Drug-like antagonists of P2Y receptors - from lead identification to drug development

Sean Conroy, Nicholas Kindon, Barrie Kellam, Michael J. Stocks*

Centre for Biomolecular Sciences, University Park Nottingham, Nottingham, NG7 2RD, UK.

Abstract

P2Y receptors are expressed in virtually all cells and tissue types and mediate an astonishing array of biological functions including platelet aggregation, smooth muscle cell proliferation and immune regulation. The P2Y receptors belong to the GPCR superfamily and are composed of eight members encoded by distinct genes that can be subdivided into two groups based on their coupling to specific G-proteins. Extensive research has been undertaken to find modulators of P2Y receptors, although to date there have only been a limited number of small molecule P2Y receptor antagonists that have been approved by drug/medicines agencies. This Perspective reviews the known P2Y receptor antagonists, highlighting oral drug-like receptor antagonists, and considers future opportunities for the development of small molecules for clinical evaluation.

Introduction

Although nucleotides, such as adenosine-5'-triphosphate (ATP) and uridine triphosphate (UTP) are important intracellular molecules, they are also released into extracellular fluids by various mechanisms. The receptors for these extracellular nucleotides have been progressively characterized and classified as purinergic receptors.[1] The subdivision of these purinergic receptors into P1 (adenosine) and P2 (ATP, ADP) was originally proposed by Burnstock in 1978[2] and further subdivision of the P2 receptors between P2X and P2Y was made in 1985.[3] It is now well established that signalling by extracellular nucleotides is mediated by two families of receptors, where P2Y receptors are metabotropic G protein-

coupled receptors (GPCRs) and P2X receptors are trimeric ion channels.[4] The P2Y receptors belong to the GPCR superfamily and possess the characteristic molecular topology of an extracellular *N*-terminus, an intracellular *C*-terminus and seven transmembrane spanning loops.[5] However, in comparison to other GPCR families, P2Y receptors show limited sequence homology; possibly explaining the differences in ligand selectivity and specificity for the relevant G-protein they signal through. In addition, P2Y receptors are found to be expressed in virtually all cells, in all tissue types and mediate an astounding array of biological functions including platelet aggregation, smooth muscle cell proliferation and immune regulation. The discovery of oral, drug-like[6-7] compounds with favourable ADME (absorption, distribution, metabolism, and excretion) properties[8] for these receptors, specifically antagonists of the $P2Y_{12}$ receptor that have proven therapeutic benefit in various disease states, could address many unmet areas of clinical need.[9]

The P2Y family is composed of eight members encoded by distinct genes that can be subdivided into two groups based on their primary signalling through specific coupled G-proteins. From a phylogenetic and structural (i.e. protein sequence) perspective, two distinct P2Y receptor sub-groups have been characterized by the relatively high level of sequence divergence. The first sub-group includes the $P2Y_{1,2,4,6,11}$ receptors and the second sub-group encompasses the $P2Y_{12,13,14}$ receptor subtypes. In addition, the various P2Y receptors differ by their selectivity for distinct nucleotides (Table 1). $P2Y_{11}$ is an ATP receptor, whereas $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ are ADP receptors. $P2Y_4$ and $P2Y_6$ are pyrimidine nucleotide receptors activated by UTP and UDP respectively. $P2Y_2$ is a dual ATP and UTP receptor. $P2Y_{14}$ is a receptor for UDP-glucose and other nucleotide sugars as well as for UDP itself.

Table 1: Properties of human P2Y receptors.

Receptor	**Tissue distribution**	**Agonist**	**G Protein**
$P2Y_1$	wide, including platelets, CNS, heart, skeletal	ADP	G_q

	muscle, GI tract		
$P2Y_2$	wide, including lung, heart, skeletal muscle, kidney, brain	ATP=UTP	G_q
$P2Y_4$	placenta, lung, vascular smooth muscle	UTP	G_q
$P2Y_6$	wide, including brain, lung, heart, placenta, spleen, intestine	UDP	G_q
$P2Y_{11}$	spleen, intestine, dendritic cells	ATP	$G_q + G_s$
$P2Y_{12}$	platelets, brain	ADP	G_i
$P2Y_{13}$	brain, spleen	ADP	G_i
$P2Y_{14}$	wide including brain, heart, adipose tissue, placenta, intestine and hematopoietic cells	UDP-glucose/UDP	G_i

For further information relating to the specificity of P2YRs see Alexander *et al*[10]

This perspective aims to examine the various approaches used to discover drug-like antagonists of P2Y receptors and builds on recent reviews in the area concentrating on P2Y receptor agonists and the early nucleotide containing modulators.[11] Recently, X-ray crystal structures of ligand-bound $P2Y_1$[12] and $P2Y_{12}$[13-14] receptors have been published. It is hoped that structural information resulting from these disclosures can assist in the design of new modulators for P2Y receptor subtypes, as many of the reported modulators still lack the potency and selectivity for viable chemical drug-like leads. Oral drug design has been hampered by the importance of retaining the negatively charged phosphates of nucleotides for their interaction with the P2Y receptors. This presents huge issues for drug development because of their metabolic instability, low bioavailability, nonspecific binding to biological membranes, and chemically due to their demanding purification. To date, the only clinically proven role for P2Y receptor antagonists lays in the antithrombotic area, where extracellular nucleotide signalling has been shown to be involved in platelet aggregation. Extracellular ADP has a crucial role in platelet activation through activation of cell surface $P2Y_1$ and $P2Y_{12}$ receptors, resulting in adherence to sites of damage when blood vessel injury occurs. In so

doing, platelets secrete several factors that initiate clot formation and recruit other platelets to the point of injury. Based on this, several antagonists for each of these receptors show anti-thrombotic activities. However, to date only $P2Y_{12}$ receptor antagonists have been successfully developed for clinical use.

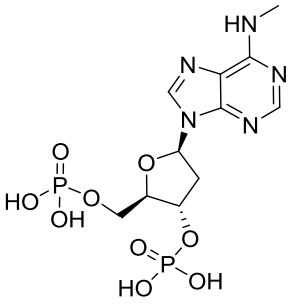
The development of clinically relevant $P2Y_{12}$ receptor antagonists and further case histories for the discovery of other P2Y receptor subtype antagonists will be presented in this perspective. Each sub-section, containing a brief introduction to the underlying pharmacology and potential clinical benefit for the P2Y receptor antagonist, is followed by a detailed study of the search for effective orally bioavailable, drug-like molecules.

Drug-like antagonists of the $P2Y_1$ receptor ($P2Y_1R$)

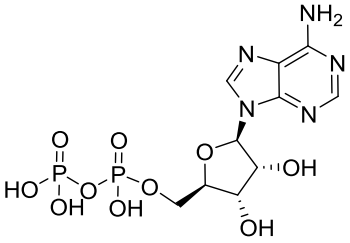
Advances in platelet biology[15] revealed that ADP played a crucial role in platelet function. Therefore, receptor antagonists of the two ADP receptors, $P2Y_1$ and $P2Y_{12}$, may provide antithrombotic efficacy[16] with reduced bleeding liabilities. Through a G_i-dependent pathway, $P2Y_{12}R$ activation is responsible for mediating the inhibition of adenylyl cyclase, which sustains platelet aggregation. Through a G_q-dependent pathway, $P2Y_1R$ activation is responsible for calcium mobilisation, which causes platelet shape change and initiates reversible aggregation.[17] It has been demonstrated in pre-clinical studies using $P2Y_1$-deficient ($P2Y_1^{-/-}$) mice, and by using selective nucleotide-derived $P2Y_1R$ antagonists such as **1** (MRS2179)[18] (Figure 1), that a complete blockade of ADP-induced platelet aggregation occurs leading to an effective reduction of arterial thrombosis with a moderate prolongation of the bleeding time.[19-20,21,22] Inhibition of the $P2Y_1R$ is therefore a promising approach for the discovery of new antithrombotic drugs.[23-24]

In the search for selective antagonists of the $P2Y_1R$, structural modifications were made on the natural agonist **2** (adenosine diphosphate, ADP). In the first reported work in this field, building on the observation that **3** was a weak partial agonist,[25] **4** was shown to be an antagonist of the $P2Y_1R$.[25] Substitution of the ribose with an acyclic group afforded **5**, a

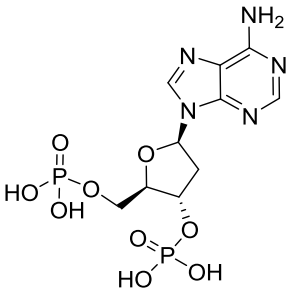
potent bisphosphate antagonist (Figure 1).[26] These compounds proved useful tools for interrogating the scope of modulating the $P2Y_1R$. However, their pharmacokinetic properties may not make them ideal oral, drug-like candidates. A series of adenosine derivatives **5a-b** were shown to have weak antagonist activity when linked to aspartic acid through a carbamate.[27] Further studies in these areas delivered **6** (MRS2279) and **7** (MRS2500)[28] as useful tool compounds to probe the mechanism and highlight the potential clinical significance of $P2Y_1R$ antagonism.[29]



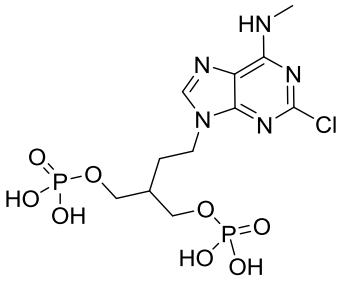
1 *h*$P2Y_1$ IC_{50} 0.33 μM



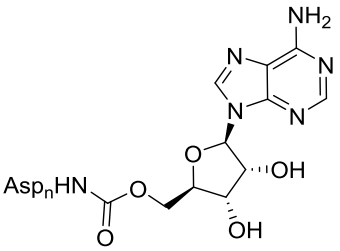
2



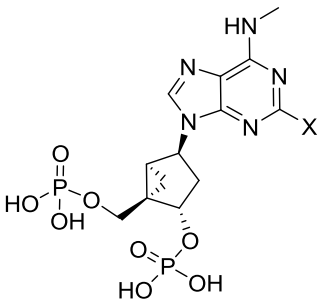
3 *h*$P2Y_1$ EC_{50} 6.3μM; IC_{50} 5.8μM



4 *h*$P2Y_1$ IC_{50} 0.84 μM



5a-b *h*$P2Y_1$ n = 1-2
pA_2 = 5.4 for n = 2

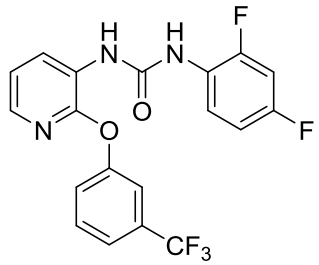


6 X=Cl
7 X=I

Figure 1: The natural agonist **2** (ADP) and a selection of nucleoside-based $P2Y_1R$ antagonists.

The following section focuses on the study of $P2Y_1R$ antagonists specifically aimed at the identification and development of oral drugs.

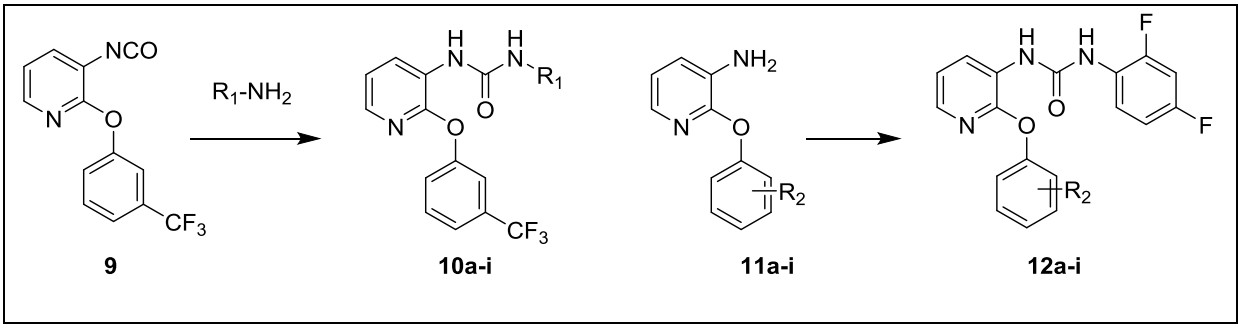
Scientists at Bristol-Myers Squibb (BMS) have reported the discovery and development of a series of potent, selective and bioavailable diarylurea $P2Y_1R$ antagonists. In their discovery program a high throughput screening (HTS) effort, using >1 million compounds against the human $P2Y_1R$, revealed that diarylurea **8**[30] had good affinity and binding selectivity toward the $P2Y_1R$ versus $P2Y_{12}R$ ($P2Y_1$ K_i = 75 nM, $P2Y_{12}$ K_i > 70 μM).



8

In light of its profile, compound **8** was chosen as the starting point for further modification. The hit series was amenable to rapid exploration by parallel synthesis, initially through substitution of the phenyl ring attached to the urea group. In this synthetic strategy, isocyanate **9** was reacted with a diverse array of 96 amines to afford a series of urea derivatives (such as **10a-i**) that rapidly generated a structure-activity relationship (SAR). To explore substitution of the phenyl ether group, the 2,4-difluorophenyl urea was kept constant and a range of substituted 3-aminopyridines **11a-i** were condensed with 2,4-difluorophenyl isocyanate, key results from these arrays are summarized in Table 2.

Table 2: Parallel synthesis and exploration of initial hit **8**.

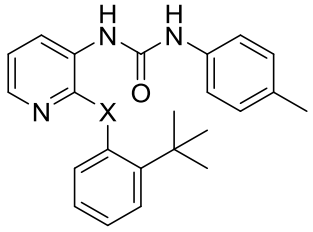


Compound	R_1	*h*$P2Y_1$ K_i	Compound	R_2	*h*$P2Y_1$ K_i

		(nM)[a]			(nM)[a]
10a	Ph	171	**12a**	3-CF_3	75
10b	2-F-Ph	47	**12b**	H	2000
10c	2-Cl-Ph	>5000	**12c**	2-*t*-Bu	18
10d	3-F-Ph	607	**12d**	3-*t*-Bu	33
10e	4-Cl-Ph	69	**12e**	2-*i*-Pr	28
10f	4-MePh	48	**12f**	2-Et	77
10g	4-OCF_3-Ph	313	**12g**	2-CF_3	172
10h	CH_2Ph	>5000	**12h**	4-CF_3	>5000
10i	CH_2CH_2Ph	>5000	**12i**	4-Me	>5000

[a]Measured by displacement of [β^{-33}P]-2-MeS-ADP binding to cloned *h*P2Y_1 receptors

From this work, it was demonstrated that mono-substitution was preferred for activity at the distal aromatic group (R_1) and that aliphatic substituents were not tolerated in this position. Data also supported the concept that lipophilicity at the *ortho*-position of the phenyl ether (R_2) was required for affinity. The importance of the ether link between the phenyl and pyridyl rings was also explored by replacing it with either a thio-ether or amino variants. Whilst substitution with sulphur afforded a 3-fold drop in affinity, replacement with nitrogen resulted in a 20-fold loss, and *N*-methylation resulted in a more significant loss (Figure 2).

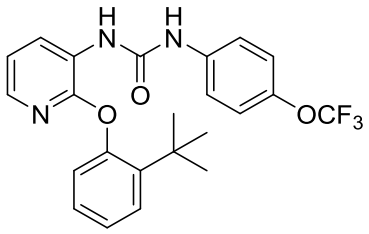


X = O *h*P2Y_1 K_i = 7 nM
X = S *h*P2Y_1 K_i = 20 nM
X = NH *h*P2Y_1 K_i = 175 nM
X = NMe *h*P2Y_1 K_i > 5000 nM

Figure 2: Structure activity relationship for the replacement of the oxygen atom with sulfur or nitrogen.

A follow-on array was then synthesized using a combination of eight preferred substituents from the phenyl ether group and the aromatic ring attached to the urea group. Using a

human platelet rich plasma assay (*h*PRP), these compounds demonstrated a clear trend that combination of a lipophile at the *ortho*-position alongside substitution of the phenyl urea at the *para*-position gave receptor antagonists, with several compounds displaying micro-molar activity. In order to ensure that the anti-platelet activity was due exclusively to $P2Y_1R$ antagonism, all compounds were tested for antagonism at other P2Y receptors such as $P2Y_2$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{14}$. Compounds with good *in vitro* $P2Y_1R$ binding affinity and functional activity were evaluated to determine their metabolic stability. From these studies, compound **13** (BPTU) showed reasonable metabolic stability in rats and was progressed to an *in vivo* rat pharmacokinetic (PK) study. This compound showed modest bioavailability (F = 18%) when dosed at 30 mg/kg and the compound was subsequently progressed to a rat antithrombotic assay (Figure 3).



13
h$P2Y_1$ K_i = 6 nM
*h*PRP IC_{50} = 2.1 μM
Rat PK
CL 16 mL/min/kg; V_{ss} 5.6 L/kg; $T_{1/2}$ 15.4 h, F 7% (1 mg/kg i.v.)
CL 13 mL/min/kg; V_{ss} 0.8 L/kg; $T_{1/2}$ 1.4 h, F 18% (30 mg/kg p.o.)

Figure 3: Rat *in vivo* PK study on compound **13.** V_{ss} is the volume of distribution at steady-state.

Compound **13** clearly improved blood flow and reduced thrombus weight in a dose dependent manner showing a maximum of 68 ± 7% thrombus weight reduction compared to vehicle using a 10 mg/kg bolus followed by 10 mg/kg/h infusion. *Ex vivo* platelet aggregation responses to ADP were also significantly inhibited at all antithrombotic doses.

Diarylurea-containing compounds of the type shown (Figures 2-3) are generally associated with poor solubility at physiological pH. BMS scientists addressed this issue by addition of amine-containing, water-solubilizing groups (R_3) onto the distal phenyl ring (Figure 4).[31]

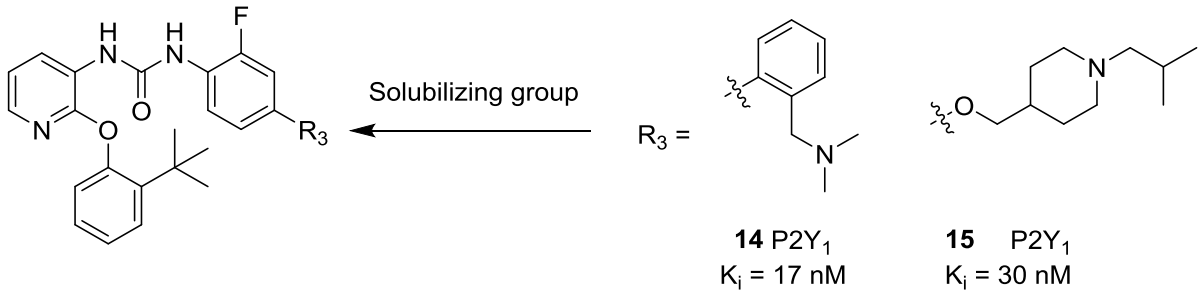


Figure 4: Incorporation of an amine as a water-solubilizing group to improve physico-chemical properties of the urea derivatives.

This afforded a series of compounds, as illustrated by **14** and **15**, with comparable binding affinities when compared to the parent **13**. Compound **14** was chosen as the new lead compound as it possessed similar levels of activity in the platelet aggregation assay (IC_{50} 2.9 μM at 25 μM ADP) but had significantly higher solubility. However, further profiling of **14** showed it was metabolically unstable in rat and human liver microsomes and displayed high inhibition against several GPCR targets [ca. 90% inhibition (at 10 μM) 5-HT2A, 5-HTT, α-2A adrenoreceptor and α-1D adrenoreceptor]. Further investigation revealed **15** as a more metabolically-stable compound with enhanced rat bioavailability (F = 40%), due in part to the increase in aqueous solubility. Unfortunately, **15** also had similar off-target activity, as well as showing activity against the *h*ERG potassium ion channel.

In order to address the poor physico-chemical properties of their lead compounds, scientists at BMS looked into bioisostere replacements of the core urea moiety. In a series of papers they explored replacement of the urea with amino thiadiazole[32] **16** and 2-aminothiazole[33] **17** mimetics (Figure 5).

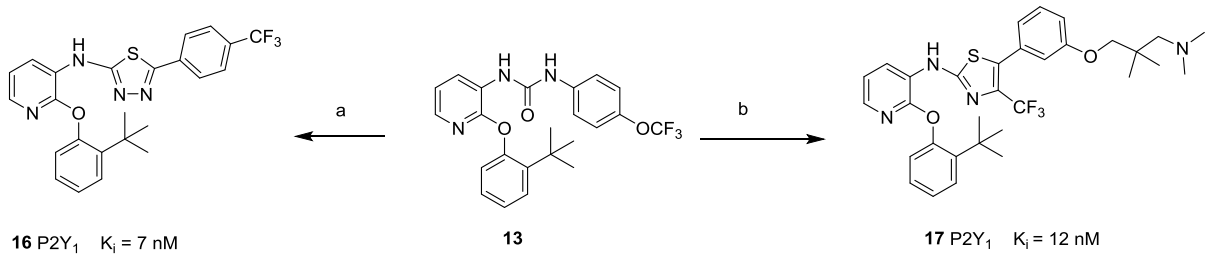
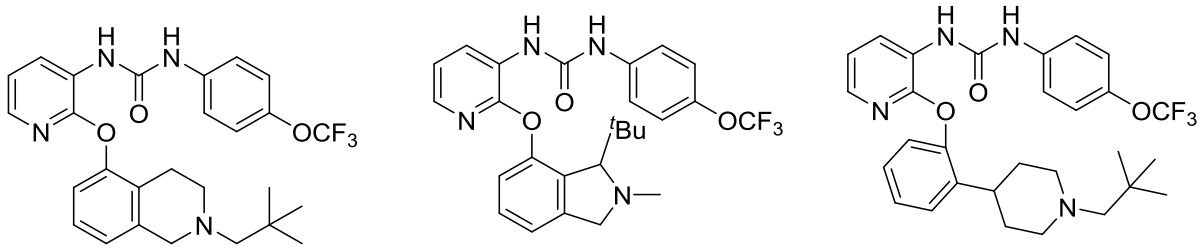


Figure 5: Bioisosteric replacement of the urea group: a) 2-amino-3,4-thiadiazole replacement and b) 2-aminothiazole replacement

High $P2Y_1R$ antagonism was demonstrated for a range of azole bioisosteric replacements (*e.g.* **16**). Several five-membered heterocycles were used as replacement for the urea moiety, with comparable levels of $P2Y_1R$ antagonism and ADP-induced aggregation activity. These receptor antagonists suffered from the same poor physico-chemical properties as the previous lead series (high lipophilicity including poor aqueous solubility <1 μg/mL at pH 6.5) and were not pursued further. In the case of the 2-aminothiazole bioisosteric replacements, a series of new analogues were discovered with excellent $P2Y_1R$ binding affinity. Several of the analogues demonstrated moderate inhibition of platelet aggregation, with compound **17** showing a reasonable rat PK profile (CL 6.7 mL/min/kg; $T_{1/2}$ 2.1 h, F 27%). However, poor solubility and high plasma protein binding (99.9% bound) may have contributed to the modest activity seen in the human serum platelet aggregation assay (IC_{50} 17 μM) and this series was not pursued.

In another approach to address the poor physico-chemical properties inherent in the biaryl urea lead compounds, incorporation of an amine containing, water-solubilizing group into the phenyl ether portion of the core structure was explored (Figure 6).



