b) (i) Et₃SiH (2 eq), BF₃·Et₂O (2 eq), CH₂Cl₂, 0°C (ii) ^tBuMe₂SiCl (1.5 eq), imidazole (1.5 eq), DMF, 70% (c) P₄S₁₀, pyridine, reflux, 68% (d) TBAF, THF, r.t., 85% (e) (i) 1.3 eq POCl₃, (1.3 eq) proton sponge, PO(OEt)₃ (ii) clodronic acid (3 eq), ⁿBu₃N (9 eq), PO(OEt)₃, 16%.

Scheme 1, which shows the synthesis of compound **13**, illustrates the general procedure for synthesising the compounds in Table 1. Selective lithiation of the 5-position of the uracil ring (**32**) using the method of Miyasaka²² followed by addition of 5-dibenzosuberone gave the 5-dibenzosuberanol (**33**). The alcohol was then reduced using triethylsilane and borontrifluoride etherate. During this reaction, there was some loss of the silyl

protecting groups, giving a mixture of mono, di and tri silylated products. Re-protection using standard procedure gave the trisilylated product (**33**). Thiation of the 4-position of the uracil was achieved using P₄S₁₀ in pyridine under reflux and removal of the silyl protecting groups using tetrabutylammonium fluoride gave the 5-substituted 4-thiouridine (**14**). Phosphorylation of the 5'-position of the ribose was achieved using phosphorus oxychloride followed by treatment with clodronic acid. The crude product was purified using DEAE Sephadex® ion exchange chromatography eluting with a 0 to 1 molar gradient of triethylammonium bicarbonate followed by elution through a Na⁺ form Dowex® ion exchange column to give the sodium salt of the product (**13**).



Scheme 2: Synthesis of AR-C118925. (a) (i) ⁿBuLi, THF, -78°C (ii) 2,8-dimethyldibenzosuberone, THF (b) AcOH, reflux, 96% over two steps (c) (i) BSTFA (2.2 eq), 1,2-dichloroethane, reflux (ii) 5-bromomethyl-2-furancarboxylic acid methyl ester, MeCN, reflux, 53% (d) Lawesson's reagent (1 eq.), 1,4-dioxane reflux, 65% (e) LiOH (5 eq.), 3:1 MeOH:H₂O, r.t., 95% (f) aminotetrazole, (1.5 eq) pybrop (1.5 eq), NEt₃ (3 eq), DMF, 60%.

The synthesis of AR-C118925 is shown in scheme 2. Bromo lithium exchange of 5-bromo-2,4-di-*tert*-butoxypyrimidine²³ followed by treatment with 2,8-dimethyldibenzosuberone gave the corresponding dibenzosuberanol product (**34**). Treatment with acetic acid under reflux gave the 5-substituted uracil (**35**) in very good yield over the two steps. *Bis*-silylation of uracil (**35**) followed by treatment with 5-bromomethyl-2-furancarboxylic acid methyl ester, selectively alkylated in the 1-position of the uracil. Treatment with Lawesson's reagent then effected the conversion to the 4-thio uracil. Hydrolysis of the methyl ester gave the carboxylic acid (**25**) in good yield. Activation of the carboxylic acid with bromo-*tris*(pyrrolidino)phosphonium hexafluorophosphate and coupling with 1-aminotetrazole gave AR-C118925 (**1**).

In conclusion, a potent and selective non nucleotide P2Y₂R antagonist, AR-C118925, was discovered using the endogenous agonist UTP as the chemical starting point for the program. AR-C118925 has reasonable physicochemical properties for a tool compound (MWt 537, LogD_{7.4} of 2.26) and was shown to have a reasonable rat *in vivo* pharmacokinetics [*i.v.* clearance (75 mL/min/Kg), V_{ss} 4.34 L/Kg, T_{1/2} 2.12 h] but was not bioavailable (F 0%). This approach of starting with the endogenous ligand has also been used in the P2Y₁₂ program²⁴ and complimented the alternative, HTS – Active to Hit, Hit to Lead approach to initiating receptor agonist/antagonist projects at AstraZeneca.²⁵⁻²⁷ Utilization of AR-C118925 in the chemical literature supporting future potential clinical applications for P2Y₂R antagonists has necessitated this present disclosure and we will be reporting further studies in due course.