18 **19** **20**

Figure 6: Changes to the phenyl ether portion of **13** to increase water solubility

Compounds (**18**-**20**) displayed similar *h*P2Y$_1$R antagonism to that of **13,** however improved physico-chemical properties resulted in good functional activities as well as improved aqueous solubility. From this set of compounds, piperidine **20** showed the best overall profile and favourable PK parameters (Table 3).[34]

Table 3: *In vitro* data and rat PK profile for the diarylureas **18-20**.

Compound	*h*P2Y$_1$ K_i (nM)	PA[a] IC_{50} (μM)	Solubility (μg/mL)	CL (mL/min/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	F (%)	PPB[b] (% bound)
18[c]	11	2.5	3	48	22	14	16	100
19[c]	57	1.2	270	84	10	1.7	13	99.7
20[d]	16	2.4	105	18	14	11	89	99.9

[a]Platelet aggregation (PA) was tested with 2.5 μM of ADP. [b]Plasma protein binding (PPB). [c] Cassette dosed 0.5 mg/kg (i.v./p.o.) [d]2 mg/kg (i.v.), 5 mg/kg (p.o.)

In a continuing theme, a range of conformationally-constrained, anilino-substituted diarylureas were synthesized to generate a new series of potent and selective P2Y$_1$R antagonists. These compounds possessed an oral antithrombotic effect with mild bleeding liability in rat thrombosis and homeostasis models (Figure 7).[35]

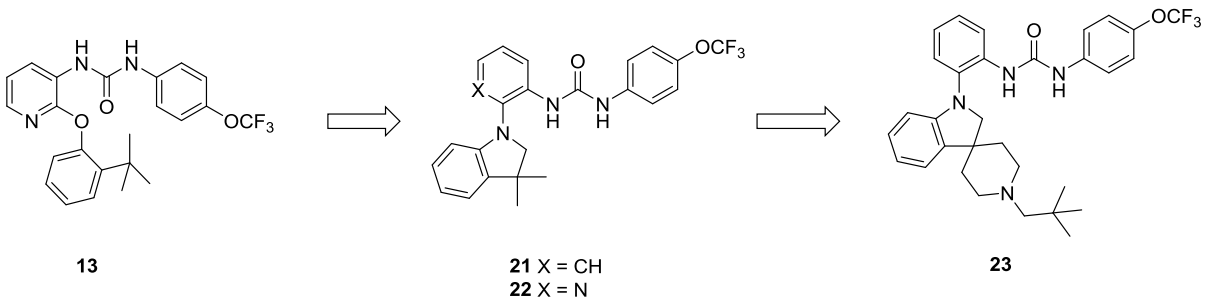
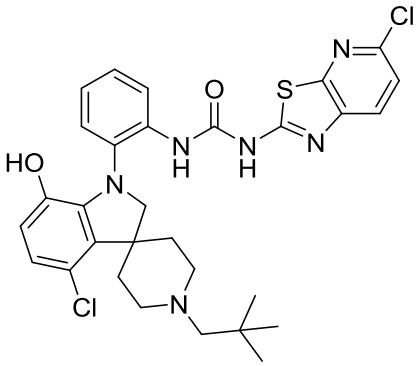


Figure 7: Evolution of new conformational constrained, *ortho*-anilino diarylurea **23**.

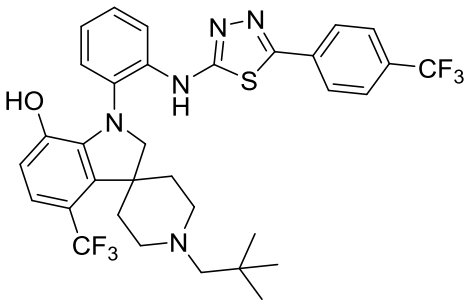
In the evolution of compound **23**, compounds **21**-**22** demonstrated good $P2Y_1R$ antagonism (**21** *h*$P2Y_1$ K_i = 8.7 nM, **22** *h*$P2Y_1$ K_i = 20.3 nM). It is interesting to note that among these ring-constrained analogues, the aryl compound **21** had slightly enhanced activity at the $P2Y_1R$ and this seemed to be a general trend observed within this series of compounds. After further optimization, **23** was identified, with an excellent overall profile (**23**, *h*$P2Y_1$ K_i = 4.3 nM, *h*$P2Y_1$; PA = 4.9 μM (at 10μM ADP); solubility 4 μg/mL; rat PK: CL 9.1 mL/min/kg; $T_{1/2}$ 7.5 h; F 32%). Compound **23** inhibited arterial thrombus formations and improved blood flow in a dose-dependent manner when administered by either *IV* or *PO* routes. Maximum thrombus weight reductions of 66% and 64% were observed in comparison to vehicle at doses of 0.6 mg/kg + 2.25 mg/kg/h (*IV*) and 10 mg/kg (*PO*), respectively. In these same rat models, a 20 mg/kg oral dose of clopidogrel reduced carotid artery thrombus weight by 67% and prolonged mesenteric bleeding time by 8.2-fold. These results suggested that inhibition of $P2Y_1$ by **23** compared favourably to the antithrombotic and haemostatic effects of clopidogrel - a clinically established $P2Y_{12}R$ antagonist.

In a further disclosure, potency in the spiropiperidine indoline-substituted diarylureas was enhanced through the introduction of a 7-hydroxyl substitution on the spiropiperidinyl indoline chemotype. Further SAR studies were conducted to improve pharmacokinetics and potency, resulting in the identification of **24** ($P2Y_1$ calcium mobilization assay = 0.12 nM; PA = 0.13 μM (at 10μM ADP); solubility 680 μg/mL; CL 7.3 mL/min/kg; $T_{1/2}$ 8.4 h; F 23%), a compound that demonstrated a robust antithrombotic effect *in vivo* and improved bleeding

risk profile compared to the $P2Y_{12}R$ antagonist clopidogrel in a rat bleeding model.[36] This exciting improvement in activity led to investigation of a series of urea bioisosteres of the diarylurea lead **24,** culminating in the discovery of a series of 2-amino-1,3,4-thiadiazoles, such as compound **25 (**$P2Y_1$ K_i 16 nM; PA 0.45 μM (at 10 μM ADP); rat PK: CL 4.0 mL/min/kg; $T_{1/2}$ 24 h; F 22% (cassette dosed 0.5 mg/kg (p.o.)), as potent $P2Y_1R$ antagonists; the first series of non-urea containing $P2Y_1R$ antagonists.[37]



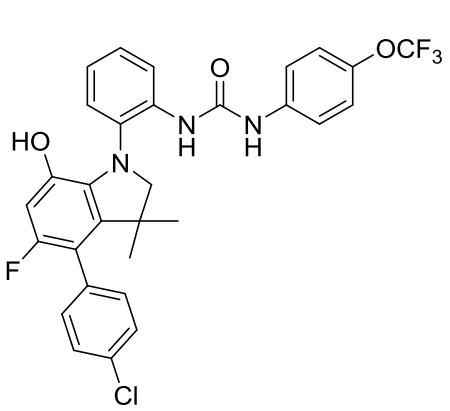
24



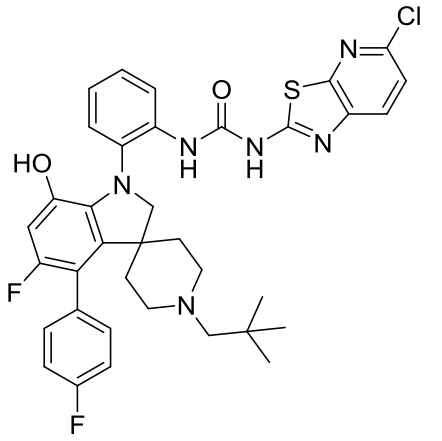
25

Several analogs of **25** were shown to have more favourable PK profiles, such as higher maximal trough concentration (C_{trough}), lower CL, smaller V_{ss}, and similar bioavailability compared with **25**.

Further optimization of **25**, by introducing 4-aryl groups at the hydroxylindoline in two neutral and basic sub-series, have been described.[38-39] In the neutral series, **26** had excellent potency and a highly desirable PK profile. Meanwhile, in the basic series, **27** (BMS-884775) had an improved PK profile (Figure 8).



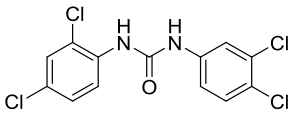
26
*h*P2Y$_1$ FLIPR = 1.1 nM
hPA IC_{50} = 0.24 μM



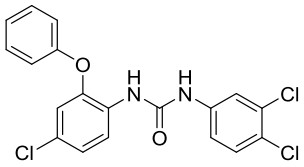
27
*h*P2Y$_1$ FLIPR = 0.12 nM
hPA IC_{50} = 0.18 μM
Rat PK
CL 4 mL/min/Kg; V_{ss} 2.1 L/Kg; F 30%

Figure 8: Discovery of **27** – a potent and orally bioavailable P2Y$_1$R antagonist (n.b. no dose given for the PK studies).

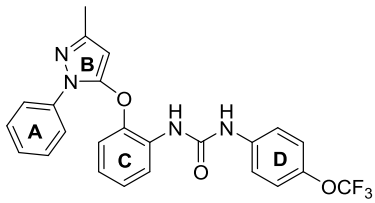
Scientists at Pfizer have reported a series of potent and orally bioavailable diarylurea P2Y$_1$R antagonists[40] resulting from an HTS campaign. The HTS hits (**28** and **29**) were further explored to deliver aryloxypyrazole **30** as a suitable template for further elaboration (Figure 9).



28 HTS Hit
*h*P2Y$_1$ K_i = 4.9 μM



29 HTS Hit
*h*P2Y$_1$ K_i = 1.8 μM



30
*h*P2Y$_1$ K_i = 0.29 μM

Figure 9: Discovery of the aryloxypyrazole template (such as **30**) for further elaboration

During their optimization project, attention was paid to the A- and B-rings of the template. Interestingly, substitution in the *ortho*-position of the A-ring increased binding affinity and was preferred over *meta*- and *para*-substitution, with either small lipophilic or halogen substituents being optimal. Limited examination of the B-ring was undertaken at this time. However, it was noted that larger lipophilic substituents in the pyrazole 3-position led to

inactivity, thus the 3-methyl substitution was preferred. Subsequent optimization then focussed on the D-ring while maintaining the optimal substitution on the A- and B-rings (Figure 10).

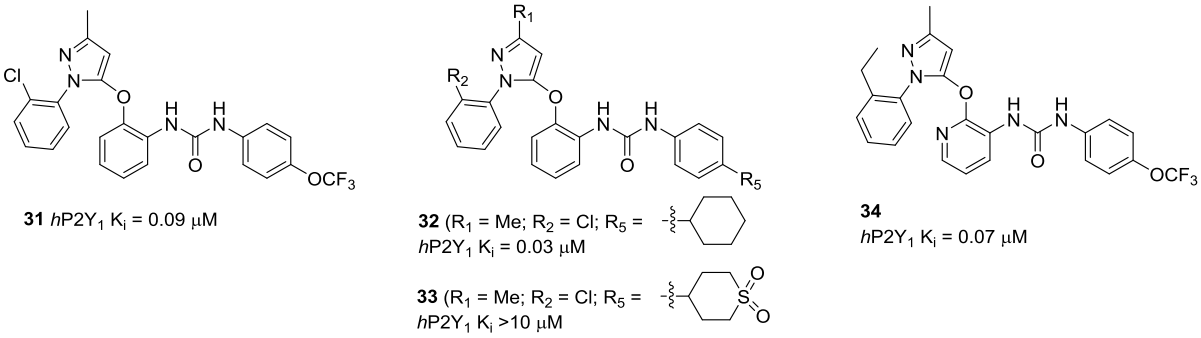
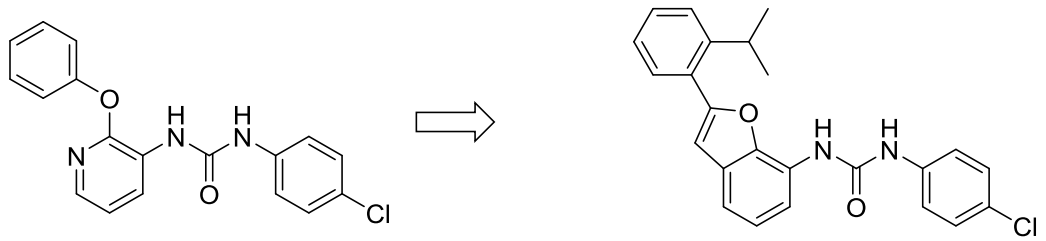


Figure 10: Lead optimization of the aryloxypyrazole template

Compound **31** had good *in vitro* metabolic stability and was further evaluated for its rat PK where it demonstrated a very favourable profile (CL 81 mL/min/kg; V_{ds} 15.7 (L/kg); $T_{1/2}$ 2.8 h; F 59%). This compound was progressed to a rat arterial injury model where it resulted in a dose-dependent decrease in both the incidence of occlusive thrombus formation and the average time to occlusion. It is interesting to note that high affinity was achieved when lipophilic groups were incorporated at the *para*-position of the D-ring (**32,** *h*$P2Y_1$ K_i = 0.03 μM), whereas a more polar substituent (**33,** *h*$P2Y_1$ K_i > 10 μM) led to a substantial loss in activity. When heterocyclic replacements were made in the C-ring, it was discovered that substitution with a pyridyl group afforded compounds (e.g. **34**) with reasonable activity.

A series of HTS-derived diarylurea $P2Y_1R$ antagonists were reported by scientists at GlaxoSmithKline (GSK).[40] In their work, they described the discovery of a hit (**35**) which was optimized to give a series of benzofuran-substituted urea derivatives *e.g.* **36** (Figure 11).

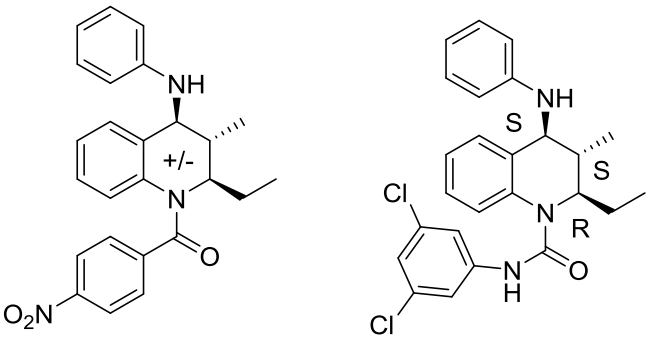


35 HTS Lead
*h*P2Y$_1$ FLIPR IC_{50} = 0.79 μM

36
*h*P2Y$_1$ Ki = 0.76 μM

Figure 11: A series of HTS-derived diarylurea P2Y$_1$R antagonists

Scientists at GSK also reported on a non-urea containing series of receptor antagonists, once more obtained as a result of HTS.[41] A high throughput calcium mobilization assay employing a fluorescent imaging plate reader (FLIPR) identified tetrahydro-4-quinolinamine **37** as an antagonist of *h*P2Y$_1$R. Exploration of this screening hit led to the identification of urea **38** where the importance of the absolute stereochemistry was highlighted through the separation of the enantiomers through chiral HPLC and it was discovered that all P2Y$_1$R antagonism was derived from a single enantiomer (Figure 12).



37 HTS Lead
*h*P2Y$_1$ FLIPR IC_{50} = 1.6 μM
*h*P2Y$_1$ Ki = 0.5 μM

38
*h*P2Y$_1$ FLIPR IC_{50} = 0.6 μM
*h*P2Y$_1$ Ki = 0.07 μM

Figure 12: Evolution of the HTS-derived lead to the active single enantiomer **38**

Compound **38** was progressed to a platelet aggregation assay and was shown to inhibit ADP-induced aggregation with an IC_{50} = 504 nM.

In a seminal paper, researchers have established the X-ray co-crystallised structure of inhibitors bound into the *h*P2Y$_1$R.[12] Structures of the *h*P2Y$_1$R in complex with either a nucleotide antagonist **1** (2.7Å resolution) or a non-nucleotide antagonist **13** (2.2Å resolution) reveals two distinct ligand-binding sites. Interestingly **13** was originally identified by a radio-ligand binding assay and described as a competitive antagonist. Antagonist **1** occupies a binding site within the seven transmembrane bundles of the P2Y$_1$R, and **13** binds to an allosteric pocket on the external receptor interface with the lipid bilayer (Figure 13).

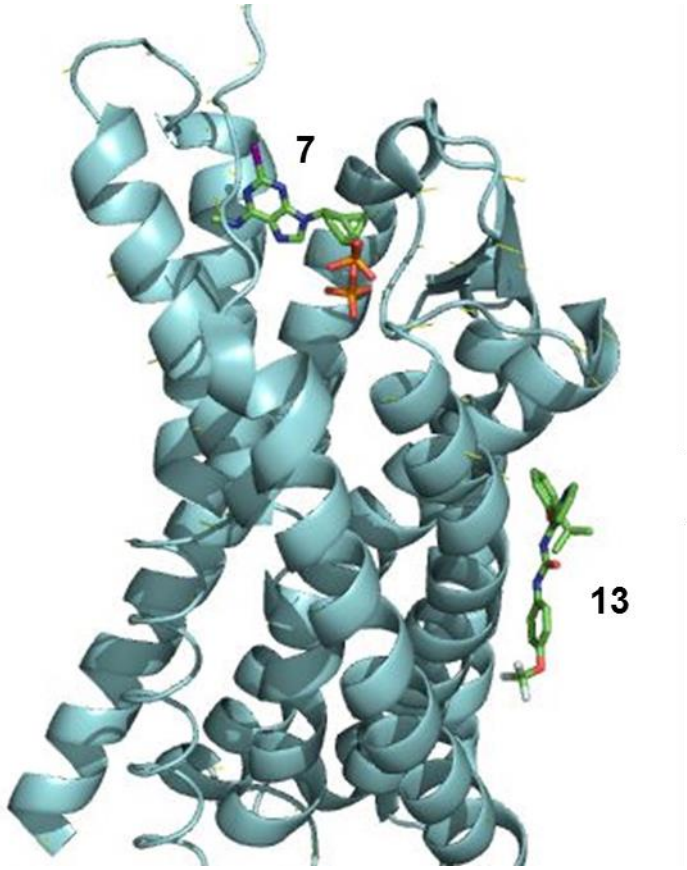
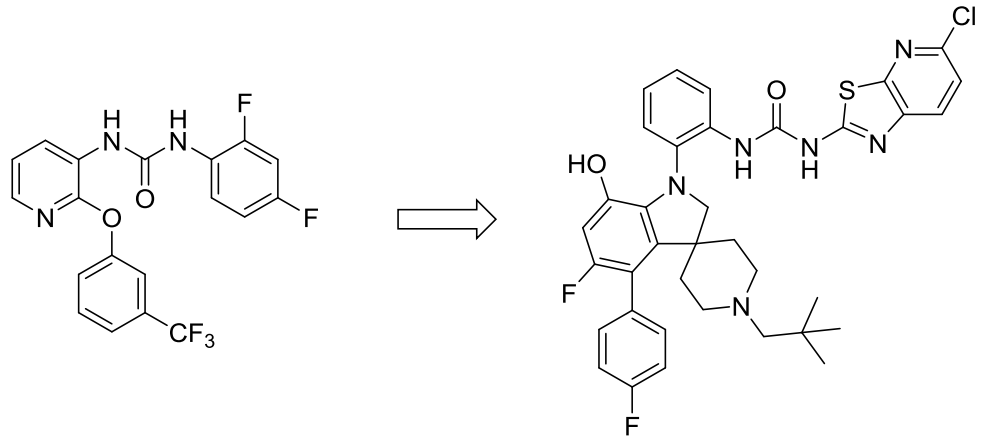


Figure 13: Compounds **7** and **13** co-crystallised into the *h*P2Y$_1$R showing the two distinct binding sites. An overlay of structures pdb codes 4XNV and 4XNW visualised with PyMOL Molecular Graphics System, Version 1.3. Schrödinger, LLC.

Perspective comment

The story of the discovery of **27**, covered over several papers, illustrates a classical medicinal chemistry project where core pharmacological optimisation ($P2Y_1R$ antagonism) was run in multi-parameter optimisation to improve physico-chemical properties (such as solubility and pharmacokinetics) of the lead compounds. It is also important to note that during this optimisation phase, consideration of all parts of the structure was examined including the utilisation of bioisosteric replacements (Figure 14).



8
*h*P2Y$_1$ K_i = 75nM
cLogD >5, MWt 409

27
*h*P2Y$_1$ FLIPR = 0.12 nM
cLogD >6; MWt 688

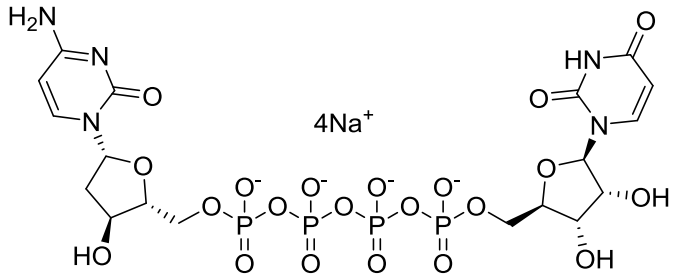
Figure 14: Summary of the discovery of **27**. cLogD is the calculated distribution coefficient.

While this optimisation process led to a dramatic increase in biological activity and improvement in solubility, increases in the core physical properties, such as MWt and lipophilicity, would lead to future issues in the development of a pre-clinical candidate.[42] Hopefully, elucidation of the co-crystal structure of the $P2Y_1R$ antagonist ligands will enable structure-based drug design of future $P2Y_1R$ antagonists, facilitating discovery of compounds with physical properties for improved oral absorption.

Drug-like antagonists of the $P2Y_2$ receptor ($P2Y_2R$)

The $P2Y_2R$ is activated by endogenous agonists UTP (*h*$P2Y_2$, EC_{50} = 140 nM) and ATP (*h*$P2Y_2$, EC_{50} = 230 nM) with almost equipotent activity in a transfected 1321N1 human

astrocytoma cell line.[43] The $P2Y_2R$ is predominately G_q-coupled and receptor agonism leads to activation of phospholipase C (PLC), IP_3 release and elevation of intracellular Ca^{2+} concentration, as well as the activation of protein kinase C (PKC) and activation of the mitogen activated protein kinase (MAPK) cascade.[44]

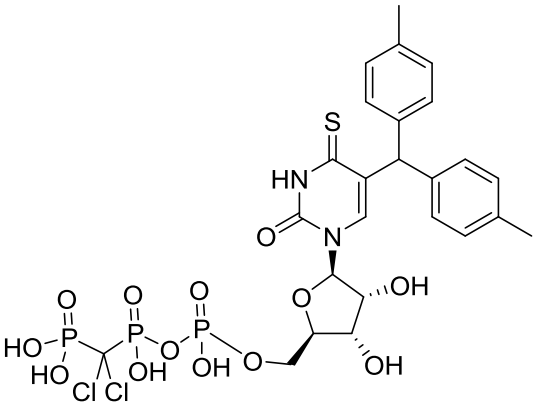


39

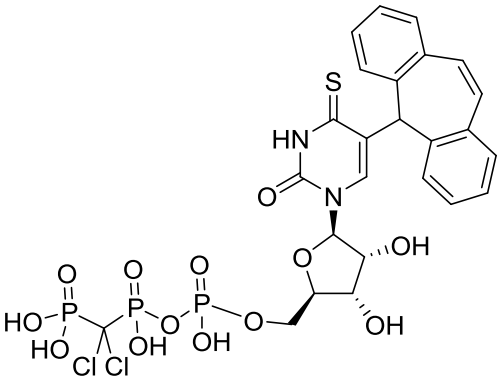
$P2Y_2R$ agonism has shown potential as a treatment for cystic fibrosis (CF), as defective chloride secretion in the respiratory epithelium of CF patients can be compensated by activation of chloride secretion channels. Through a knockout study,[45] comparison of $P2Y_2R^{(+/+)}$ with $P2Y_2R^{(-/-)}$ mice revealed that the $P2Y_2R$ mediated 85-95% of nucleotide-stimulated chloride secretion in the trachea, and agonist **39** (INS37217**,** *h*$P2Y_2$, EC_{50} = 220 nM) was shown to increase chloride and water secretion, as well as an increased mucin release of tracheal tissue in an *ex vivo* model.[46] It has also been shown that receptor agonism of the $P2Y_2R$ leads to keratinocyte proliferation[47] and neutrophil migration,[48] suggesting that receptor antagonists could show promise in treating psoriasis. Offermanns *et al*[49] reported that ATP released from tumor cell-activated platelets and induced the opening of the endothelial barrier, leading to migration of tumor cells and hence cancer-proliferation. More importantly they identified $P2Y_2Rs$ as the primary mediator of this effect; demonstrating a strong reduction of tumor cell metastasis in $P2Y_2R$ deficient mice and showing the therapeutic potential of $P2Y_2R$ antagonists as anti-metastatic agents.

The only reported development of $P2Y_2R$ antagonists from an industrial research group are compounds patented by scientists at AstraZeneca in the late 1990s (formerly Fison's and Astra Pharmaceuticals). Unfortunately, no absolute potency values for the compounds were

reported and so a detailed SAR cannot be given here. However, looking chronologically at the patents, it is possible to see the sequential development of several structurally distinct classes of receptor antagonists, culminating in compounds which could be classed as drug-like.



40
cLogP = 2.73
MWt 761



41
cLogP = 2.46
MWt 757

Figure 15: $P2Y_2R$ antagonists patented by AstraZeneca. All exemplified compounds were reported as being $P2Y_2R$ antagonists with a $pA_2 > 4.0$ in *h*$P2Y_2R$ transfected Jurkat cells. cLogP was calculated using instant J Chem.[50]

Within the first patent, a series of triphosphate receptor antagonists were reported (Figure 15).[51] It was shown, in an analogous series of $P2Y_2R$ agonists, that taking UTP as the starting point and replacing the βγ-oxygen with a dichloromethylene group, along with conversion to a 4-thiouridine, induced enhanced metabolic stability as well as maintaining full agonist activity.[52] Conversion to a $P2Y_2R$ antagonism profile was achieved through the introduction of a large, lipophilic group at the C-5 position of the uridine. This key substitution was extensively exemplified with a biphenyl and dibenzo-suberenyl substituent **40** and **41** respectively; however further substituents are reported throughout the patent.[51] The *in vivo* use of these triphosphate analogues would probably be limited to *intravenous* and topical administration. With the scope of developing oral, drug-like compounds, there is a desire to move away from excessively high molecular weight and highly-charged triphosphate

moieties. To this extent, a subsequent patent[53] was published detailing acidic, non-phosphate $P2Y_2R$ antagonists, exemplified by **42** and **43** (Figure 16).

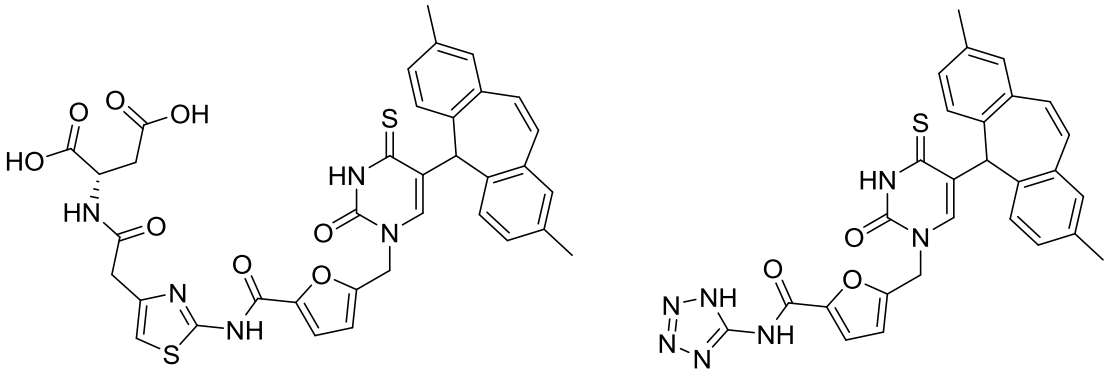
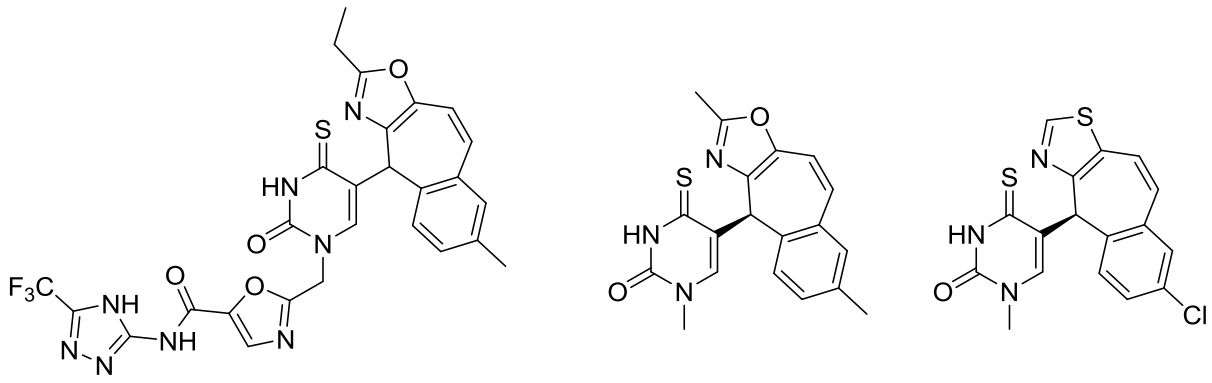


Figure 16: $P2Y_2R$ antagonist patented by AstraZeneca. All exemplified compounds were reported as being $P2Y_2R$ antagonists with a $pA_2 > 4.0$ in *h*$P2Y_2R$ transfected Jurkat cells.

Within this second series of acidic receptor antagonists, it is possible to see distinct structural changes. Across the majority of the exemplified compounds, the core 4-thiouridine was maintained along with the dibenzo-suberenyl substituent, now further substituted with two additional methyl groups. Presumably these features were required to maintain or enhance the activity seen in the first series of compounds.

In this series, the ribose group was replaced with a simpler 5-membered heterocycle, in most cases a substituted furan ring. In addition to this modification, a further notable structural change was replacement of the triphosphate with an acidic phosphate mimetic. This modification was reported as *L*-aspartic acid derivative **42** and the acyltetrazole analogue **43** (AR-C118925XX), along with other bioisosteric replacements. However, in achieving this structural change, a substantial increase in the overall lipophilicity was observed that would compromise their physico-chemical properties.



44
cLogP = 3.30
MWt 511

45
cLogP = 3.24
MWt 351

46
cLogP = 3.86
MWt 374

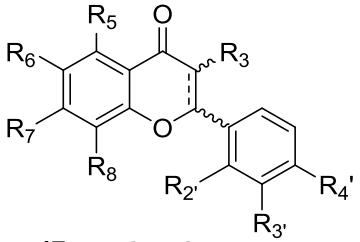
Figure 17: $P2Y_2R$ antagonists patented by AstraZeneca including acidic (such as **44**) and neutral receptor antagonists (such as **45**-**46**).

A series of less lipophilic receptor antagonists were reported in the final patent and a selection of these compounds is shown (Figure 17).[54] The key strategy appeared to be replacement of one of the phenyl rings in the dibenzo-suberenyl substituent with a 5-membered heterocyclic ring. This can be shown through considering **44** (cLogP = 3.3) which is appreciably less lipophilic than **43** (cLogP = 5.3) whilst remaining structurally similarity. More importantly, this change allowed for generation of a neutral receptor antagonist series as exemplified by **45** and **46.** Although there have been no further reports on the activity of these compounds, structurally they appear to be more drug-like than the compounds in the previous patent applications.

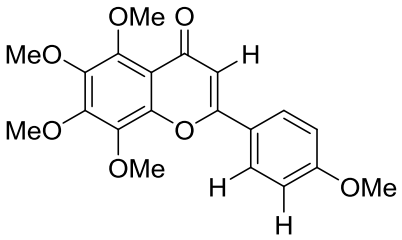
Since disclosure of these $P2Y_2R$ antagonists from AstraZeneca, several studies using **43** with success in both *in vivo* and *ex vivo* models have been reported; further validating the therapeutic benefit of $P2Y_2R$ antagonists. In 2004, scientists at Novartis[55] showed that **43** was inactive at 10 μM concentrations against a panel of 37 receptors (except for *h*$P2Y_2$). In an *ex vivo* model **43** concentration-dependently antagonized ATPγS-induced mucin secretion in human bronchial epithelial cells (IC_{50} ~ 1 μM). In 2015, Ceruti *et al*[56] showed that in an *in vivo* model, **43** completely inhibited satellite glial cell activation and cross talk

with trigeminal neurons, exerting a potent anti-allodynia effect. This suggests $P2Y_2R$ antagonists also have therapeutic potential for management of trigeminal neuralgia.

It has been reported[57] that several members of the flavonoid family displayed $P2Y_2R$ antagonism (*m*$P2Y_2R$, IC_{50} ≈ 10-50 µM). When screening a series of 40 flavonoids (Figure 18), **48** (Tangeretin, *m*$P2Y_2R$, IC_{50} = 12 µM) reduced the amplitude of the $P2Y_2R$ response to UTP but not the EC_{50} value, indicating it could be acting as an allosteric $P2Y_2R$ antagonist.



47 R = OH, OMe, Me, H

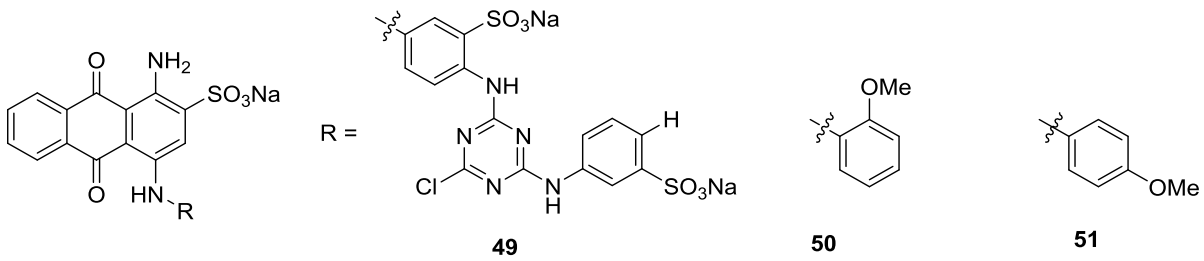


48

Figure 18: A series of 40 flavonoids **47** (generic structure) and **48**

More recently it has been suggested[58] that amphiphilic phytochemicals, similar to these flavonoids, appear to function indirectly through disruption of cell membranes in which the transmembrane proteins reside. This might explain the non-competitive antagonism observed for **48** and the difficultly encountered in extracting meaningful SAR from the compound series. However more importantly, the promiscuity in developing polyphenolic compounds is well known and is generally avoided when selecting lead-like compounds.

From the unselective, broad-spectrum P2YR antagonist **49** (Reactive Blue-2, *h*$P2Y_2R$, IC_{50} =1.85 µM), a series of receptor antagonists were reported (Figure 19).[59] The most potent (**50** *h*$P2Y_2R$, IC_{50} = 9.82 µM), although unpublished, 'appeared to be selective versus other P2Y subtypes as well as nucleotide-metabolizing enzymes'.



49 **50** **51**

Figure 19: Evolution of **50** (PSB-716) and **51** (PSB-416) from **49**

To develop a greater understanding for the binding of these $P2Y_2R$ antagonists, work was reported[60] on site-directed mutagenesis studies, to understand the binding modes of UTP, **49** and **50** (*h*$P2Y_2R$, IC_{50} = 11.5 μM). It is worth highlighting several of these point mutations as they provide insight into ligand binding within the $P2Y_2R$.

Table 4: Mutagenesis data for selected $P2Y_2R$ antagonists

Mutant	UTP	**49**	**51**
	EC_{50}/μM	IC_{50}/μM	IC_{50}/μM
wt4	0.0590	1.85	21.7
Y114A	0.0372	**>>100**	24.6
R272A	**20.60**	nd	nd
C278S	**2.09**	**1.05**	**3.44**
wt3	0.0804	1.62	21.9
Y198A	0.108	**9.30**	21.1
S296A	**>>300**	nd	nd

With respect to the receptor expression, mutants Y114A, R272A and C278S can be compared to wt4; mutants Y198A and S296A can be compared to wt3.

Mutation Y114A on the TM3 domain and Y198A on the TM5 domain did not significantly affect the UTP response, but did affect the antagonistic response of **49** but not **51**. Mutation S296A on the TM7 domain significantly affected the agonistic response to UTP and similarly mutation R272A on the extracellular loop 3 (EL3) in close proximity to the TM6 domain, resulted in a significant loss of agonist response to UTP. The mutation C278S significantly affected agonist response to UTP. It was proposed that this mutation inhibited formation of a key Cys^{25}-Cys^{278} disulfide bridge, which is thought to be important for the increase in receptor antagonism observed for **49** and **51**.

From this mutagenesis data, a bovine rhodopsin-based $P2Y_2R$ homology model was developed. It was hypothesized that Arg^{272} has a 'gatekeeper' role, helping to 'guide' the phosphates of the agonists into the receptor. Ser^{296} is located at the bottom of the binding pocket where it forms key hydrogen bonding networks with the nucleobase of UTP and ATP. A docked antagonist-binding pose of **50** was reported. However, even with the publication of this homology model in 2009 no further development of these compounds has been reported.

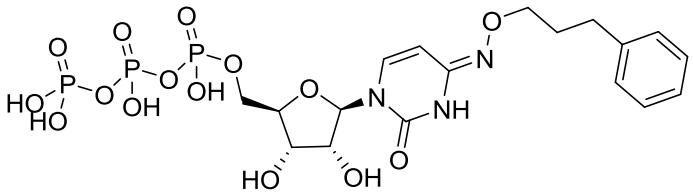
Recent crystal structures of $P2Y_{12}R$[10] show significant difference in antagonist and agonist bound conformations. In the absence of an X-ray crystal structure for the $P2Y_2R$, further work into developing $P2Y_2R$ homology models, focusing on antagonist receptor binding could prove invaluable for future development of drug-like $P2Y_2R$ antagonists.

Antagonists of the $P2Y_4$ receptor ($P2Y_4R$)

The *h*$P2Y_4R$ is fully activated by the endogenous agonist UTP (*h*$P2Y_4$, EC_{50} = 550 nM, *r*$P2Y_4$, EC_{50} = 200 nM) in transfected 1321N1 cells and is considerably more potent than ADP and UDP. ADP was shown to be a weak, partial agonist (*h*$P2Y_4$, 15% max at 100 μM, *r*$P2Y_4$, 34% max at 100 μM) and the weak agonist activity, sometimes observed for UDP, can be attributed to contaminating UTP. Interestingly, there are disparate differences when comparing the pharmacological properties of ATP on human and rat $P2Y_4$Rs. In human,

ATP acts as a competitive antagonist (*h*P2Y$_4$, pA$_2$ = 6.2) whereas in rat, ATP acts as a full agonist (*r*P2Y$_4$, EC$_{50}$ = 1.17 μM).[61] Mutagenesis work has shown that residues on the second extracellular loop (EL2) of the P2Y$_4$R were responsible for ATP's ability to act as an agonist in rat and an antagonist in human. In the triple *h*P2Y$_4$R mutant (S177N, V183I, R190L), ATP was an equipotent agonist compared to UTP.[62]

The first selective P2Y$_4$R agonist **52** (*h*P2Y$_4$, EC$_{50}$ = 23 nM) was reported in 2011 and showed a 28-fold and 32-fold selectivity versus the P2Y$_2$R and P2Y$_6$R respectively (*h*P2Y$_2$, EC$_{50}$ = 640 nM; *h*P2Y$_6$, EC$_{50}$ = 740 nM). Through development of CXCR4-derived P2Y$_4$R homology models, the selectivity gain was thought to be due to unconserved residues in the EL2 domain.[63]

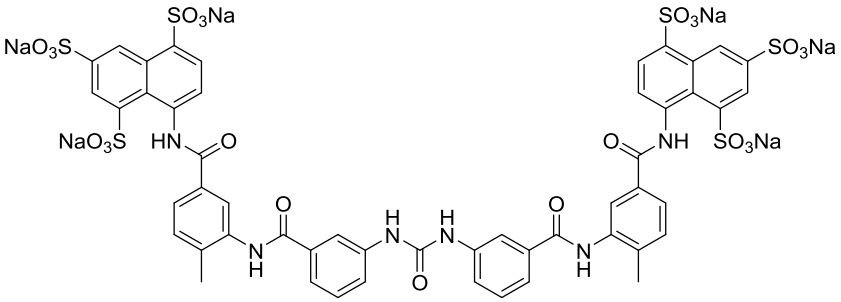


52

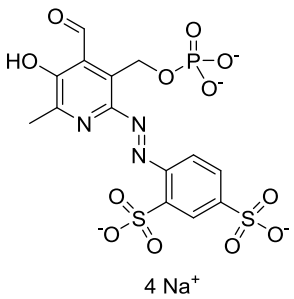
In a similar manner to the P2Y$_2$R, activation of the G$_q$-coupled dependent pathways of P2Y$_4$R leads to stimulation of chloride secretion channels and water secretion. Work with P2Y$_4$-deficient mice showed P2Y$_4$R expression in liver, stomach and intestine.[64] It was shown that the P2Y$_4$R was the primary mediator of UTP-stimulated chloride secretion in both small and large intestines, suggesting the potential use for the development of P2Y$_4$R antagonists as anti-diarrheal agents.[65]

Unlike most P2Y receptors, the P2Y$_4$R does not show the same susceptibility to broad-spectrum P2YR antagonists and is only antagonized by **49**. Using *h*P2Y$_4$R stably transfected into 1321NI cells, **53** (suramin) gave no antagonistic response and **54** (pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) tetrasodium salt hydrate (PPADS)) was shown to be relatively ineffective.[66] Compound **49** was demonstrated to be a competitive P2Y$_4$R antagonist in rat transfected *Xenopus* oocytes (*r*P2Y$_4$, pA$_2$ = 6.43).[67] It is thought that the

$P2Y_4R$ may play a causative role in hypoglycemic cell death and, using **49**, it was shown that $P2Y_4R$ antagonism increased cell survival in both cerebellar granule neurons and HEK-293 cells under hyperglycemic conditions.[68] This gives scope for future $P2Y_4R$ antagonists to be used as neuroprotective agents in diabetes. As with $P2Y_2$, there is scope to use **49** as a starting point for the development of selective anthraquinone-based $P2Y_4R$ antagonists.



53

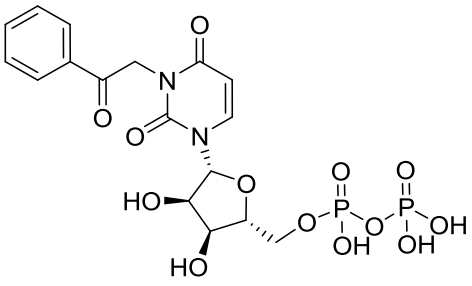


54

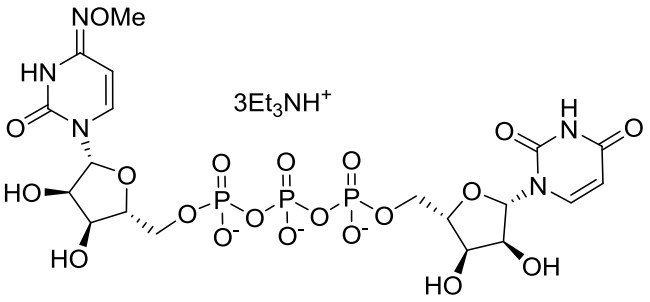
However, there has been no further published work on any $P2Y_4R$ antagonists. Hence, there remains a large scope and unmet need for the development of lead- and drug-like $P2Y_4R$ antagonists.

Antagonists of the $P2Y_6$ receptor ($P2Y_6R$)

The $P2Y_6R$ receptor is activated by UDP (EC_{50} = 0.30 μM). Its transcript has been detected in numerous organs, including spleen, thymus, intestine, blood leukocytes and the aorta. The majority of research has been performed on agonists of this receptor as typified by **55** (PSB0474)[69] and **56** (MRS2957).



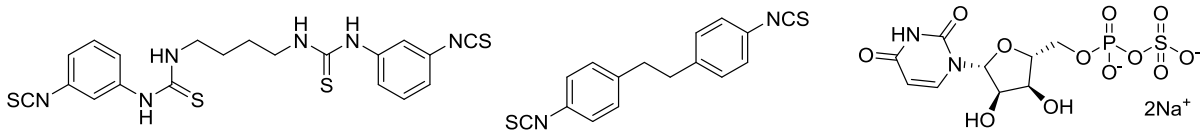
55



56

From these studies, it was shown that activation of the $P2Y_6R$ amplifies mucosal ATP release underlying bladder over-activity in patients with benign prostatic hyperplasia. Therefore, a potential use of a selective $P2Y_6R$ antagonist could be as a novel strategy to control persistent storage symptoms in obstructed patients.[70] In a recent article, Hirota *et al* suggested that a $P2Y_6R$ antagonist could have benefit in *Clostridium difficile* toxin-induced CXCL8/IL-8 production and intestinal epithelial barrier dysfunction and may therefore provide a new therapeutic target for the treatment of *C*. *difficile* infections.[71] In a further recent study, it was shown that endothelium-dependent relaxation of the aorta by UDP was abolished in $P2Y_6$ KO mice with the authors postulating that these observations might be relevant to several physio-pathological conditions, such as atherosclerosis or hypertension.[72] Inhibition of $P2Y_6R$ has also been suggested as a potential therapeutic target for monosodium urate (MSU)-associated inflammatory diseases, such as tophaceous gout.[73] With these and other[5] recent observations, inhibition of the $P2Y_6R$ could prove a viable therapeutic mechanism in many potential disease areas. Unfortunately, there are only a few known non drug-like inhibitors of the $P2Y_6R$ (**57**-**58**) available.[74,75,76]

Fischer *et al.* recently described the SAR of uracil nucleotide derivatives and found that uridyl phosphosulfate (**59**) was a weak $P2Y_6R$ antagonist (Figure 20).[75]



57
h$P2Y_6$ IC_{50} = 37 nM

58
h$P2Y_6$ IC_{50} ~ 100 nM

59
h$P2Y_6$ IC_{50} = 112 μM

Figure 20: Known $P2Y_6R$ antagonists; **57** (MRS2578), **58** (MRS2567) and **59**

Even though these compounds have been used to study the potential therapeutic role of $P2Y_6R$ antagonism, the combination of their chemical functionality and bulk physical properties make them less than optimal starting points for oral drug discovery programs.[77]

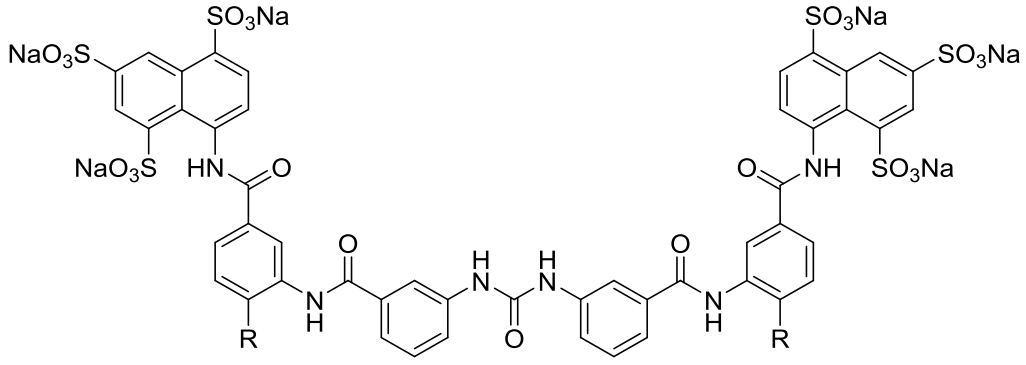
Antagonists of the $P2Y_{11}$ receptor ($P2Y_{11}R$)

The $P2Y_{11}R$ was cloned from human placenta and genomic DNA libraries.[78] It is coupled to both G_q and G_s with its activation leading to increases in cAMP and IP_3 levels. The rank order of potency of a series of nucleotides for the $P2Y_{11}R$, transfected into two cell lines, 1321N1 astrocytoma cells and CHO-K1 cells, is: ATPγS ~ BzATP>dATP>ATP>$ADP_{\beta}S$>2MeSATP.[79] $P2Y_{11}$ mRNA has been found to be highly expressed in macrophages, platelets, lymphocytes, neutrophils, megakaryocytic cells and has also been detected in neuronal-derived and glial-derived cell lines.[80]

The $P2Y_{11}R$ is expressed on some leukocyte types including monocytes,[81] neutrophils,[82] lymphocytes and monocyte-derived dendritic cells.[83] It has been found to mediate ATP-induced maturation and regulate trafficking of specific dendritic cell types,[84–86] regulate macrophage activation[87] and regulate release of cytokines such as IL-6,[88,89] IL-8 and the production of IL-12.[90] It has also been shown that inhibition of neutrophil apoptosis by ATP is mediated by the $P2Y_{11}R$.[91]

The SAR of a series of suramin-derived $P2Y_{11}R$ antagonists have been investigated.[92] In this study, the methyl groups of (**53**) were replaced by a range of substituents (Table 5).