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References and notes

- (1) Burnstock, G. Purinergic Nerves. *Pharmacol. Rev.* **1972**, *24* (3), 509–581.
- (2) Burnstock, G.; Kennedy, C. Is There a Basis for Distinguishing Two Types of P₂-Purinoreceptor? *Gen. Pharmacol.* **1985**, *16* (5), 433–440.
- (3) Alexander, S.; Mathie, A.; Peters, J. Guide to Receptors and Channels (GRAC), 5th Edition. *Br. J. Pharmacol.* **2011**, *164* (November), S1–S2.
- (4) Cressman, V. L.; Lazarowski, E.; Homolya, L.; Boucher, R. C.; Koller, B. H.; Grubb, B. R. Effect of Loss of P₂Y₂ Receptor Gene Expression on Nucleotide Regulation of Murine Epithelial Cl⁻ Transport. *J. Biol. Chem.* **1999**, *274* (37), 26461–26468.
- (5) Yerxa, B. R. Pharmacology of INS37217 [P₁-(Uridine 5')-P₄- (2'-deoxycytidine 5')tetraphosphate, Tetrasodium Salt], a Next-Generation P₂Y₂ Receptor Agonist for the Treatment of Cystic Fibrosis. *J. Pharmacol. Exp. Ther.* **2002**, *302* (3), 871–880.
- (6) Keating, G. M. Diquafosol Ophthalmic Solution 3 %: A Review of Its Use in Dry Eye. *Drugs* **2015**, *75* (8), 911–922.
- (7) Chen, Y.; Corriden, R.; Inoue, Y.; Yip, L.; Hashiguchi, N.; Zinkernagel, A.; Nizet, V.; Insel, P. A.; Junger, W. G. ATP Release Guides Neutrophil Chemotaxis via P₂Y₂ and A₃ Receptors. *Science* (80-.). **2006**, *314* (5806), 1792–1795.
- (8) Schumacher, D.; Strilic, B.; Sivaraj, K. K.; Wetttschurek, N.; Offermanns, S. Platelet-Derived Nucleotides Promote Tumor-Cell Transendothelial Migration and Metastasis via P₂Y₂ Receptor. *Cancer Cell* **2013**, *24* (1), 130–137.
- (9) Weyler, S.; Baqi, Y.; Hillmann, P.; Kaulich, M.; Hunder, A. M.; Müller, I. A.; Müller, C. E. Combinatorial Synthesis of Anilinoanthraquinone Derivatives and Evaluation as Non-Nucleotide-Derived P₂Y₂ Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18* (1), 223–227.
- (10) Kaulich, M.; Streicher, F.; Mayer, R.; Müller, I.; Müller, C. E. Flavonoids - Novel Lead Compounds for the Development of P₂Y₂ Receptor Antagonists. *Drug Dev. Res.* **2003**, *59* (1), 72–81.
- (11) Sauer, R.; El-Tayeb, A.; Kaulich, M.; Müller, C. E. Synthesis of Uracil Nucleotide Analogs with a Modified, Acyclic Ribose Moiety as P₂Y₂ Receptor Antagonists. *Bioorg. Med. Chem.* **2009**, *17* (14), 5071–5079.
- (12) Conroy, S.; Kindon, N.; Kellam, B.; Stocks, M. J. Drug-like Antagonists of P₂Y Receptors—From Lead Identification to Drug Development. *J. Med. Chem.* **2016**, *59* (22), 9981–10005.
- (13) Kindon, N.; Meghani, P.; Thom, S. N. C. Novel Compounds. WO1999002501 A1 1998.
- (14) Kindon, N.; Meghani, P.; Thom, S. N. C. Novel Compounds. WO1999002501 A1 1999.
- (15) Kemp, P. A.; Sugar, R. A.; Jackson, A. D. Nucleotide-Mediated Mucin Secretion from Differentiated Human Bronchial Epithelial Cells. *Am. J. Respir. Cell Mol. Biol.* **2004**, *31* (4), 446–455.
- (16) Cosentino, S.; Banfi, C.; Burbiel, J. C.; Luo, H.; Tremoli, E.; Abbracchio, M. P. Cardiomyocyte Death Induced by Ischaemic/hypoxic Stress Is Differentially Affected by Distinct Purinergic P₂ Receptors. *J. Cell. Mol. Med.* **2012**, *16* (5), 1074–1084.