Table 5: Structure activity relationship of suramin analogues



R	$P2Y_1$*	$P2Y_2$*	$P2Y_{11}$ pK_i **
H	59.3	31.6	6.95

CH_3 (**53**)	54.5	50.5	6.52
C_2H_5	48.7	48.8	6.00
$CH(CH_3)_2$	45.0	31.1	6.35
Ph	75.1	73.2	6.52
F (**60**, NF157)	63.3	42.8	7.35
Cl	40.6	55.2	6.97
OMe	23.2	44.9	7.12
CH_2OCH_3	47.5	44.3	5.62

*Percentage inhibition by a single dose (100 μM) of agonist-induced calcium mobilization of native $P2Y_1R$ and $P2Y_2R$ in HEK293 cells and at $P2Y_{11}R$ recombinantly-expressed in 1321N1 astrocytoma cells). **The pK_i values for $P2Y_{11}R$ on 1321N1 astrocytoma cells are shown.

Interestingly, the same changes to structurally simpler compounds (**61a**-**h**) gave compounds with little or no antagonism at $P2Y_{11}$ (Figure 21).

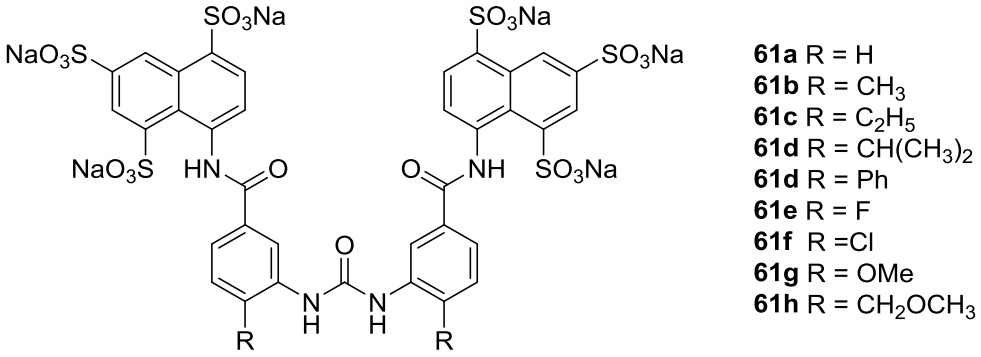
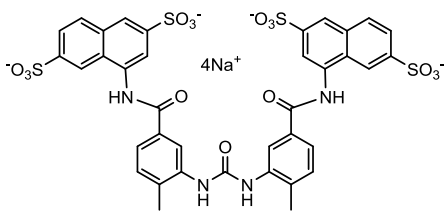


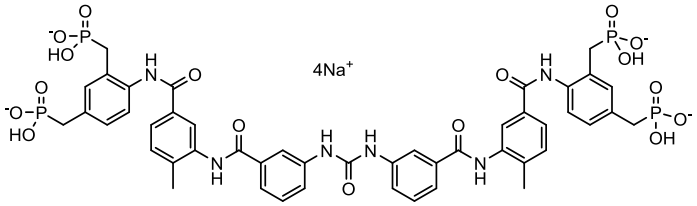
Figure 21: Synthetic changes to **61a**-**h** gave compounds with little or no activity at $P2Y_{11}$

A wide range of substituents were tolerated with the difluoro-substituted analogue, with compound **60** being the most potent ($P2Y_{11}$, pK_i = 7.35). This analogue, as well as having significant selectivity over $P2Y_1R$ and $P2Y_2R$ (> 650-fold), has 3- to > 67-fold selectivity over $P2X_{2,3,4,7}$.

A systematic screening of a library of naphthalene phosphonic acid and sulfonic acid compounds led to the identification of **62** ($P2Y_{11}$, pK_i = 7.8). This compound was 2.6-fold more potent than **60** and almost 1000-fold more potent than its very close structural analogue **61b**. At concentrations up to 100 μM there were no observed effects at the $P2Y_1R$, $P2Y_2R$ and $P2Y_6R$ (expressed in 1321N1 astrocytoma cells).



62



63

A rat formalin-induced inflammatory pain model, using the selective $P2Y_{11}R$ antagonist **62** (NF340) and agonist **63** (NF546), suggested that activation of the receptor, along with activation of the $P2Y_1R$ and $P2Y_6R$, lead to nociception.[92-93]

A computational model using the β_1-bovine rhodopsin crystallographic structure as a template in combination with site-directed mutagenesis was used to deduce the key ATP-binding site interactions. ATP was 10-fold less potent at the E186A mutant $P2Y_{11}R$ and 1000-fold less potent at the R268A mutant, whilst being functionally inactive at the R106A and R307A mutants. It is proposed that Arg^{106}, Arg^{268}, Arg^{307} and Glu^{186} are involved in ionic interactions with the tri-phosphate chain. Arg^{307} may also be H-bonded to the adenine N^6. Computational modelling also suggests Ser^{206} is involved in an interaction with the γ-phosphate of ATP and Met^{310} interacts with the adenine ring.[94-95]

Drug-like antagonists of the $P2Y_{12}$ receptor ($P2Y_{12}R$)

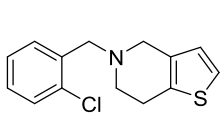
The $P2Y_{12}R$ is found on many cell types including megakaryocytes and platelets. The endogenous agonist is ADP and, following a thrombotic event, activation of the $P2Y_{12}R$ amplifies platelet activation and aggregation, leading to thrombus formation.[96] Although the involvement of ADP in platelet aggregation was first discovered in the 1960s,[96-97] it was not

until 2001 that the $P2Y_{12}R$ was structurally characterized.[98-99] Activation of both $P2Y_1R$ and $P2Y_{12}R$ are important for platelet activation and thrombus formation. However, the wide distribution of the $P2Y_1R$ amongst different cell types compared with the $P2Y_{12}R$ have made $P2Y_1R$ antagonists a less desirable target in drug discovery for treatment and prevention of thrombosis.

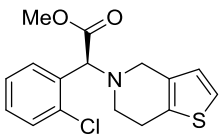
The thienopyridine class of irreversible binders for the $P2Y_{12}R$ was discovered in the 1970s by researchers at Sanofi, whilst screening compounds with structural similarity to the nonsteroidal anti-inflammatory drug tinoridine; although it is interesting to note that the actual drug target was not known at this time.[100-101] It was shown that some of these compounds did not exhibit anti-inflammatory properties but did have an anti-thrombotic effect after oral dosing in a rat model. One of the most effective compounds **64 (**ticlodipine) was chosen for development and was launched in 1978 as an anti-thrombotic agent for a range of high risk patients. Unfortunately, (**64**) was found to suffer from a poor benefit/risk ratio and a backup program resulted in the launch of **65** (clopidogrel) in 1998, which had a much improved safety profile. Interestingly, **65** was initially tested as a racemate, but it was eventually found that the anti-thrombotic activity resided with the *S*-enantiomer.[101]

This class of inhibitor required activation through conversion to the active metabolite **66,** which binds irreversibly to the receptor. Binding to the $P2Y_{12}R$ was confirmed in 2001[102] and site-directed mutagenesis identified two extracellular cysteine residues, Cys^{17} and Cys^{270} as candidates for disulfide bond formation with this active metabolite[103] which was later confirmed when the X-ray crystal structure was published in 2014.[13]

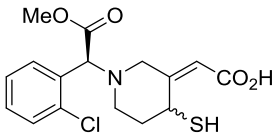
In 2009, another irreversible inhibitor within this class **67** (prasugrel - a racemate) was launched.[104]



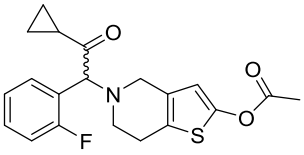
64



65

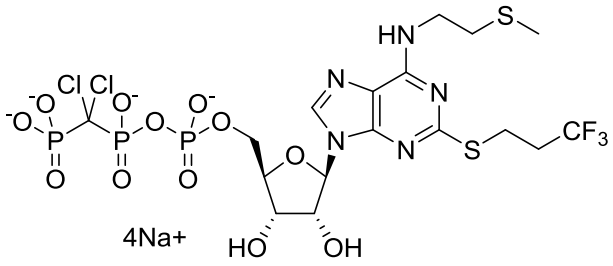


66



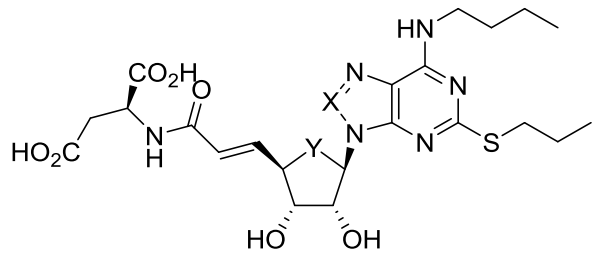
67

AstraZeneca scientists took the weak $P2Y_{12}R$ antagonist ATP as the starting point for their discovery project. Replacement of the βγ-oxygen with a dihalomethylene group and installation of substituents on the adenine ring gave a series of high affinity reversible antagonists.[105] One of these, **68** (cangrelor, $P2Y_{12}$ IC_{50} = 0.4 nM), was approved in 2015 for use as a quick onset/quickly reversed intravenous antiplatelet drug, over twenty years from its discovery.



68

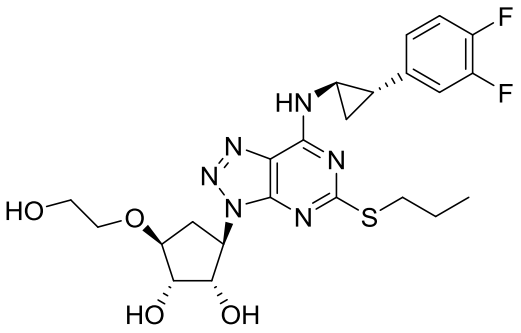
The next objective was to develop a drug suitable for oral administration. This required a move away from compounds containing highly acidic groups.[101] As ADP is an agonist for the $P2Y_{12}R$ and ATP an antagonist, it was hypothesized that the terminal phosphate was important for receptor antagonism. Effort was therefore directed at finding alternative acidic groups to the triphosphate chain, in particular the terminal phosphate. This work led to the aspartic acid-derived analogues **69**-**71**. Within this series, conversion of the purine ring to a triazolopyrimidine **70** gave a > 100-fold increase in binding affinity over the purine analogue. Replacement of the ribose with a cyclopentyl ring gave a series of compounds, such as **71**, with similar activity and increased metabolic stability (Figure 22).



69 X = C, Y = O hP2Y$_{12}$ = pIC$_{50}$ 7.0
70 X = N, Y = O hP2Y$_{12}$ = pIC$_{50}$ 9.5
71 X = N, Y = CH_2 hP2Y$_{12}$ = pIC$_{50}$ 9.3

Figure 22: Key SAR of glycoside analogues showing the evolution to the potent cyclopentyl analogue **71**. Affinity pIC$_{50}$ values were derived from an ADP-induced aggregation assay using washed platelets.

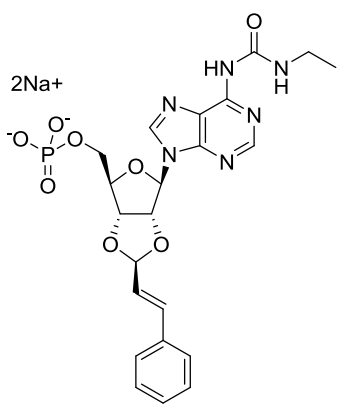
The physical properties of these molecules (MW>500, >5 H-bond donor groups and the dicarboxylic acid moiety) were not conducive to good oral absorption. Replacement of the butylamine group on the triazolopyrimidine ring with a 1*R*,2*S*-*trans*-phenylcyclopropylamine gave an increase in potency across a number of analogs. Further modifications aimed at finding a suitable replacement for the dicarboxylic acid led, with fluorine fine tuning, to **72** (ticagrelor),[106] an orally active reversible P2Y$_{12}$R antagonist, which was launched in Europe in 2010 (hP2Y$_{12}$ pK$_i$ 8.7; rat PK CL 21 mL/min/kg; Vss 3.8 L/kg; T$_{1/2}$ 2.6 h, F 24%).



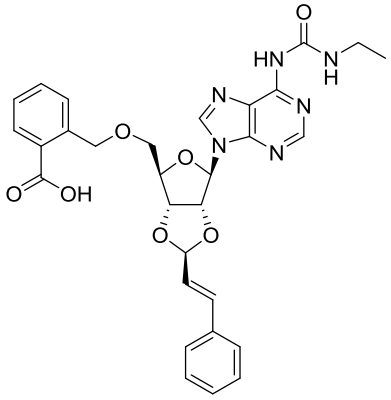
72

Outside of the work patented by AstraZeneca other companies have developed other nucleotide-based P2Y$_{12}$R antagonists. Inspire Pharmaceuticals reported the monophosphate, **73** (INS50589, IC$_{50}$ 16 nM) that showed inhibition of platelet aggregation in a washed human platelet assay. Unfortunately, this compound proved unsuccessful in clinical trials as an intravenously-delivered drug.[107] Further carboxylic acid replacements for

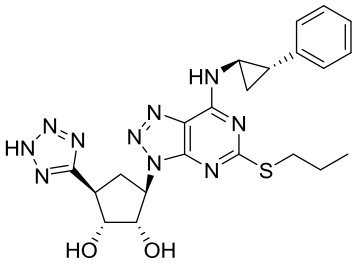
the phosphate groups were investigated with the 2-carboxybenzyl analogue **74** (*h*P2Y$_{12}$, IC$_{50}$ = 40 nM) being the most potent of this series.



73



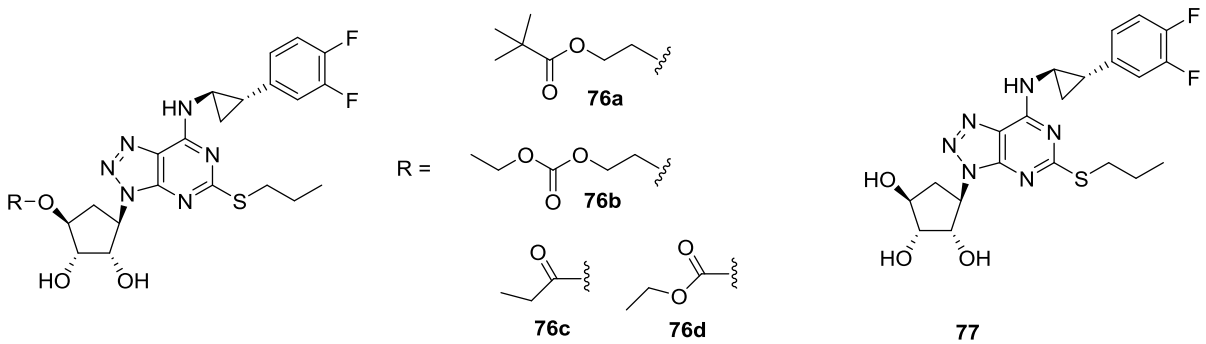
74



75

A series of analogues of **72**, primarily focussed on replacing the hydroxyethoxy substituent with a heterocycle, was reported by researchers at Johnson & Johnson. Compounds containing the 1*H*-tetrazol-5-yl group were the most potent (e.g. **75**, *h*P2Y$_{12}$, K_i = 2 nM). No *in vivo* data was reported.[108]

A number of ester and carbonic ester derivatives (**76a**-**d**) of **72** and **77** (a major metabolite of **72** with similar binding affinity) have a comparable effect to **72** and **77** on platelet aggregation after oral dosing (5 mg/kg) to rats. No *in vitro* data was given and the activity is assumed to be due to hydrolysis (presumably metabolic) to **72** and **77** (Figure 23).[109]



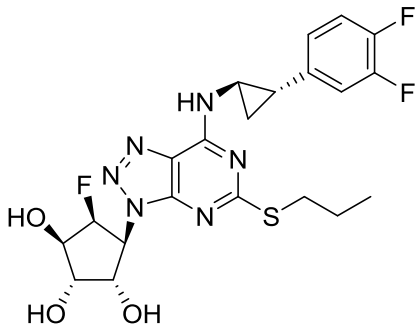
76a **76b** **76c** **76d**

77

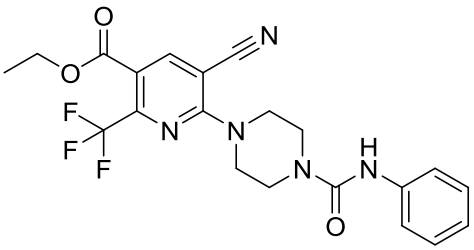
Figure 23: Derivatives of **72, 76a**-**d** and **77** - the active metabolite of **72**.

More recently,[110] researchers at Shanghai Hengrui Pharmaceutical Co Ltd. described their SAR exploration of the cyclopentyl ring and cyclopropylamino groups of **72**. Compound **78**, a fluorinated analogue of **77**, was more potent than **72** *in vitro* and *in vivo*, with a satisfactory PK profile and a shorter bleeding time observed.

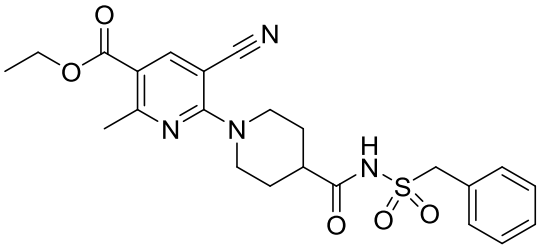
With the scope of developing non-nucleotide antagonists, high throughput screening of the AstraZeneca compound bank identified piperazinyl-pyridine, **79** as a hit (*h*P2Y$_{12}$ IC$_{50}$ = 330 nM). SAR investigations were amenable to parallel synthesis and this eventually led to acyl sulfonamide **80** (AZD1283, *h*P2Y$_{12}$ IC$_{50}$ = 11 nM), a compound with a PK profile suitable for progression into human clinical trials.[111–113] It is interesting to note that an acyl sulphonamide group is also present in **81** (elinogrel), a P2Y$_{12}$R antagonist which progressed into clinical trials supported by Portola Pharmaceuticals,[114] then by Novartis. However, development of this compound was terminated in 2012.



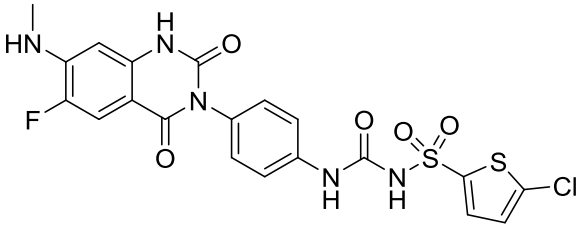
78



79



80



81

With the use of fusion protein, the crystal bound structures of the P2Y$_{12}$R with full agonist 2-methylthio-adenosine-5'-diphosphate (2-MeSADP), 2-methylthio-adenosine-5'-triphosphate

(2-MeSATP) and **80** have been published.[13-14] Both 2-MeSADP and **80** bind in the same pocket with the adenine group of 2-MeSADP and the nicotinate group of **80** forming similar $\pi-\pi$ interactions with Tyr^{105}. However, the pocket shape in these structures is different and 2-MeSADP and **80** display only a partial overlap. It is interesting to note the movement in TM7 observed for binding of **80** in the antagonist-bound $P2Y_{12}R$ state compared to that of the agonist-bound $P2Y_{12}R$ state (Figure 24).

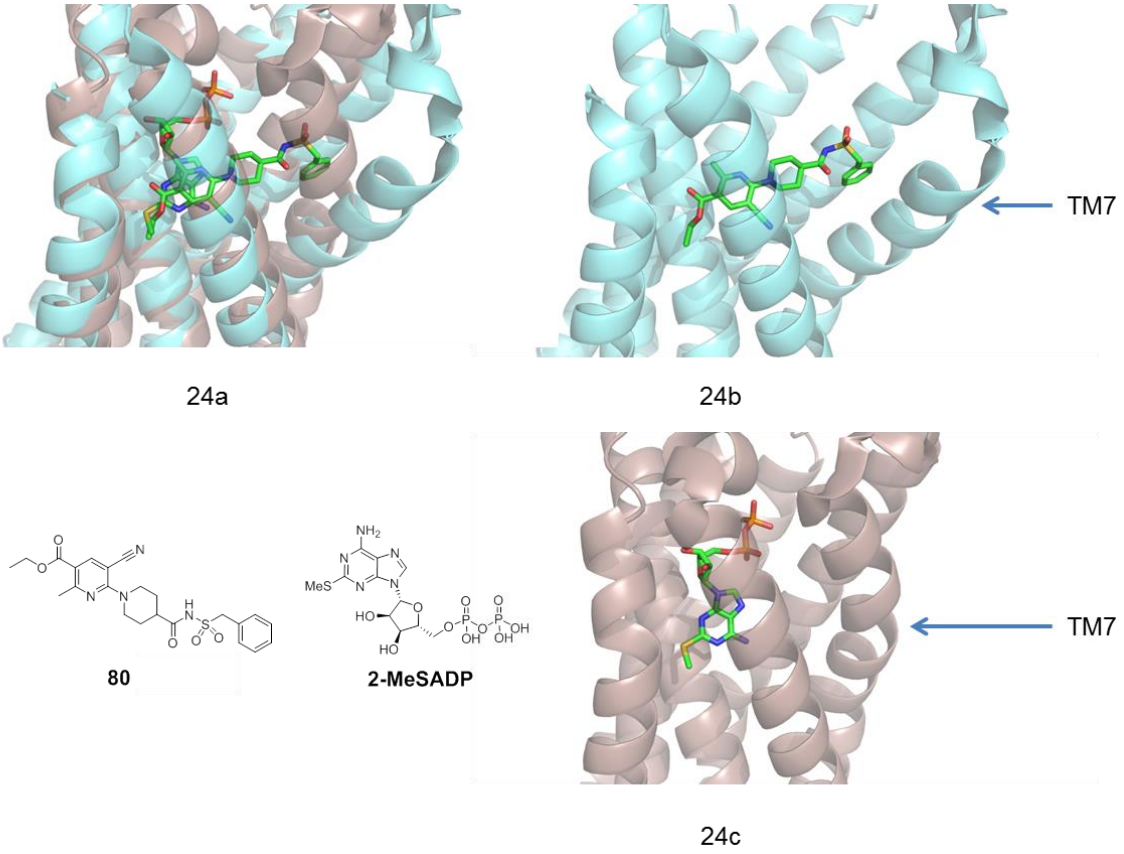
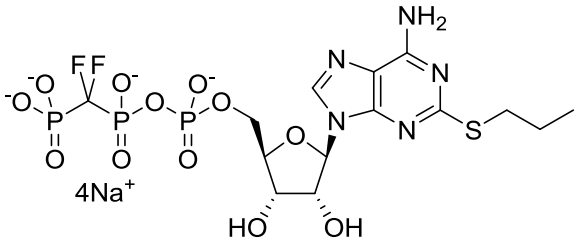
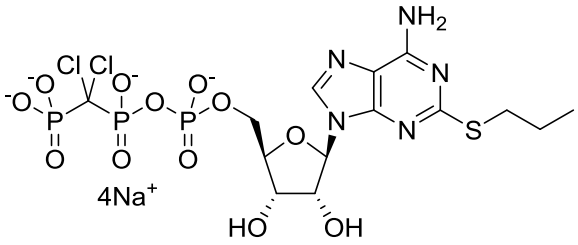


Figure 24: a) Overlay of agonist (cyan) and antagonist (brown) bound $P2Y_{12}Rs$ with **80** and 2MeSADP. b) Crystal structure of **80** in an antagonist-bound state $P2Y_{12}R$ c) Methylthio-adenosine-5'-diphosphate (2-MeSADP) in the agonist-bound state $P2Y_{12}R$**.** An overlay of structures pdb codes 4PXZ (brown) and 4NTJ (cyan) visualised with PyMOL Molecular Graphics System, Version 1.3. Schrödinger, LLC.

The 2-thioether group occupies a lipophilic pocket formed by Phe[106], Lys[155], Ser[156] and Asn[159], providing an explanation for the affinity enhancing effect of the alkylthio group. The 2-MeSATP structure has a similar conformation to 2-MeSADP. Furthermore, **82**, **83** and **68**, with the modified triphosphate chain and larger 2-alkylthio substituent, can be docked in a similar way to 2-MeSADP within the binding cavity of $P2Y_{12}$R-2-MeSADP, suggesting the reason for receptor agonism and antagonism is unclear. The docking of **72**, with the large N^6 substituent, requires conformational change of the receptor in order to be accommodated.

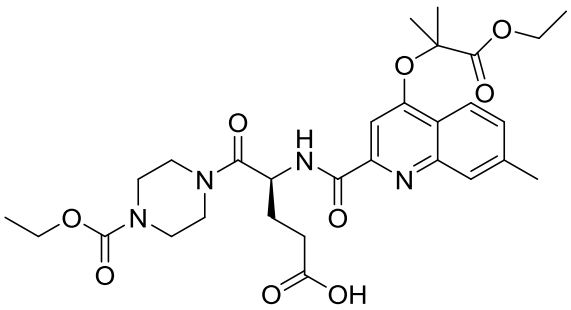


82

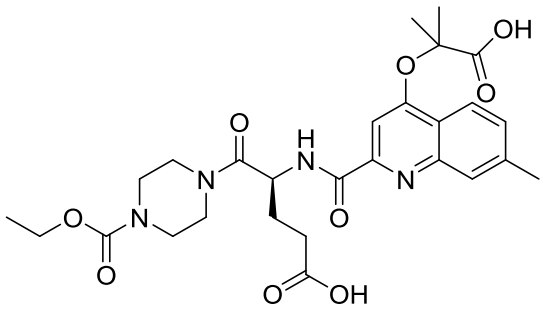


83

Berlex Biosciences reported [115] that the piperazinyl-glutamate **84** (BX-667, *h*$P2Y_{12}$ IC_{50} 129.9 nM) and its active metabolite **85** (BX-048, IC_{50} 8.3 nM), are reversible antagonists for the $P2Y_{12}$R in an *in vitro* ADP-induced washed platelet aggregation assay.[116] The compounds were shown to inhibit platelet aggregation and thrombus formation *in vivo* in both rat and dog models.[117]

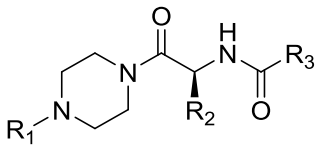


84



85

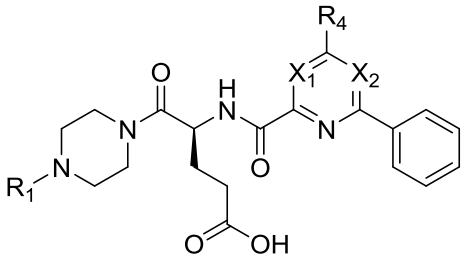
Taking **84** as a starting point, scientists at Pfizer used parallel synthesis to find a replacement for the quinoline ring (Figure 25).[118,119,120]



R_1 = EtOC(O), R_2 = H
R_1 = EtOC(O), R_2 = $CH_2CH_2CO_2H$
R_1 = mTolyl, R_2 = $CH_2CH_2CO_2H$
R_3 = Aryl/Heteroaryl groups

Figure 25: Chemical expansion of **84** illustrating the range of compounds prepared

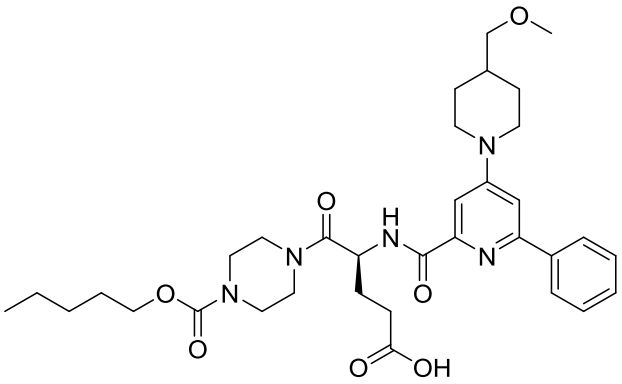
A large number of substituted heterocycles were evaluated (group R_3 in Figure 25) and it was found that a nitrogen atom in the ring *ortho*- to the amide was preferred along with a phenyl ring *ortho*- to the same nitrogen (Figure 26).



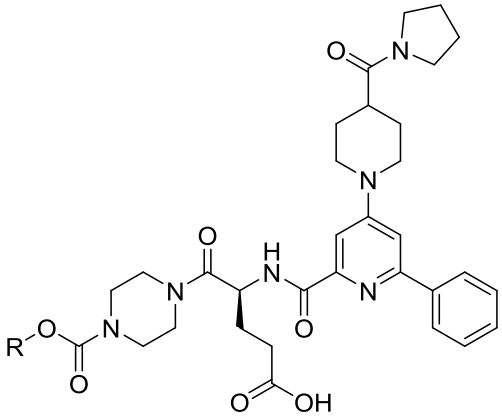
R_1 = EtOC(O), *m*Tolyl
X_1, X_2 = N or CH

Figure 26: A series of substituted heterocyclic $P2Y_{12}R$ antagonists

Optimization showed that R_4 could be a wide variety of substituents and did not need to contain an acidic group. Compound **86** (*h*$P2Y_{12}$, K_i = 15 nM), which contains only one carboxylic acid group, was found to have the best overall profile, with a PK and safety profile suitable for pre-clinical evaluation. Further refinement[121] gave the closely related compounds **87**-**88**, with activity (**87** *h*$P2Y_{12}$ K_i 2.1 nM, **88** *h*K_i 2.8 nM) and PK profiles suitable for clinical backups.

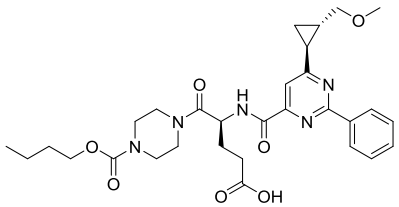


86

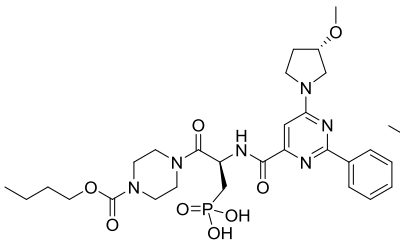


87 (R = ^{n}Bu)
88 (R = ^{n}Pent)

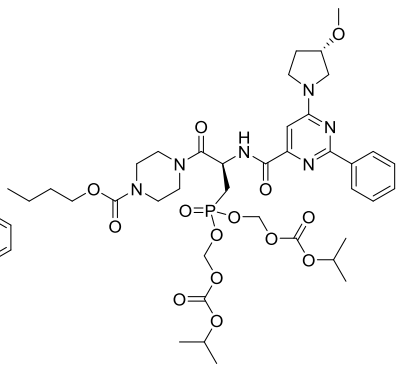
Actelion[122] reported the development of P2Y$_{12}$R antagonists using **85** as the starting point, leading to **89**, which is structurally similar to **86**-**88**. This compound has very good activity (*h*P2Y$_{12}$R IC$_{50}$ = 4.8 nM) and a PK profile suitable for further evaluation. In a later paper[123] replacement of the carboxylic acid was investigated. A number of acidic carboxylic acid bioisosteres, including acyl sulphonamides, tetrazole and 3-hydroxyisoxazole exhibited similar potencies to the parent compound, with the phosphonic acid derivative, **90** (ACT246475) chosen as the pre-clinical candidate. Unfortunately, the bioavailability was found to be low in both rat and dog. This led to the development of the *bis*((isopropoxycarbonyl)oxy)methyl ester pro-drug **91** (ACT281959)**.**



89



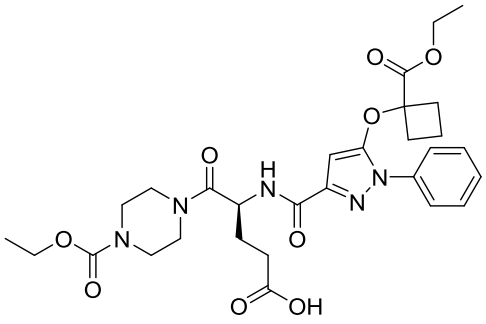
90



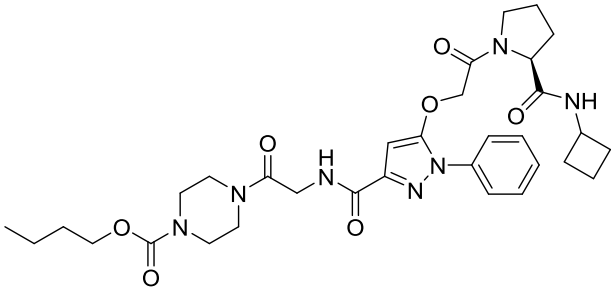
91

Sanofi-Aventis[124] found the 1-phenylpyrazolone scaffold **92** (*h*P2Y12 K$_i$ = 139 nM) as a suitable replacement for the quinoline ring of **84**. A comparison of **92** and **84** docked into a

$P2Y_{12}$ homology model showed the 1-phenylpyrazolone moiety of **92** occupies the same hydrophobic pocket as the quinoline group of **84**. The 5-substituent of the 1-phenylpyrazolone, which did not overlay with the 4-substituent of quinoline of **84**, was selected for systematic change; followed by variations to the piperazine ethyl carbamate and the central amino acid group. Optimization led to the neutral antagonist **93** (*h*$P2Y_{12}$ K_i = 7.7 nM). Removal of the acidic moiety led to a substantial reduction of PPB to give high activity in the human platelet rich plasma assay (*h*PRP IC_{50} = 30 nM). Compound **93** showed anti-aggregator activity in an *ex-vivo* dog model (10 mg/kg, *p.o.*), showing the potential for pre-clinical development.

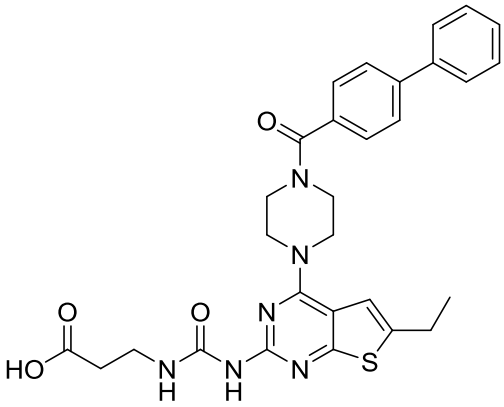


92

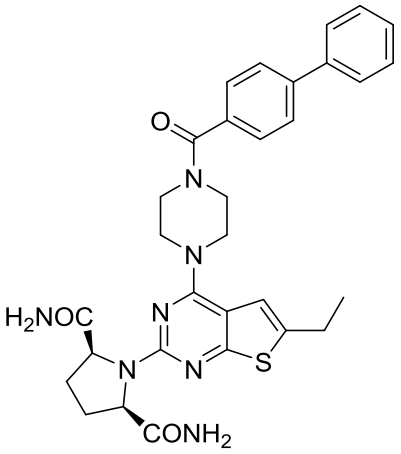


93

Pfizer reported thienopyrimidine-based $P2Y_{12}$R antagonists[125] with the most potent examples being **94** (*h*$P2Y_{12}$ K_i = 4 nM) and **95** (*h*$P2Y_{12}$ K_i = 3 nM). However, a significant drop in activity was observed in the *h*PRP assay (3 and 5 μM respectively), which was attributed to the highly hydrophobic biphenyl group.

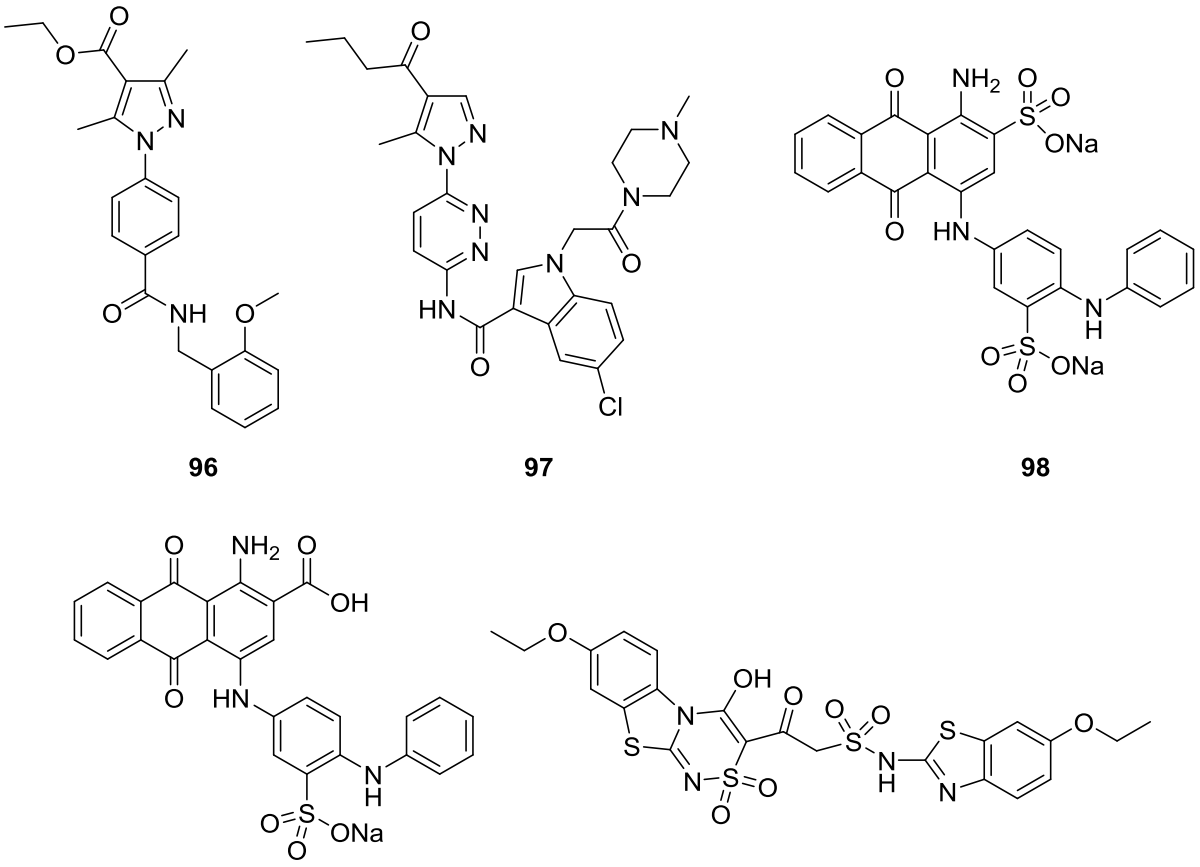


94



95

Taking the HTS hit **96** ($P2Y_{12}$ IC_{50} = 1.5 μM) as a starting point, Sanofi-Aventis developed **97** (SAR216471) as a pre-clinical candidate.[126] With high binding affinity (IC_{50} = 17 nM), the compound exhibited a small activity drop-off in the *h*PRP assay (IC_{50} = 100 nM) resulting in good activity in the *ex-vivo* rat platelet aggregation model (ED_{50} = 2.75 mg/kg *p.o.*). A number of anthraquinone derivatives have been found to be selective, high affinity $P2Y_{12}R$ antagonists, the most potent of which are **98** (*h*$P2Y_{12}$ K_i = 25 nM) and **99** (*h*$P2Y_{12}$ K_i = 21 nM).[127,128] Compound **100** (*h*$P2Y_{12}$ IC_{50} = 170 nM) was found after screening the COR compound library.[129] Limited investigation of the SAR through simultaneous changing of the ethoxy groups failed to find more potent analogues (Figure 27).



96 **97** **98**

99 **100**

Figure 27: A selection of $P2Y_{12}R$ antagonists from Sanofi-Aventis and **100** (CT50547) discovered through screening the COR screening collection.

Perspective comment

The important role the $P2Y_{12}R$ plays in thrombus formation and the huge commercial success of clopidrogrel, has meant that a number of pharma companies have invested significantly in $P2Y_{12}R$ antagonist programmes.

The thienopyridine class of irreversible binders was discovered incidentally in rat and mouse *in vivo* and *ex-vivo* anti-inflammatory screens. The requirement for metabolic transformation to an active species for $P2Y_{12}R$ activity means that compounds, within this series, would be inactive in either a cellular or membrane-based HTS assay. Furthermore, the active metabolite **66** is chemically unstable and would not therefore be present in a compound screening library.

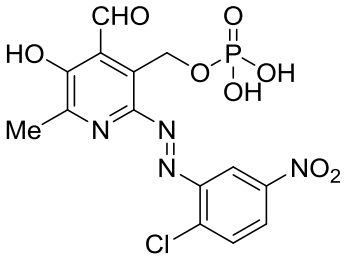
The risks involved in developing an irreversible antagonist and its potential for deleterious binding to proteins could be seen as being prohibitive to most pharmaceutical groups. As a consequence, $P2Y_{12}R$ antagonist programmes have focussed on finding reversible receptor antagonists. The first of these company programs, Fison's Pharmaceuticals, before the advent of high throughput screening, used ATP as a starting point. It is commendable that these investigators were able to develop an oral drug, ticagrelor, from such a non-lead-like chemical starting point.

Antagonists of the $P2Y_{13}$ receptor ($P2Y_{13}R$)

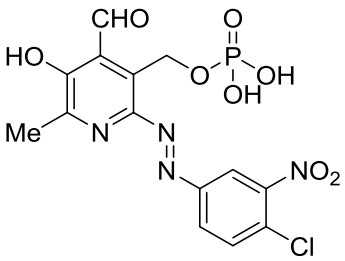
The $P2Y_{13}R$ was first identified at the turn of this century,[130,131] with its primary sequence demonstrating a 48% amino acid homology with the $P2Y_{12}R$. Coupled to G_i, it is activated by a range of diphosphate adenine nucleotides (ADP, 2MeSADP, ADPβS). A more thorough evaluation of the receptor revealed a number of key similarities and differences between the $P2Y_{13}$ form of the receptor and its closely related $P2Y_{12}$ subtype.[132] $P2Y_{13}$ is less sensitive to activation *via* triphosphate nucleotides, 2MeSADP displays a superior or equal potency to that of ADP depending upon the endpoint measurement and the receptor is only weakly antagonized by **83**.[105] In addition, the $P2Y_{13}R$ is not antagonized by the active metabolite of **66** at concentrations up to 2 μM.[132] Meanwhile, the similarities between the two receptor subtypes were also revealing; both subtypes, for example, are antagonized by 2MeSAMP, diadenosine tetraphosphate (Ap_4A) and most interestingly **68** , with the latter behaving in a non-competitive manner with respect to the $P2Y_{13}R$. It was further noted that ADP activation of the $P2Y_{13}R$ could be antagonized by **49** (IC_{50} = 1.9 μM), **53** (IC_{50} = 2.3 μM) and **54** (IC_{50} = 11.6 μM). The $P2Y_{13}R$ is expressed in the brain, spleen lymph nodes and bone marrow where it is also expressed on hemopoietic cells.[133]

To date, there remains a paucity of $P2Y_{13}$ selective antagonists, with the main structural contribution arising from derivatives of **54**.[134] Recognizing that the pyridine derivative **54** had been previously identified as a low potency antagonist of the $P2Y_{13}R$,[132] Kim *et al* embarked

upon the design and synthesis of a range of new derivatives, assessing their pharmacology through attenuation of ADP-induced PLC activity in human astrocytoma 1321NI-Gα_{16} cells stably transfected with human P2Y$_{13}$Rs (*h*P2Y$_{13}$-1321N1- Gα_{16}).