- (17) Rafehi, M.; Burbiel, J. C.; Attah, I. Y.; Abdelrahman, A.; Müller, C. E. Synthesis, Characterization, and in Vitro Evaluation of the Selective P₂Y₂ Receptor Antagonist AR-C118925. *Purinergic Signal.* **2017**, *13* (1), 89–103.
- (18) Mohamady, S.; Jakeman, D. L. An Improved Method for the Synthesis of Nucleoside Triphosphate Analogues. *J. Org. Chem.* **2005**, *70* (25), 10588–10591.
- (19) El-Tayeb, A.; Qi, A.; Müller, C. E. Synthesis and Structure–Activity Relationships of Uracil Nucleotide Derivatives and Analogues as Agonists at Human P₂Y₂, P₂Y₄, and P₂Y₆ Receptors. *J. Med. Chem.* **2006**, *49* (24), 7076–7087.
- (20) El-Tayeb, A.; Qi, A.; Nicholas, R. A.; Müller, C. E. Structural Modifications of UMP, UDP, and UTP Leading to Subtype-Selective Agonists for P₂Y₂, P₂Y₄, and P₂Y₆ Receptors. *J. Med. Chem.* **2011**, *54* (8), 2878–2890.
- (21) Leeson, P. D.; Springthorpe, B. The Influence of Drug-like Concepts on Decision-Making in Medicinal Chemistry. *Nat. Rev. Drug Discov.* **2007**, *6* (11), 881–890.
- (22) Hayakawa, H.; Tanaka, H.; Obi, K.; Itoh, M.; Miyasaka, T. A Simple and General Entry to 5-Substituted Uridines Based on the Regioselective Lithiation Controlled by a Protecting Group in the Sugar Moiety. *Tetrahedron Lett.* **1987**, *28* (1), 87–90.
- (23) Lerch, U.; Burdon, M. G.; Moffatt, J. G. C-Glycosyl Nucleosides. I. Synthesis of Pseudouridine and Related Compounds. *J. Org. Chem.* **1971**, *36* (11), 1507–1513.
- (24) Springthorpe, B.; Bailey, A.; Barton, P.; Birkinshaw, T. N.; Bonnett, R. V.; Brown, R. C.; Chapman, D.; Dixon, J.; Guile, S. D.; Humphries, R. G.; Hunt, S. F.; Ince, F.; Ingall, A. H.; Kirk, I. P.; Leeson, P. D.; Leff, P.; Lewis, R. J.; Martin, B. P.; McGinnity, D. F.; Mortimore, M. P.; Paine, S. W.; Paireudeau, G.; Patel, A.; Rigby, A. J.; Riley, R. J.; Teobald, B. J.; Tomlinson, W.; Webborn, P. J. H.; Willis, P. a. From ATP to AZD6140: The Discovery of an Orally Active Reversible P₂Y₁₂ Receptor Antagonist for the Prevention of Thrombosis. *Bioorg. Med. Chem. Lett.* **2007**, *17* (21), 6013–6018.
- (25) Baxter, A.; Cooper, A.; Kinchin, E.; Moakes, K.; Unitt, J.; Wallace, A. Hit-to-Lead Studies: The Discovery of Potent, Orally Bioavailable Thiazolopyrimidine CXCR2 Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2006**, *16* (4), 960–963.
- (26) Baxter, A.; Brough, S.; Cooper, A.; Floettmann, E.; Foster, S.; Harding, C.; Kettle, J.; McNally, T.; Martin, C.; Mobbs, M.; Needham, M.; Newham, P.; Paine, S.; St-Gallay, S.; Salter, S.; Unitt, J.; Xue, Y. Hit-to-Lead Studies: The Discovery of Potent, Orally Active, Thiophenecarboxamide IKK-2 Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14* (11), 2817–2822.
- (27) Baxter, A.; Bent, J.; Bowers, K.; Braddock, M.; Brough, S.; Fagura, M.; Lawson, M.; McNally, T.; Mortimore, M.; Robertson, M.; Weaver, R.; Webborn, P. Hit-to-Lead Studies: The Discovery of Potent Adamantane Amide P₂X₇ Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2003**, *13* (22), 4047–4050.