101



102

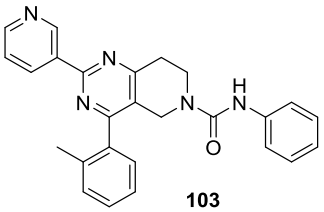
The most potent compounds generated from this synthesis campaign were **101** (MRS2211 *h*P2Y$_{13}$ pIC$_{50}$ = 5.97) and **102** (MRS2603 *h*P2Y$_{13}$ pIC$_{50}$ = 6.18). **102** demonstrated a competitive antagonism (*h*P2Y$_{13}$ pA$_2$ = 6.3) alongside a >20-fold selectivity towards the P2Y$_{13}$R when compared to P2Y$_1$ and P2Y$_{12}$. Its subsequent commercial availability has made it the molecular probe of choice for further investigation of P2Y$_{13}$R subtype pharmacology.[135–144]

Drug-like antagonists of the P2Y$_{14}$ receptor (P2Y$_{14}$R)

The P2Y$_{14}$R was, at its time of identification, the eighth member of the P2Y receptor family.[145] It is a G$_i$-coupled receptor, which is activated by at least four naturally occurring UDP-sugars. These include UDP-galactose, UDP-glucuronic acid, UDP-*N*-acetylglucosamine and UDP-glucose (UDP-Glc), with the latter acting as the most potent agonist. P2Y$_{14}$ mRNA has been detected in a range of human tissues including the stomach, intestine, adipose, brain, lung, spleen, heart and placenta.[133] Additionally, this receptor has been found in neutrophils.[146] Since UDP-Glc promoted signalling in multiple types of immune cells has been described; the P2Y$_{14}$R may possess a role in regulation of immune system homeostasis.[147] More recently, experiments with P2Y$_{14}$ knockout mice have highlighted the potential of P2Y$_{14}$R antagonism in the treatment of diabetes.[148]

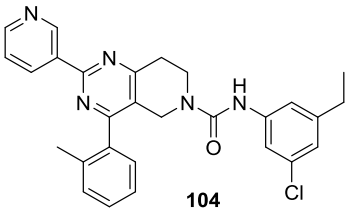
Using a yeast model system, it was demonstrated that UDP antagonized the UDP-Glc promoted $P2Y_{14}R$ activation in a concentration-dependent manner.[149] Cognisant of this report, Harden *et al* undertook a more detailed study to ascertain that UDP acted as a competitive antagonist at the human $P2Y_{14}R$ (pK_B = 7.8).[150] A similar level of receptor antagonism was observed using uridine 5'-*O*-thiodiphosphate whereas ADP, CDP and GDP produced no antagonist activity. An interesting observation from this study identified a striking ortholog variance, whereby UDP was demonstrated to act as a potent agonist (*r*$P2Y_{14}R$ EC_{50} = 0.35 μM) highlighting a polarized pharmacological difference in ligand behaviour. The authors then reassessed the action of UDP in three different cell lines; human embryonic kidney (HEK) 293, C6 glioma, and Chinese hamster ovary (CHO) cells, all stably expressing the human form of the $P2Y_{14}R$.[151] This series of experiments ultimately concluded that UDP was in fact acting as an agonist with EC_{50} values of 74, 29 and 33 nM respectively in the previously highlighted cell lines.

The first non-nucleotide based antagonists for the $P2Y_{14}R$ were originally reported by Merck and comprised a series of compounds based around a tetrahydropyrido[4,3-*d*]pyrimidine scaffold.[152] The initial hit **103** was identified *via* an high-throughput calcium^{2+} mobilization assay screen of the Merck sample collection. Whilst displaying only modest potency (*m*$P2Y_{14}$ IC_{50} = 4.9 μM), the scaffold was seen as attractive to pursue a hit-to-lead optimization programme; comparing activity at both the mouse and chimpanzee orthologs of the $P2Y_{14}R$. The authors comment that the selection of chimpanzee $P2Y_{14}$ (99% sequence homology to human) for screening was driven by the larger window (signal/background noise) with this receptor over the human $P2Y_{14}$, while the mouse receptor (83% identical to human) was used to enable future evaluation of the compounds in mouse models of disease. Systematic modification of the substitution pattern on the phenyl urea moiety eventually revealed that a 3,4- or 3,5-disubstitution pattern afforded the most potent antagonists as long as the 3-position substituent was an ethyl group, *e.g.* **104** (Figure 28).



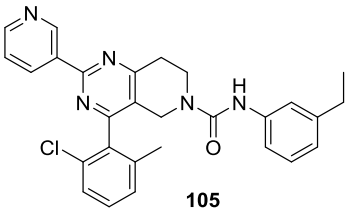
103

mouse $P2Y_{14}$
IC_{50} = 4.9 μM



104

mouse $P2Y_{14}$ IC_{50} = 0.16 μM
chimpanzee $P2Y_{14}$ IC_{50} = 0.017 μM



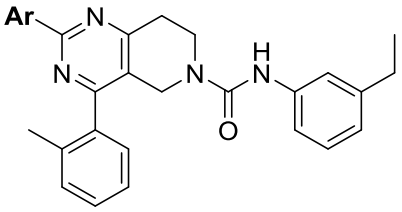
105

mouse $P2Y_{14}$ IC_{50} = 0.050 μM
chimpanzee $P2Y_{14}$ IC_{50} = 0.016 μM

Figure 28: The first non-nucleotide based antagonists for the $P2Y_{14}R$.

Turning their attention to the *o*-tolyl group at the 4-position of the heterocylic core generated a series of analogs highlighting that either *ortho*-positions could be substituted by either a methyl or chloro group, maintaining very good activity at both receptors, *e.g.* **105**. The final series of analogs retained the 3-ethylphenyl urea and the *o*-tolyl moieties and then altered the 3-pyridyl group to produce nineteen new compounds of which five are highlighted (Table 6).

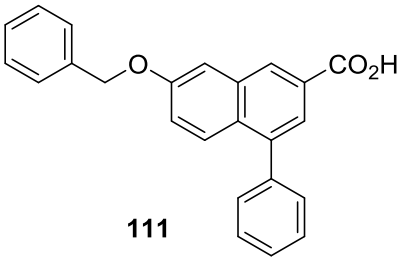
Table 6: Antagonist potency of 2-aryl substituted tetrahydropyrido[4,3-*d*]pyrimidines on the mouse and chimpanzee $P2Y_{14}Rs$.[152]



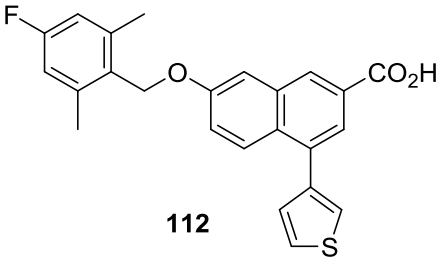
Compound	Ar	Mouse $P2Y_{14}$ IC_{50} (μM)	Chimpanzee $P2Y_{14}$ IC_{50} (μM)
106	3,4-(OCH_2O)-Ph	0.010	0.081
107	4-(CN)-3-Pyr	0.003	0.004
108	5-pyrimidyl	0.012	0.008
109	2-(CN)-5-pyrimidyl	0.001	0.001
110	Me_2-4-isoxazolyl	0.004	0.002

Compounds **107** and **109** were not progressed due to potent *h*ERG potassium ion channel binding (<200 nM), whereas compounds **106**, **108** and **110** displayed reduced *h*ERG binding (>5 μM). Preliminary *in vivo* analysis of these compounds when dosed as suspensions (50 mg/kg p.o. or *iv* at 5 mg/kg *i.v.*) in C57B6 mice revealed compounds with high volumes of distribution and extended half-lives. Compound **106** gave the best overall PK parameters and was considered suitable for further *in vivo* experiments to interrogate the pharmacological role of $P2Y_{14}$. Further characterization of **106** revealed that its antagonistic activity was mediated through a non-competitive manner with respect to UDP-mediated receptor activation.

The Merck group followed this report with a back-to-back manuscript highlighting a different scaffold, a 4,7-disubstituted naphthoic acid **111**, which had been identified as displaying antagonism of UDP-Glc activation (IC_{50} = 3.5 μM) of both the mouse and chimpanzee $P2Y_{14}R$ variants using the same assay methodology.[153-154] Unlike **106**, this compound exhibited competitive antagonism of ^{3}H-UDP in a recombinant simian $P2Y_{14}$ binding assay (K_i 0.16 μM). A comprehensive SAR study focused upon substituents in the 3-, 4- and 7-positions of the naphthoic acid scaffold and revealed **112** as the most potent compound whilst confirming the essential role of aryl substituents at the 4- and 7-positions.



111

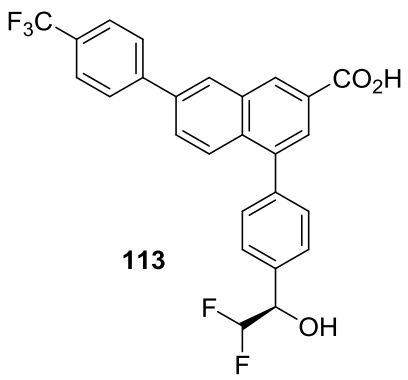


112

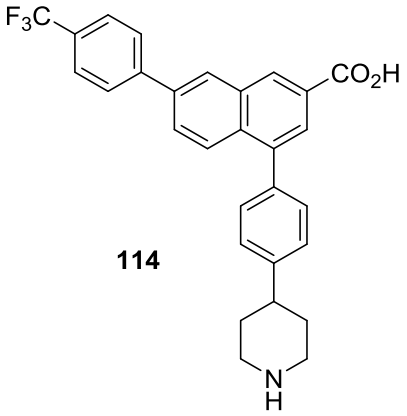
Early PK assessment of **112** was undertaken through oral dosing of C57BL/6 mice at 50 mg/kg and *i.v.* administration at 2 mg/kg. (F = 12%, $T_{1/2}$ = 2.7 h). Further studies identified that **112** was extensively metabolized *via* phase II metabolism (>99%) to the glucuronide and excreted *via* the bile.

A second round of synthetic variants were synthesized in an attempt to attenuate this level of

glucuronidation through decreasing the electron density of the carboxylic acid moiety. This ultimately led to identification of **113** as the best molecule in terms of its potency and PK profile (K_i = 4 nM, F = 67% (mouse) CL = 1.6 ml/min/kg, $T_{½}$ = 3.0 h, C_{max} = 113 μM).



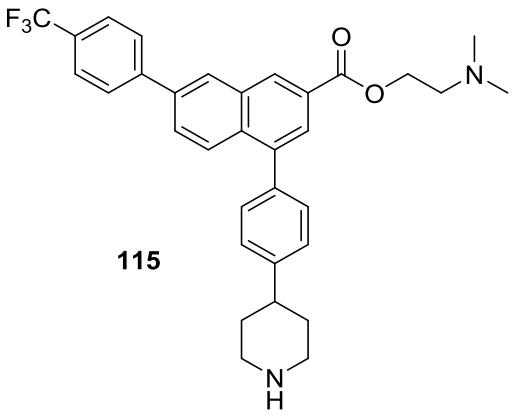
113



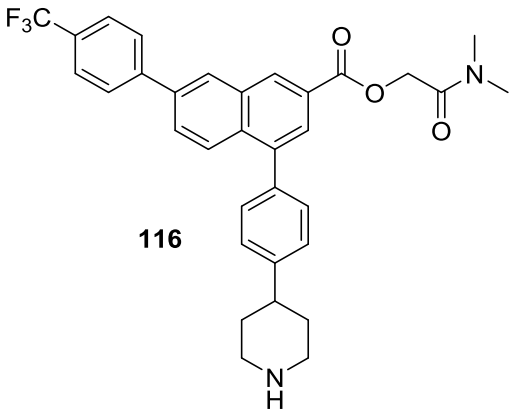
114

However, it was identified that **113** displayed high affinity for plasma proteins, being >99% bound in the presence of 4.5% human serum albumin (HSA).[155] Further synthetic efforts identified zwitterion **114** as a compound which retained its pharmacological potency (K_i = 1.9 nM in the chimpanzee $P2Y_{14}$ binding assay) as well as displaying acceptable levels of HSA binding. The latter assay was determined by using a binding assay incorporating 2% HSA for antagonist measurements. Harden *et al* subsequently reported a more thorough evaluation of the pharmacology of **114**.[156] They successfully demonstrated that in C6 glioma cells stably expressing the $P2Y_{14}R$, the concentration-effect curve of UDP-Glc for promoting inhibition of adenylyl cyclase was right-shifted in a concentration-dependent manner by **114**. In addition, at a concentration of 1 μM **114** exhibited no agonist or antagonist effect at the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ or $P2Y_{13}$Rs.

The zwitterionic nature of **114** imbued detrimental effects upon its oral bioavailability (*F* = 5%, C_{24h} = 0.68 μM). Therefore a series of ester derivatives were synthesized to investigate whether a pro-drug strategy would be beneficial. Of the ten esters described, four displayed good lability to afford high conversion rates to the parent drug **114** in the presence of whole blood. However of these, only **115** and **116** were able to replicate this in an *in vivo* setting with **116** being identified as the most promising candidate.



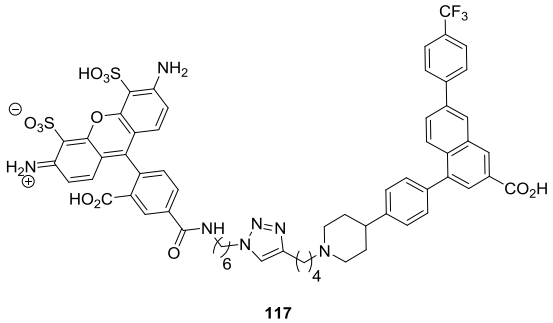
115



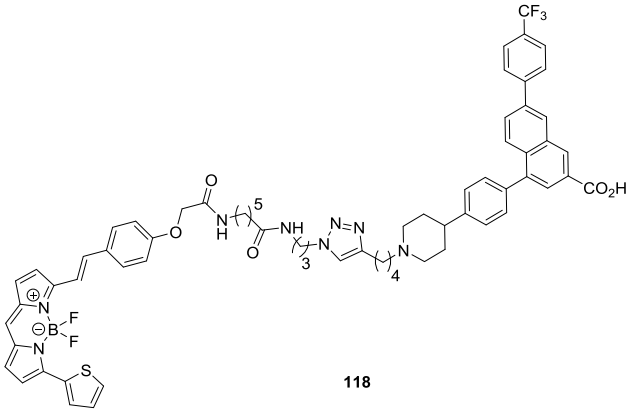
116

Compound **116,** when orally dosed in mice at 50 mg/kg, delivered plasma levels of **114** ($F_{virtual}$ = 67%, C_{24h} = 2.28 μM, AUC = 54 μM h and CL = 1.6 mL/min/kg) which were significantly higher than when **114** was administered as the free zwitterion (F = 5%, C_{24h} = 0.68 μM, AUC = 23 μM h).

As a pharmacological tool, **114** was also identified as a potential congener for the development of a fluorescent tool for receptor interrogation.[157] Molecular modelling studies of **114** bound to a homology model of the $P2Y_{14}R$ were undertaken, the model being constructed on the basis of X-ray crystal structures of the $P2Y_{12}R$.[13-14] Modelling established that the piperidyl nitrogen was suggested to be pointing towards the extracellular opening of the ligand binding pocket such that the charged piperidine nitrogen is solvent exposed and therefore a potential tether point on which to build a linker moiety. To this end an alkyl acetylene linker was appended to the piperidyl nitrogen to afford a congener primed for conjugation with an azide modified fluorophore *via* a copper-catalyzed [2+3] cycloaddition, ultimately affording Alexafluor488 **117** and a BODIPY 630/650-X **118** fluorescent conjugates.



117



118

117 exhibited exceptionally high affinity towards the $P2Y_{14}R$ (K_i = 80 pM) whereas **118** displayed a much weaker interaction (K_i >100 nM) illustrating the critical influence of both the linker and the fluorophore on the final pharmacology of the conjugate.[158] Fluorescent conjugate **117** was employed in flow cytometry studies to quantify specific binding to the $P2Y_{14}R$ and further utilized to confirm the binding selectivity's and affinities of established P2Y receptor ligands.

Conclusion

Within the past 2 decades, numerous P2Y receptor antagonists have been reported in both peer-reviewed and patent literature. The therapeutic relevance and efficacy of some of these antagonists has been proven *in vitro*, *ex vivo and in vivo*. The most clinically relevant and successful antagonists to-date have centered on new anti-thrombotics targeting the $P2Y_{12}R$. The serendipitous discovery of the thienopyridine class of irreversible $P2Y_{12}R$ binders led to the development and commercial success of clopidogrel (launched in 1998),[101] followed by prasugrel (2009).[104] Their successes encouraged numerous pharma-companies to invest into $P2Y_{12}R$ antagonist programs. It is commendable that taking ATP as a starting point, scientists at AstraZeneca developed the oral drug ticagrelor (2011);[106] a program which has also led to the approval of cangrelor (2015).[105] $P2Y_1R$ antagonists have also showed promise as anti-thrombotics and substantial research into developing suitable clinical candidates has been reported by scientists at BMS, Pfizer and GSK. To-date, it remains that no compounds have progressed to FDA approval. Notwithstanding this fact, drug-like

compounds (e.g. **27** and **31**) are available to further optimize, however the core physico chemical properties of these $P2Y_1R$ antagonists have no doubt hindered their clinical development. Outside of anti-thrombotics, antagonists of the other P2Y receptors show promise for the treatment of a variety of diseases. $P2Y_{14}R$ antagonists show potential as preventative agents for type-2 diabetes, with Merck reporting both potent competitive and non-competitive antagonists, with the pro-drug **116** being the most advanced and promising clinical candidate.[155] Antagonists of the $P2Y_2R$ look to be therapeutically viable in a variety of conditions, with a growing body of evidence to support their use as anti-metastatic agents.[49] Several patents published by AstraZeneca reported the most relevant $P2Y_2R$ antagonists; with **43** being used by many research groups in *in vivo* and *ex vivo* studies.[55,56] There is a need to develop drug-like $P2Y_2R$ antagonists which would further validate the therapeutic potential of $P2Y_2R$ antagonism in many disease states.

In our opinion there are at present no drug-like $P2Y_4R$, $P2Y_6R$, $P2Y_{11}R$ and $P2Y_{13}R$ antagonists in the published or patent literature. The structural class of the majority of these antagonists can, with exceptions, be derived from the broad-spectrum P2 receptor antagonists: **49**, **53** and **54**. Although some of these have shown potency and selectivity, their overall physico chemical properties are not commensurate with good oral absorption and metabolic stability. With growing evidence for these receptors having a fundamental role in a variety of disease states, the identification of new lead-like compounds, will hopefully afford new P2YR clinical candidates.

Throughout the identification and optimization of $P2Y_1$, $P2Y_{12}$ and $P2Y_{14}R$ antagonists, radio-ligand binding and Ca^{2+} mobilization (FLIPR-based) HTS assays have been reported, that helped identify new lead-like receptor antagonists. Importantly, several fluorescent ligands have recently been reported for the $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{14}$ receptors.[157,159,160] We feel that these, and future fluorescent ligands, could underpin fragment-based approaches to identify new lead-like antagonists of the P2Y receptors. These fluorescent ligands will also be of huge importance in interrogating the pharmacology of these receptors in healthy and diseased cell lines. The most significant recent development towards understanding the

binding of agonists and antagonists to P2YRs, has been the publication of ligand-bound $P2Y_1R$[12] and $P2Y_{12}R$[13,14] X-ray crystal structures. The homology models that have been reported for P2Y receptors have predominately focused on agonist-nucleotide binding. With this new structural information, and *in-lieu* of structural information for all P2Y receptors, there is now scope to develop antagonist-focused homology models across the two subsets of $P2Y_1$-like and $P2Y_{12}$-like receptors. This is something which will undoubtedly aid identification and optimization of new lead-like antagonists of P2Y receptors.

Biographies

Sean Conroy received his Master's in Chemistry (MChem) from The University of Southampton (U.K.) in 2013. During which, he also worked in industry on the development of oral small molecule plasma kallikrein inhibitors. Currently he is a doctoral student within the Division of Medicinal Chemistry and Structural Biology at the University of Nottingham (U.K.), under the supervision of Dr. Michael Stocks, Prof. Barrie Kellam and Prof. Stephen Hill with his research focusing on the development of P2Y receptor antagonists.

Nicholas Kindon received his degree in Natural Sciences from Cambridge University in 1984 and stayed on to do a PhD in Organic Chemistry under the supervision of Dr. Ian Fleming, completing in 1987. He then undertook 2 years of post-doctoral research with Professor Steven D. Burke at the University of Wisconsin. Upon his return to the UK he started his industrial career at Fison's Pharmaceuticals, later to become Astra then AstraZeneca where he remained for over 20 years. He is currently a research fellow in the Centre for Biomolecular Sciences at the University of Nottingham.

Barrie Kellam is Professor of Medicinal Chemistry in the School of Pharmacy and his research is focused on the synthesis of novel molecular entities and their application to a wide arena of biological and pharmacological problems. Most recently his research has centred on the medicinal chemistry of G- Protein Coupled Receptors (GPCRs) and current work is focused on the beta-adrenoceptor, adenosine, cannabinoid, orexin, P2Y and

histamine families. His research also involves the area of fluorescent ligand design. This work has produced numerous papers and patents and also resulted in the successful formation of the University of Nottingham spinout company CellAura Technologies Ltd.

Michael Stocks was appointed as an Associate Professor in Medicinal Chemistry in 2012 within the School of Pharmacy at The University of Nottingham. He has over 20 years of industrial experience in drug discovery within AstraZeneca and during his industrial career; he was both the lead scientist and project leader on multiple pre-clinical research projects as well as being the synthetic medicinal chemistry lead of the AstraZeneca compound enhancement initiative. Since joining the School of Pharmacy in 2012, he has grown his research group and his research has focused on the medicinal chemistry design of compounds to study and modulate the function of biological targets.

Acknowledgment: The authors thank the Medical Research Council for financial support to Sean Conroy and Nicholas Kindon (MRC grant code: MR/L016389/1).

Corresponding Author Information: Michael.stocks@nottingham.ac.uk. Tel +44 (0)115 951 5151

Abbreviations Used: ADME absorption, distribution, metabolism, and excretion; Ap_4A diadenosine tetraphosphate; ATP adenosine-5'-diphosphate; CF cystic fibrosis; CHO Chinese Hamster Ovary; CL clearance; FLIPR fluorescent imaging plate reader; F bioavailability; GPCR G protein-coupled receptor; HEK human embryonic kidney; *h*ERG human ether-à-go-go-related gene; *h*PRP human platelet rich plasma; HTS high throughput screening; PA platelet aggregation; PK pharmacokinetics; PPB plasma protein binding; SAR structure activity relationship; $T_{1/2}$ terminal half-life; UTP uridine triphosphate; V_{ss} volume of distribution.

References:

(1) Burnstock, G. Introduction: P2 Receptors. *Curr. Top. Med. Chem.* **2004**, *4* (8), 793–

803.

(2) Burnstock, G. Purinergic Nerves. *Pharmacol. Rev.* **1972**, *24* (3), 509–581.

(3) Burnstock, G.; Kennedy, C. Is There a Basis for Distinguishing Two Types of P2-Purinoceptor? *Gen. Pharmacol.* **1985**, *16* (5), 433–440.

(4) Burnstock, G. Purine and Pyrimidine Receptors. *Cell. Mol. Life Sci.* **2007**, *64* (12), 1471–1483.

(5) Boeynaems, J.-M.; Robaye, B.; Janssens, R.; Suarez-Huerta, N.; Communi, D. Overview of P2Y Receptors as Therapeutic Targets. *Drug Dev. Res.* **2001**, *52* (1-2), 187–189.

(6) Lipinski, C. A. Drug-like Properties and the Causes of Poor Solubility and Poor Permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44* (1), 235–249.

(7) Di, L.; Kerns, E.; Carter, G. Drug-Like Property Concepts in Pharmaceutical Design. *Curr. Pharm. Des.* **2009**, *15* (19), 2184–2194.

(8) Navia, M.; Chaturvedi, P. Design Principles for Orally Bioavailable Drugs. *Drug Discov. Today* **1996**, *1* (5), 179–189.

(9) Boeynaems, J.-M.; Communi, D.; Robaye, B. Overview of the Pharmacology and Physiological Roles of P2Y Receptors. *Wiley Interdiscip. Rev. Membr. Transp. Signal.* **2012**, *1* (5), 581–588.

(10) Alexander, S.; Mathie, A.; Peters, J. Guide to Receptors and Channels (GRAC), 5th Edition. *Br. J. Pharmacol.* **2011**, *164* (November), S1–S2.

(11) Jacobson, K. A.; Ivanov, A. A.; de Castro, S.; Harden, T. K.; Ko, H. Development of Selective Agonists and Antagonists of P2Y Receptors. *Purinergic Signal.* **2009**, *5* (1), 75–89.

(12) Zhang, D.; Gao, Z.-G.; Zhang, K.; Kiselev, E.; Crane, S.; Wang, J.; Paoletta, S.; Yi, C.; Ma, L.; Zhang, W.; Han, G. W.; Liu, H.; Cherezov, V.; Katritch, V.; Jiang, H.;

Stevens, R. C.; Jacobson, K. A.; Zhao, Q.; Wu, B. Two Disparate Ligand-Binding Sites in the Human $P2Y_1$ Receptor. *Nature* **2015**, *520* (7547), 317–321.

(13) Zhang, K.; Zhang, J.; Gao, Z.-G.; Zhang, D.; Zhu, L.; Han, G. W.; Moss, S. M.; Paoletta, S.; Kiselev, E.; Lu, W.; Fenalti, G.; Zhang, W.; Müller, C. E.; Yang, H.; Jiang, H.; Cherezov, V.; Katritch, V.; Jacobson, K. A.; Stevens, R. C.; Wu, B.; Zhao, Q. Structure of the Human $P2Y_{12}$ Receptor in Complex with an Antithrombotic Drug. *Nature* **2014**, *509* (7498), 115–118.

(14) Zhang, J.; Zhang, K.; Gao, Z.-G.; Paoletta, S.; Zhang, D.; Han, G. W.; Li, T.; Ma, L.; Zhang, W.; Müller, C. E.; Yang, H.; Jiang, H.; Cherezov, V.; Katritch, V.; Jacobson, K. A.; Stevens, R. C.; Wu, B.; Zhao, Q. Agonist-Bound Structure of the Human $P2Y_{12}$ Receptor. *Nature* **2014**, *509* (7498), 119–122.

(15) Jacobson, K. A.; Deflorian, F.; Mishra, S.; Costanzi, S. Pharmacochemistry of the Platelet Purinergic Receptors. *Purinergic Signal.* **2011**, *7* (3), 305–324.

(16) Gachet, C. P2 Receptors, Platelet Function and Pharmacological Implications. *Thromb. Haemost.* **2008**, *99*, 466–472.

(17) Jin, J.; Daniel, J. L.; Kunapuli, S. P. Molecular Basis for ADP-Induced Platelet Activation: II. The $P2Y_1$ Receptor Mediates ADP-Induced Intracellular Calcium Mobilization and Shape Change in Platelets. *J. Biol. Chem.* **1998**, *273* (4), 2030–2034.

(18) Brennan, C. A.; Gumport, R. I. T4 RNA Ligase Catalyzed Synthesis of Base Analogue-Containing Oligodeoxyribonucleotides and a Characterization of Their Thermal Stabilities. *Nucleic Acids Res.* **1985**, *13* (24), 8665–8684.

(19) Lenain, N.; Freund, M.; Léon, C.; Cazenave, J.-P.; Gachet, C. Inhibition of Localized Thrombosis in $P2Y_1$-Deficient Mice and Rodents Treated with MRS2179, a $P2Y_1$ Receptor Antagonist. *J. Thromb. Haemost.* **2003**, *1* (6), 1144–1149.

(20) Leon, C.; Freund, M.; Ravanat, C.; Baurand, A.; Cazenave, J.-P.; Gachet, C. Key Role of the $P2Y_1$ Receptor in Tissue Factor-Induced Thrombin-Dependent Acute Thromboembolism : Studies in $P2Y_1$-Knockout Mice and Mice Treated With a $P2Y_1$ Antagonist. *Circulation* **2001**, *103* (5), 718–723.

(21) Fabre, J. E.; Nguyen, M.; Latour, A.; Keifer, J. A.; Audoly, L. P.; Coffman, T. M.; Koller, B. H. Decreased Platelet Aggregation, Increased Bleeding Time and Resistance to Thromboembolism in $P2Y_1$-Deficient Mice. *Nat. Med.* **1999**, *5* (10), 1199–1202.

(22) Schumacher, W. A.; Bostwick, J. S.; Ogletree, M. L.; Stewart, A. B.; Steinbacher, T. E.; Hua, J.; Price, L. A.; Wong, P. C.; Rehfuss, R. P. Biomarker Optimization to Track the Antithrombotic and Hemostatic Effects of Clopidogrel in Rats. *J. Pharmacol. Exp. Ther.* **2007**, *322* (1), 369–377.

(23) Hechler, B.; Gachet, C. P2 Receptors and Platelet Function. *Purinergic Signal.* **2011**, *7* (3), 293–303.

(24) Cattaneo, M. Platelet P2 Receptors: Old and New Targets for Antithrombotic Drugs. *Expert Rev. Cardiovasc. Ther.* **2007**, *5* (1), 45–55.

(25) Camaioni, E.; Boyer, J. L.; Mohanram, A.; Harden, T. K.; Jacobson, K. A. Deoxyadenosine Bisphosphate Derivatives as Potent Antagonists at $P2Y_1$ Receptors. *J. Med. Chem.* **1998**, *41* (2), 183–190.

(26) Kim, Y.-C.; Gallo-Rodriguez, C.; Jang, S.-Y.; Nandanan, E.; Adams, M.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. Acyclic Analogues of Deoxyadenosine 3‘,5‘-Bisphosphates as $P2Y_1$ Receptor Antagonists. *J. Med. Chem.* **2000**, *43* (4), 746–755.

(27) Sak, K.; Uri, A.; Enkvist, E.; Raidaru, G.; Subbi, J.; Kelve, M.; Järv, J. Adenosine-Derived Non-Phosphate Antagonists for $P2Y_1$ Purinoceptors. *Biochem. Biophys. Res. Commun.* **2000**, *272* (2), 327–331.

(28) Ohno, M.; Costanzi, S.; Kim, H. S.; Kempeneers, V.; Vastmans, K.; Herdewijn, P.; Maddileti, S.; Gao, Z.-G.; Harden, T. K.; Jacobson, K. A. Nucleotide Analogues Containing 2-Oxa-bicyclo[2.2.1]heptane and L-α-Threofuranosyl Ring Systems: Interactions with P2Y Receptors. *Bioorg. Med. Chem.* **2004**, *12* (21), 5619–5630.

(29) Baurand, A.; Gachet, C. The P2Y(1) Receptor as a Target for New Antithrombotic Drugs: A Review of the P2Y(1) Antagonist MRS-2179. *Cardiovasc. Drug Rev.* **2003**, *21* (1), 67–76.

(30) Chao, H.; Turdi, H.; Herpin, T. F.; Roberge, J. Y.; Liu, Y.; Schnur, D. M.; Poss, M. a.; Rehfuss, R.; Hua, J.; Wu, Q.; Price, L. A.; Abell, L. M.; Schumacher, W. A.; Bostwick, J. S.; Steinbacher, T. E.; Stewart, A. B.; Ogletree, M. L.; Huang, C. S.; Chang, M.; Cacace, A. M.; Arcuri, M. J.; Celani, D.; Wexler, R. R.; Lawrence, R. M. Discovery of 2-(Phenoxypyridine)-3-Phenylureas as Small Molecule $P2Y_1$ Antagonists. *J. Med. Chem.* **2013**, *56* (4), 1704–1714.

(31) Wang, T. C.; Qiao, J. X.; Clark, C. G.; Jua, J.; Price, L. A.; Wu, Q.; Chang, M.; Zheng, J.; Huang, C. S.; Everlof, G.; Schumacher, W. A.; Wong, P. C.; Seiffert, D. A.; Stewart, A. B.; Bostwick, J. S.; Crain, E. J.; Watson, C. A.; Rehfuss, R.; Wexler, R. R.; Lam, P. Y. S. Discovery of Diarylurea $P2Y_1$ Antagonists with Improved Aqueous Solubility. *Bioorg. Med. Chem. Lett.* **2013**, *23* (11), 3239–3243.

(32) Ruel, R.; L'Heureux, A.; Thibeault, C.; Daris, J.-P.; Martel, A.; Price, L.; Wu, Q.; Hua, J.; Wexler, R. R.; Rehfuss, R.; Lam, P. Y. S. New Azole Antagonists with High Affinity for the $P2Y_1$ Receptor. *Bioorg. Med. Chem. Lett.* **2013**, *23* (12), 3519–3522.

(33) Pi, Z.; Sutton, J.; Lloyd, J.; Hua, J.; Price, L.; Wu, Q.; Chang, M.; Zheng, J.; Rehfuss, R.; Huang, C. S.; Wexler, R. R.; Lam, P. Y. S. 2-Aminothiazole Based $P2Y_1$ Antagonists as Novel Antiplatelet Agents. *Bioorg. Med. Chem. Lett.* **2013**, *23* (14), 4206–4209.

(34) Ruel, R.; L'Heureux, A.; Thibeault, C.; Lapointe, P.; Martel, A.; Qiao, J. X.; Hua, J.;

Price, L. A.; Wu, Q.; Chang, M.; Zheng, J.; Huang, C. S.; Wexler, R. R.; Rehfuss, R.; Lam, P. Y. S. Potent $P2Y_1$ Urea Antagonists Bearing Various Cyclic Amine Scaffolds. *Bioorg. Med. Chem. Lett.* **2013**, *23* (24), 6825–6828.

(35) Qiao, J. X.; Wang, T. C.; Ruel, R.; Thibeault, C.; L'Heureux, A.; Schumacher, W. a; Spronk, S. a; Hiebert, S.; Bouthillier, G.; Lloyd, J.; Pi, Z.; Schnur, D. M.; Abell, L. M.; Hua, J.; Price, L. a; Liu, E.; Wu, Q.; Steinbacher, T. E.; Bostwick, J. S.; Chang, M.; Zheng, J.; Gao, Q.; Ma, B.; McDonnell, P. A.; Huang, C. S.; Rehfuss, R.; Wexler, R. R.; Lam, P. Y. S. Conformationally Constrained Ortho- Anilino Diaryl Ureas: Discovery of 1-(2-(1′-Neopentylspiro[indoline-3,4′-Piperidine]-1-Yl)phenyl)-3-(4-(Trifluoromethoxy)phenyl)urea, a Potent, Selective, and Bioavailable $P2Y_1$ Antagonist. *J. Med. Chem.* **2013**, *56* (22), 9275–9295.

(36) Jeon, Y. T.; Yang, W.; Qiao, J. X.; Li, L.; Ruel, R.; Thibeault, C.; Hiebert, S.; Wang, T. C.; Wang, Y.; Liu, Y.; Clark, C. G.; Wong, H. S.; Zhu, J.; Wu, D.-R.; Sun, D.; Chen, B.-C.; Mathur, A.; Chacko, S. A.; Malley, M.; Chen, X.-Q.; Shen, H.; Huang, C. S.; Schumacher, W. A.; Bostwick, J. S.; Stewart, A. B.; Price, L. A.; Hua, J.; Li, D.; Levesque, P. C.; Seiffert, D. A.; Rehfuss, R.; Wexler, R. R.; Lam, P. Y. S. Identification of 1-{2-[4-Chloro-1′-(2,2-Dimethylpropyl)-7-Hydroxy-1,2-Dihydrospiro[indole-3,4′-Piperidine]-1-Yl]phenyl}-3-{5-Chloro-[1,3]thiazolo[5,4-B]pyridin-2-Yl}urea, a Potent, Efficacious and Orally Bioavailable $P2Y_1$ Antagonist as an Antiplatelet Ag. *Bioorg. Med. Chem. Lett.* **2014**, *24* (5), 1294–1298.

(37) Hu, C. H.; Qiao, J. X.; Han, Y.; Wang, T. C.; Hua, J.; Price, L. A.; Wu, Q.; Shen, H.; Huang, C. S.; Rehfuss, R.; Wexler, R. R.; Lam, P. Y. S. 2-Amino-1,3,4-Thiadiazoles in the 7-Hydroxy-N-Neopentyl Spiropiperidine Indolinyl Series as Potent $P2Y_1$ Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2014**, *24* (11), 2481–2485.

(38) Yang, W.; Wang, Y.; Lai, A.; Qiao, J. X.; Wang, T. C.; Hua, J.; Price, L. A; Shen, H.; Chen, X.; Wong, P.; Crain, E.; Watson, C.; Huang, C. S.; Seiffert, D. A; Rehfuss, R.;

Wexler, R. R.; Lam, P. Y. S. Discovery of 4-Aryl-7-Hydroxyindoline-Based $P2Y_1$ Antagonists as Novel Antiplatelet Agents. *J. Med. Chem.* **2014**, *57* (14), 6150–6164.

(39) Qiao, J. X.; Wang, T. C.; Hiebert, S.; Hu, C. H.; Schumacher, W.; Spronk, S.; Clark, C. G.; Han, Y.; Hua, J.; Price, L.; Shen, H.; Chacko, S.; Everlof, G.; Bostwick, J. S.; Steinbacher, T. E.; Li, Y.-X.; Huang, C. S.; Seiffert, D.; Rehfuss, R.; Wexler, R. R.; Lam, P. Y. S. 4-Benzothiazole-7-Hydroxyindolinyl Diaryl Ureas Are Potent $P2Y_1$ Antagonists with Favorable Pharmacokinetics: Low Clearance and Small Volume of Distribution. *ChemMedChem* **2014**, *9* (10), 2327–2343.

(40) Pfefferkorn, J.; Choi, C.; Winters, T.; Kennedy, R.; Chi, L.; Perrin, L.; Lu, G.; Ping, Y.-W.; McClanahan, T.; Schroeder, R.; Leininger, M. T.; Geyer, A.; Schefzick, S.; Atherton, J. $P2Y_1$ Receptor Antagonists as Novel Antithrombotic Agents. *Bioorg. Med. Chem. Lett.* **2008**, *18* (11), 3338–3343.

(41) Morales-Ramos, Á. I.; Mecom, J. S.; Kiesow, T. J.; Graybill, T. L.; Brown, G. D.; Aiyar, N. V.; Davenport, E. A.; Kallal, L. A.; Knapp-Reed, B. A.; Li, P.; Londregan, A. T.; Morrow, D. M.; Senadhi, S.; Thalji, R. K.; Zhao, S.; Burns-Kurtis, C. L.; Marino, J. P. Tetrahydro-4-Quinolinamines Identified as Novel $P2Y_1$ Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18* (23), 6222–6226.

(42) Hann, M. M. Molecular Obesity, Potency and Other Addictions in Drug Discovery. *Medchemcomm* **2011**, *2* (5), 349.

(43) Lazarowski, E. R.; Watt, W. C.; Stutts, M. J.; Boucher, R. C.; Harden, T. K. Pharmacological Selectivity of the Cloned Human P2U-Purinoceptor: Potent Activation by Diadenosine Tetraphosphate. *Br. J. Pharmacol.* **1995**, *116* (1), 1619–1627.

(44) Boarder, M. R.; Hourani, S. M. O. The Regulation of Vascular Function by P2 Receptors: Multiple Sites and Multiple Receptors. *Trends Pharmacol. Sci.* **1998**, *19* (3), 99–107.

(45) Cressman, V. L.; Lazarowski, E.; Homolya, L.; Boucher, R. C.; Koller, B. H.; Grubb, B. R. Effect of Loss of $P2Y_2$ Receptor Gene Expression on Nucleotide Regulation of Murine Epithelial Cl-Transport. *J. Biol. Chem.* **1999**, *274* (37), 26461–26468.

(46) Yerxa, B. R. Pharmacology of INS37217 [P1-(Uridine 5')-P4- (2'-deoxycytidine 5')tetraphosphate, Tetrasodium Salt], a Next-Generation $P2Y_2$ Receptor Agonist for the Treatment of Cystic Fibrosis. *J. Pharmacol. Exp. Ther.* **2002**, *302* (3), 871–880.

(47) Dixon, C. J.; Bowler, W. B.; Littlewood-Evans, A.; Dillon, J. P.; Bilbe, G.; Sharpe, G. R.; Gallagher, J. A. Regulation of Epidermal Homeostasis through $P2Y_2$ Receptors. *Br. J. Pharmacol.* **1999**, *127* (7), 1680–1686.

(48) 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 $P2Y_2$ and A3 Receptors. *Science (80-.).* **2006**, *314* (5806), 1792–1795.

(49) Schumacher, D.; Strilic, B.; Sivaraj, K. K.; Wettschureck, N.; Offermanns, S. Platelet-Derived Nucleotides Promote Tumor-Cell Transendothelial Migration and Metastasis via $P2Y_2$ Receptor. *Cancer Cell* **2013**, *24* (1), 130–137.

(50) Instant JChem Was Used for Structure Database Management, Search and Prediction, Instant JChem 16.2.15.0 2016, ChemAxon (Http://www.chemaxon.com).

(51) Kindon, N.; Meghani, P.; Thom, S. Novel Phosphate Compounds and Their Use as Medicaments. WO1998045309. **1999**.

(52) El-Tayeb, A.; Qi, A.; Nicholas, R. A.; Müller, C. E. Structural Modifications of UMP, UDP, and UTP Leading to Subtype-Selective Agonists for $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors. *J. Med. Chem.* **2011**, *54* (8), 2878–2890.

(53) Kindon, N.; Meghani, P.; Thom, S. N. C. Novel Compounds. WO1999002501 A1 **1998**.

(54) Kindon, N.; Meghani, P.; Thom, S. New Compounds which are P2-purinoceptor 7-

Transmembrane (tm) G-protein Coupled Receptor Antagonists. WO1999026944 A1 **1999**.

(55) 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.

(56) Magni, G.; Merli, D.; Verderio, C.; Abbracchio, M. P.; Ceruti, S. $P2Y_2$ Receptor Antagonists as Anti-Allodynic Agents in Acute and Sub-Chronic Trigeminal Sensitization: Role of Satellite Glial Cells. *Glia* **2015**, *63* (7), 1256–1269.

(57) Kaulich, M.; Streicher, F.; Mayer, R.; Müller, I.; Müller, C. E. Flavonoids - Novel Lead Compounds for the Development of $P2Y_2$ Receptor Antagonists. *Drug Dev. Res.* **2003**, *59* (1), 72–81.

(58) Ingólfsson, H. I.; Thakur, P.; Herold, K. F.; Hobart, E. A.; Ramsey, N. B.; Periole, X.; de Jong, D. H.; Zwama, M.; Yilmaz, D.; Hall, K.; Maretzky, T.; Hemmings, H. C.; Blobel, C.; Marrink, S. J.; Koçer, A.; Sack, J. T.; Andersen, O. S. Phytochemicals Perturb Membranes and Promiscuously Alter Protein Function. *ACS Chem. Biol.* **2014**, *9* (8), 1788–1798.

(59) 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 $P2Y_2$ Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18* (1), 223–227.

(60) Hillmann, P.; Ko, G.; Spinrath, A.; Raulf, A.; von Kügelgen, I.; Wolff, S. C.; Nicholas, R. A.; Kostenis, E.; Höltje, H.-D.; Müller, C. E. Key Determinants of Nucleotide-Activated G Protein-Coupled $P2Y_2$ Receptor Function Revealed by Chemical and Pharmacological Experiments, Mutagenesis and Homology Modeling. *J. Med. Chem.* **2009**, *52* (9), 2762–2775.

(61) Kennedy, C.; Qi, A. D.; Herold, C. L.; Harden, T. K.; Nicholas, R. A. ATP, an Agonist

at the Rat P2Y(4) Receptor, Is an Antagonist at the Human P2Y(4) Receptor. *Mol. Pharmacol.* **2000**, *57* (5), 926–931.

(62) Herold, C. L. Agonist Versus Antagonist Action of ATP at the $P2Y_4$ Receptor is Determined by the Second Extracellular Loop. *J. Biol. Chem.* **2004**, *279* (12), 11456–11464.

(63) Maruoka, H.; Jayasekara, M. P. S.; Barrett, M. O.; Franklin, D. A.; de Castro, S.; Kim, N.; Costanzi, S.; Harden, T. K.; Jacobson, K. A. Pyrimidine Nucleotides with 4-Alkyloxyimino and Terminal Tetraphosphate δ-Ester Modifications as Selective Agonists of the $P2Y_4$ Receptor. *J. Med. Chem.* **2011**, *54* (12), 4018–4033.

(64) Robaye, B. Loss of Nucleotide Regulation of Epithelial Chloride Transport in the Jejunum of $P2Y_4$-Null Mice. *Mol. Pharmacol.* **2003**, *63* (4), 777–783.

(65) Ghanem, E.; Robaye, B.; Leal, T.; Leipziger, J.; Driessche, W. Van; Beauwens, R.; Boeynaems, J.-M. The Role of Epithelial $P2Y_2$ and $P2Y_4$ Receptors in the Regulation of Intestinal Chloride Secretion. *Br. J. Pharmacol.* **2005**, *146* (3), 364–369.

(66) Charlton, S. J.; Brown, C. A.; Weisman, G. A.; Turner, J. T.; Erb, L.; Boarder, M. R. Cloned and Transfected $P2Y_4$ Receptors: Characterization of a Suramin and PPADS-Insensitive Response to UTP. *Br. J. Pharmacol.* **1996**, *119* (7), 1301–1303.

(67) Wildman, S. S.; Unwin, R. J.; King, B. F. Extended Pharmacological Profiles of Rat $P2Y_2$ and Rat $P2Y_4$ Receptors and Their Sensitivity to Extracellular H^+ and Zn^{2+} Ions. *Br. J. Pharmacol.* **2003**, *140* (7), 1177–1186.

(68) Cavaliere, F.; Amadio, S.; Angelini, D. F.; Sancesario, G.; Bernardi, G.; Volonté, C. Role of the Metabotropic $P2Y_4$ Receptor during Hypoglycemia: Cross Talk with the Ionotropic NMDAR1 Receptor. *Exp. Cell Res.* **2004**, *300* (1), 149–158.

(69) Maruoka, H.; Barrett, M. O.; Ko, H.; Tosh, D. K.; Melman, A.; Burianek, L. E.; Balasubramanian, R.; Berk, B.; Costanzi, S.; Harden, T. K.; Jacobson, K. A.

Pyrimidine Ribonucleotides with Enhanced Selectivity as $P2Y_6$ Receptor Agonists: Novel 4-Alkyloxyimino, (S)-Methanocarba, and 5′-Triphosphate γ-Ester Modifications. *J. Med. Chem.* **2010**, *53* (11), 4488–4501.

(70) Silva, I.; Ferreirinha, F.; Magalhães-Cardoso, M. T.; Silva-Ramos, M.; Correia-de-Sá, P. Activation of $P2Y_6$ Receptors Facilitates Nonneuronal Adenosine Triphosphate and Acetylcholine Release from Urothelium with the Lamina Propria of Men with Bladder Outlet Obstruction. *J. Urol.* **2015**, *194* (4), 1146–1154.

(71) Hansen, A.; Alston, L.; Tulk, S. E.; Schenck, L. P.; Grassie, M. E.; Alhassan, B. F.; Veermalla, A. T.; Al-Bashir, S.; Gendron, F.-P.; Altier, C.; MacDonald, J. A.; Beck, P. L.; Hirota, S. A. The $P2Y_6$ Receptor Mediates Clostridium Difficile Toxin-Induced CXCL8/IL-8 Production and Intestinal Epithelial Barrier Dysfunction. *PLoS One* **2013**, *8* (11), e81491.

(72) Bar, I.; Guns, P.; Metallo, J.; Cammarata, D.; Wilkin, F.; Boeynams, J.-M.; Bult, H.; Robaye, B. Knockout Mice Reveal a Role for $P2Y_6$ Receptor in Macrophages, Endothelial Cells, and Vascular Smooth Muscle Cells. *Mol. Pharmacol.* **2008**, *74* (3), 777–784.

(73) Uratsuji, H.; Tada, Y.; Kawashima, T.; Kamata, M.; Hau, C. S.; Asano, Y.; Sugaya, M.; Kadono, T.; Asahina, A.; Sato, S.; Tamaki, K. $P2Y_6$ Receptor Signaling Pathway Mediates Inflammatory Responses Induced by Monosodium Urate Crystals. *J. Immunol.* **2012**, *188* (1), 436–444.

(74) Mamedova, L. K.; Joshi, B. V; Gao, Z.-G.; von Kügelgen, I.; Jacobson, K. A. Diisothiocyanate Derivatives as Potent, Insurmountable Antagonists of $P2Y_6$ Nucleotide Receptors. *Biochem. Pharmacol.* **2004**, *67* (9), 1763–1770.

(75) Meltzer, D.; Ethan, O.; Arguin, G.; Nadel, Y.; Danino, O.; Lecka, J.; Sévigny, J.; Gendron, F.-P.; Fischer, B. Synthesis and Structure–activity Relationship of Uracil Nucleotide Derivatives towards the Identification of Human $P2Y_6$ Receptor

Antagonists. *Bioorg. Med. Chem.* **2015**, *23* (17), 5764–5773.

(76) Riegel, A.-K.; Faigle, M.; Zug, S.; Rosenberger, P.; Robaye, B.; Boeynaems, J.-M.; Idzko, M.; Eltzschig, H. K. Selective Induction of Endothelial $P2Y_6$ Nucleotide Receptor Promotes Vascular Inflammation. *Blood* **2011**, *117* (8), 2548–2555.

(77) Oprea, T. I.; Allu, T. K.; Fara, D. C.; Rad, R. F.; Ostopovici, L.; Bologa, C. G. Lead-Like, Drug-like or "Pub-Like": How Different Are They? *J. Comput. Aided. Mol. Des.* **2007**, *21* (1-3), 113–119.

(78) Communi, D.; Govaerts, C.; Parmentier, M.; Boeynaems, J. M. Cloning of a Human Purinergic P2Y Receptor Coupled to Phospholipase C and Adenylyl Cyclase. *J. Biol. Chem.* **1997**, *272* (51), 31969–31973.

(79) Communi, D.; Robaye, B.; Boeynaems, J.-M. Pharmacological Characterization of the Human $P2Y_{11}$ Receptor. *Br. J. Pharmacol.* **1999**, *128* (6), 1199–1206.

(80) Moore, D. J.; Chambers, J. K.; Wahlin, J. P.; Tan, K. B.; Moore, G. B.; Jenkins, O.; Emson, P. C.; Murdock, P. R. Expression Pattern of Human P2Y Receptor Subtypes: A Quantitative Reverse Transcription-Polymerase Chain Reaction Study. *Biochim. Biophys. Acta* **2001**, *1521* (1-3), 107–119.

(81) Berchtold, S.; Ogilvie, A. L. J.; Bogdan, C.; Mühl-Zürbes, P.; Ogilvie, A.; Schuler, G.; Steinkasserer, A. Human Monocyte Derived Dendritic Cells Express Functional P2X and P2Y Receptors as Well as Ecto-Nucleotidases. *FEBS Lett.* **1999**, *458* (3), 424–428.

(82) Chen, Y. A Putative Osmoreceptor System That Controls Neutrophil Function through the Release of ATP, Its Conversion to Adenosine, and Activation of A2 Adenosine and P2 Receptors. *J. Leukoc. Biol.* **2004**, *76* (1), 245–253.

(83) Conigrave, A. D.; Fernando, K. C.; Gu, B.; Tasevski, V.; Zhang, W.; Luttrell, B. M.; Wiley, J. S. $P2Y_{11}$ Receptor Expression by Human Lymphocytes: Evidence for Two

cAMP-Linked Purinoceptors. *Eur. J. Pharmacol.* **2001**, *426* (3), 157–163.

(84) Wilkin, F.; Duhant, X.; Bruyns, C.; Suarez-Huerta, N.; Boeynaems, J.-M.; Robaye, B. The $P2Y_{11}$ Receptor Mediates the ATP-Induced Maturation of Human Monocyte-Derived Dendritic Cells. *J. Immunol.* **2001**, *166* (12), 7172–7177.

(85) Schnurr, M. ATP Gradients Inhibit the Migratory Capacity of Specific Human Dendritic Cell Types: Implications for $P2Y_{11}$ Receptor Signaling. *Blood* **2003**, *102* (2), 613–620.

(86) Chadet, S.; Ivanes, F.; Benoist, L.; Salmon-Gandonniere, C.; Guibon, R.; Velge-Roussel, F.; Babuty, D.; Baron, C.; Roger, S.; Angoulvant, D. Hypoxia/Reoxygenation Inhibits $P2Y_{11}$ Receptor Expression and Its Immunosuppressive Activity in Human Dendritic Cells. *J. Immunol.* **2015**, *195* (2), 651–660.

(87) Sakaki, H.; Tsukimoto, M.; Harada, H.; Moriyama, Y.; Kojima, S. Autocrine Regulation of Macrophage Activation via Exocytosis of ATP and Activation of $P2Y_{11}$ Receptor. *PLoS One* **2013**, *8* (4), e59778.

(88) Ishimaru, M.; Tsukimoto, M.; Harada, H.; Kojima, S. Involvement of $P2Y_{11}$ Receptor in IFN-γ-Induced IL-6 Production in Human Keratinocytes. *Eur. J. Pharmacol.* **2013**, *703* (1-3), 67–73.

(89) Nagakura, C.; Negishi, Y.; Tsukimoto, M.; Itou, S.; Kondo, T.; Takeda, K.; Kojima, S. Involvement of $P2Y_{11}$ Receptor in Silica Nanoparticles 30-Induced IL-6 Production by Human Keratinocytes. *Toxicology* **2014**, *322*, 61–68.

(90) Meis, S.; Hamacher, A.; Hongwiset, D. NF546 [4,4'-(Carbonylbis(imino-3,1-Phenylene-Carbonylimino-3,1-(4-Methyl-Phenylene)-Carbonylimino))-bis(1,3-Xylene-Alpha,alpha'-diphosphonic Acid) Tetrasodium Salt] Is a Non-Nucleotide $P2Y_{11}$ Agonist and Stimulates Release of Interleukin-8 from Human Monoc. *J. Pharmacol. Exp. Ther.* **2010**, *332* (1), 238–247.

(91) Vaughan, K. R.; Stokes, L.; Prince, L. R.; Marriott, H. M.; Meis, S.; Kassack, M. U.;

Bingle, C. D.; Sabroe, I.; Surprenant, A.; Whyte, M. K. B. Inhibition of Neutrophil Apoptosis by ATP Is Mediated by the $P2Y_{11}$ Receptor. *J. Immunol.* **2007**, *179* (12), 8544–8553.

(92) Ullmann, H.; Meis, S.; Hongwiset, D.; Marzian, C.; Wiese, M.; Nickel, P.; Communi, D.; Boeynaems, J.-M.; Wolf, C.; Hausmann, R.; Schmalzing, G.; Kassack, M. U. Synthesis and Structure−Activity Relationships of Suramin-Derived $P2Y_{11}$ Receptor Antagonists with Nanomolar Potency. *J. Med. Chem.* **2005**, *48* (22), 7040–7048.

(93) Barragán-Iglesias, P.; Mendoza-Garcés, L.; Pineda-Farias, J. B.; Solano-Olivares, V.; Rodríguez-Silverio, J.; Flores-Murrieta, F. J.; Granados-Soto, V.; Rocha-González, H. I. Participation of Peripheral $P2Y_1$, $P2Y_6$ and $P2Y_{11}$ Receptors in Formalin-Induced Inflammatory Pain in Rats. *Pharmacol. Biochem. Behav.* **2015**, *128*, 23–32.

(94) Barragán-Iglesias, P.; Pineda-Farias, J.; Cervantes-Durán, C.; Bravo-Hernández, M.; Rocha-González, H.; Murbartián, J.; Granados-Soto, V. Role of Spinal $P2Y_6$ and $P2Y_{11}$ Receptors in Neuropathic Pain in Rats: Possible Involvement of Glial Cells. *Mol. Pain* **2014**, *10* (1), 29.

(95) Ecke, D.; Fischer, B.; Reiser, G. Diastereoselectivity of the $P2Y_{11}$ Nucleotide Receptor: Mutational Analysis. *Br. J. Pharmacol.* **2008**, *155* (8), 1250–1255.

(96) Gaarder, A.; Jonsen, J.; Laland, S.; Hellem, A.; Owren, P. A. Adenosine Diphosphate in Red Cells as a Factor in the Adhesiveness of Human Blood Platelets. *Nature* **1961**, *192*, 531–532.

(97) Born, G. V. R. Aggregation of Blood Platelets by Adenosine Diphosphate and Its Reversal. *Nature* **1962**, *194* (4832), 927–929.

(98) Hollopeter, G.; Jantzen, H. M.; Vincent, D.; Li, G.; England, L.; Ramakrishnan, V.; Yang, R. B.; Nurden, P.; Nurden, A.; Julius, D.; Conley, P. B. Identification of the Platelet ADP Receptor Targeted by Antithrombotic Drugs. *Nature* **2001**, *409* (6817), 202–207.

(99) Nicholas, R. A. Identification of the P2Y(12) Receptor: A Novel Member of the P2Y Family of Receptors Activated by Extracellular Nucleotides. *Mol. Pharmacol.* **2001**, *60* (3), 416–420.

(100) Maffrand, J. P.; Eloy, F. Synthèse de Thiénopyridines et de Furopyridines D'intérêt Thérapeutique. *Eur. J. Med. Chem. – Chim. Ther.* **1974**, *9*, 483–486.

(101) Maffrand, J. The Story of Clopidogrel and Its Predecessor, Ticlopidine: Could These Major Antiplatelet and Antithrombotic Drugs Be Discovered and Developed Today? *Comptes Rendus Chim.* **2012**, *15* (8), 737–743.

(102) Savi, P.; Labouret, C.; Delesque, N.; Guette, F.; Lupker, J.; Herbert, J. M. $P2Y_{12}$, a New Platelet ADP Receptor, Target of Clopidogrel. *Biochem. Biophys. Res. Commun.* **2001**, *283* (2), 379–383.

(103) Ding, Z. Inactivation of the Human $P2Y_{12}$ Receptor by Thiol Reagents Requires Interaction with Both Extracellular Cysteine Residues, Cys17 and Cys270. *Blood* **2003**, *101* (10), 3908–3914.

(104) Niitsu, Y.; Jakubowski, J. A.; Sugidachi, A.; Asai, F. Pharmacology of CS-747 (Prasugrel, LY640315), a Novel, Potent Antiplatelet Agent with in Vivo $P2Y_{12}$ Receptor Antagonist Activity. *Semin. Thromb. Hemost.* **2005**, *31*, 184−194.

(105) Ingall, A. H.; Dixon, J.; Bailey, A.; Coombs, M. E.; Cox, D.; McInally, J. I.; Hunt, S. F.; Kindon, N. D.; Teobald, B. J.; Willis, P. A.; Humphries, R. G.; Leff, P.; Clegg, J. A.; Smith, J. A.; Tomlinson, W. Antagonists of the Platelet P 2T Receptor: A Novel Approach to Antithrombotic Therapy. *J. Med. Chem.* **1999**, *42* (2), 213–220.

(106) Springthorpe, B.; Bailey, A.; Barton, P.; Birkinshaw, T. N.; Bonnert, 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.; Pairaudeau, 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.

(107) Douglass, J. G.; DeCamp, J. B.; Fulcher, E. H.; Jones, W.; Mahanty, S.; Morgan, A.; Smirnov, D.; Boyer, J. L.; Watson, P. S. Adenosine Analogues as Inhibitors of $P2Y_{12}$ Receptor Mediated Platelet Aggregation. *Bioorg. Med. Chem. Lett.* **2008**, *18* (6), 2167–2171.

(108) Ye, H.; Chen, C.; Zhang, H.-C.; Haertlein, B.; Parry, T. J.; Damiano, B. P.; Maryanoff, B. E. Carba-Nucleosides as Potent Antagonists of the Adenosine 5′-Diphosphate (ADP) Purinergic Receptor ($P2Y_{12}$) on Human Platelets. *ChemMedChem* **2008**, *3* (5), 732–736.

(109) Zhang, H.; Liu, J.; Zhang, L.; Kong, L.; Yao, H.; Sun, H. Synthesis and Biological Evaluation of Ticagrelor Derivatives as Novel Antiplatelet Agents. *Bioorg. Med. Chem. Lett.* **2012**, *22* (11), 3598–3602.

(110) Tu, W.; Fan, J.; Zhang, H.; Xu, G.; Liu, Z.; Qu, J.; Yang, F.; Zhang, L.; Luan, T.; Yuan, J.; Gong, A.; Feng, J.; Sun, P.; Dong, Q. Synthesis and Biological Evaluation of Cyclopentyl-Triazolol-Pyrimidine (CPTP) Based $P2Y_{12}$ Antagonists. *Bioorg. Med. Chem. Lett.* **2014**, *24* (1), 141–146.

(111) Bach, P.; Antonsson, T.; Bylund, R.; Björkman, J.-A.; Österlund, K.; Giordanetto, F.; van Giezen, J. J. J.; Andersen, S. M.; Zachrisson, H.; Zetterberg, F. Lead Optimization of Ethyl 6-Aminonicotinate Acyl Sulfonamides as Antagonists of the $P2Y_{12}$ Receptor. Separation of the Antithrombotic Effect and Bleeding for Candidate Drug AZD1283. *J. Med. Chem.* **2013**, *56* (17), 7015–7024.

(112) Bach, P.; Boström, J.; Brickmann, K.; van Giezen, J. J. J.; Groneberg, R. D.; Harvey, D. M.; O'Sullivan, M.; Zetterberg, F. Synthesis, Structure–property Relationships and Pharmacokinetic Evaluation of Ethyl 6-Aminonicotinate Sulfonylureas as Antagonists

of the $P2Y_{12}$ Receptor. *Eur. J. Med. Chem.* **2013**, *65*, 360–375.

(113) Bach, P.; Boström, J.; Brickmann, K.; van Giezen, J. J. J.; Hovland, R.; Petersson, A. U.; Ray, A.; Zetterberg, F. A Novel Series of Piperazinyl-Pyridine Ureas as Antagonists of the Purinergic $P2Y_{12}$ Receptor. *Bioorg. Med. Chem. Lett.* **2011**, *21* (10), 2877–2881.

(114) Cruskie, M.; Mehrotra, M.; Pandey, AS.; Scarborough, R. M.; White, D. C.; Yiannikouros, G. P. Substituted-(quinazolinyl)phenyl Thiophenyl-sulfonylureas, Methods for Making and Intermediates Thereof. WO2007056167, **2007**.

(115) Bryant, J. A.; Buckman, B. O.; Isla, I. ; Moha, R. ; Morrisse, M. M.; Wei, G. P. ; X, W.; Yuan, S. P. Adenosine Diphosphate Receptor Antagonists. US2003060474, **2003**.

(116) Bryant, J.; Post, J. M.; Alexander, S.; Wang, Y.; Kent, L.; Schirm, S.; Tseng, J.; Subramanyam, B.; Buckman, B.; Islam, I.; Yuan, S.; Sullivan, M. E.; Snider, M.; Morser, J. Novel $P2Y_{12}$ Adenosine Diphosphate Receptor Antagonists for Inhibition of Platelet Aggregation (I): In Vitro Effects on Platelets. *Thromb. Res.* **2008**, *122* (4), 523–532.

(117) Wang, Y.; Vincelette, J.; da Cunha, V.; Martin-McNulty, B.; Mallari, C.; Fitch, R. M.; Alexander, S.; Islam, I.; Buckman, B. O.; Yuan, S.; Post, J. M.; Subramanyam, B.; Vergona, R.; Sullivan, M. E.; Dole, W. P.; Morser, J.; Bryant, J. A Novel P2Y(12) Adenosine Diphosphate Receptor Antagonist That Inhibits Platelet Aggregation and Thrombus Formation in Rat and Dog Models. *Thromb. Haemost.* **2007**, *97* (5), 847–855.

(118) Parlow, J. J.; Burney, M. W.; Case, B. L.; Girard, T. J.; Hall, K. A.; Hiebsch, R. R.; Huff, R. M.; Lachance, R. M.; Mischke, D. A.; Rapp, S. R.; Woerndle, R. S.; Ennis, M. D. Piperazinyl-Glutamate-Pyridines as Potent Orally Bioavailable $P2Y_{12}$ Antagonists for Inhibition of Platelet Aggregation. *Bioorg. Med. Chem. Lett.* **2009**, *19* (16), 4657–4663.

(119) Parlow, J. J.; Burney, M. W.; Case, B. L.; Girard, T. J.; Hall, K. A.; Hiebsch, R. R.; Huff, R. M.; Lachance, R. M.; Mischke, D. A.; Rapp, S. R.; Woerndle, R. S.; Ennis, M. D. Piperazinyl-Glutamate-Pyrimidines as Potent $P2Y_{12}$ Antagonists for Inhibition of Platelet Aggregation. *Bioorg. Med. Chem. Lett.* **2009**, *19* (21), 6148–6156.

(120) Parlow, J. J.; Burney, M. W.; Case, B. L.; Girard, T. J.; Hall, K. A.; Harris, P. K.; Hiebsch, R. R.; Huff, R. M.; Lachance, R. M.; Mischke, D. A.; Rapp, S. R.; Woerndle, R. S.; Ennis, M. D. Piperazinyl Glutamate Pyridines as Potent Orally Bioavailable $P2Y_{12}$ Antagonists for Inhibition of Platelet Aggregation. *J. Med. Chem.* **2010**, *53* (5), 2010–2037.

(121) Parlow, J. J.; Burney, M. W.; Case, B. L.; Girard, T. J.; Hall, K. A.; Harris, P. K.; Hiebsch, R. R.; Huff, R. M.; Lachance, R. M.; Mischke, D. A.; Rapp, S. R.; Woerndle, R. S.; Ennis, M. D. Part II: Piperazinyl-Glutamate-Pyridines as Potent Orally Bioavailable $P2Y_{12}$ Antagonists for Inhibition of Platelet Aggregation. *Bioorg. Med. Chem. Lett.* **2010**, *20* (4), 1388–1394.

(122) Caroff, E.; Meyer, E.; Treiber, A.; Hilpert, K.; Riederer, M. A. Optimization of 2-Phenyl-Pyrimidine-4-Carboxamides towards Potent, Orally Bioavailable and Selective $P2Y_{12}$ Antagonists for Inhibition of Platelet Aggregation. *Bioorg. Med. Chem. Lett.* **2014**, *24* (17), 4323–4331.

(123) Caroff, E.; Hubler, F.; Meyer, E.; Renneberg, D.; Gnerre, C.; Treiber, A.; Rey, M.; Hess, P.; Steiner, B.; Hilpert, K.; Riederer, M. A. 4-((R)-2-{[6-((S)-3-Methoxypyrrolidin-1-Yl)-2-Phenylpyrimidine-4-Carbonyl]amino}-3-Phosphonopropionyl)piperazine-1-Carboxylic Acid Butyl Ester (ACT-246475) and Its Prodrug (ACT-281959), a Novel $P2Y_{12}$ Receptor Antagonist with a Wider Therapeutic Windo. *J. Med. Chem.* **2015**, *58* (23), 9133–9153.

(124) Zech, G.; Hessler, G.; Evers, A.; Weiss, T.; Florian, P.; Just, M.; Czech, J.; Czechtizky, W.; Görlitzer, J.; Ruf, S.; Kohlmann, M.; Nazaré, M. Identification of High-

Affinity $P2Y_{12}$ Antagonists Based on a Phenylpyrazole Glutamic Acid Piperazine Backbone. *J. Med. Chem.* **2012**, *55* (20), 8615–8629.

(125) Kortum, S. W.; Lachance, R. M.; Schweitzer, B. a; Yalamanchili, G.; Rahman, H.; Ennis, M. D.; Huff, R. M.; TenBrink, R. E. Thienopyrimidine-Based $P2Y_{12}$ Platelet Aggregation Inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19* (20), 5919–5923.

(126) Boldron, C.; Besse, A.; Bordes, M.-F.; Tissandié, S.; Yvon, X.; Gau, B.; Badorc, A.; Rousseaux, T.; Barré, G.; Meneyrol, J.; Zech, G.; Nazare, M.; Fossey, V.; Pflieger, A.-M.; Bonnet-Lignon, S.; Millet, L.; Briot, C.; Dol, F.; Hérault, J.-P.; Savi, P.; Lassalle, G.; Delesque, N.; Herbert, J.-M.; Bono, F. N -[6-(4-Butanoyl-5-Methyl-1 H -Pyrazol-1-Yl)pyridazin-3-Yl]-5-Chloro-1-[2-(4-Methylpiperazin-1-Yl)-2-Oxoethyl]-1 H -Indole-3-Carboxamide (SAR216471), a Novel Intravenous and Oral, Reversible, and Directly Acting $P2Y_{12}$ Antagonist. *J. Med. Chem.* **2014**, *57* (17), 7293–7316.

(127) Baqi, Y.; Atzler, K.; Köse, M.; Glänzel, M.; Müller, C. E. High-Affinity, Non-Nucleotide-Derived Competitive Antagonists of Platelet $P2Y_{12}$ Receptors. *J. Med. Chem.* **2009**, *52* (12), 3784–3793.

(128) Hoffmann, K.; Baqi, Y.; Morena, M. S.; Glanzel, M.; Muller, C. E.; von Kugelgen, I. Interaction of New, Very Potent Non-Nucleotide Antagonists with Arg256 of the Human Platelet $P2Y_{12}$ Receptor. *J. Pharmacol. Exp. Ther.* **2009**, *331* (2), 648–655.

(129) Scarborough, R. M.; Laibelman, A. M.; Clizbe, L. A.; Fretto, L. J.; Conley, P. B.; Reynolds, E. E.; Sedlock, D. M.; Jantzen, H. Novel Tricyclic benzothiazolo[2,3-C]thiadiazine Antagonists of the Platelet ADP Receptor (P2Y(12)). *Bioorg. Med. Chem. Lett.* **2001**, *11* (14), 1805–1808.

(130) Communi, D.; Paindavoine, P.; Place, G. A.; Parmentier, M.; Boeynaems, J.-M. Expression of P2Y Receptors in Cell Lines Derived from the Human Lung. *Br. J. Pharmacol.* **1999**, *127* (2), 562–568.

(131) Zhang, F. L. P2Y13: Identification and Characterization of a Novel Galpha I-Coupled

ADP Receptor from Human and Mouse. *J. Pharmacol. Exp. Ther.* **2002**, *301* (2), 705–713.

(132) Marteau, F. Pharmacological Characterization of the Human $P2Y_{13}$ Receptor. *Mol. Pharmacol.* **2003**, *64* (1), 104–112.

(133) Burnstock, G.; Knight, G. E. Cellular Distribution and Functions of P2 Receptor Subtypes in Different Systems. *Int. Rev. Cytol. - a Surv. Cell Biol. Vol. 240* **2004**, *240*, 31–304.

(134) Kim, Y.-C.; Lee, J.-S.; Sak, K.; Marteau, F.; Mamedova, L.; Boeynaems, J.-M.; Jacobson, K. A. Synthesis of Pyridoxal Phosphate Derivatives with Antagonist Activity at the $P2Y_{13}$ Receptor. *Biochem. Pharmacol.* **2005**, *70* (2), 266–274.

(135) Espada, S.; Ortega, F.; Molina-Jijón, E.; Rojo, A. I.; Pérez-Sen, R.; Pedraza-Chaverri, J.; Miras-Portugal, M. T.; Cuadrado, A. The Purinergic $P2Y_{13}$ Receptor Activates the Nrf2/HO-1 Axis and Protects against Oxidative Stress-Induced Neuronal Death. *Free Radic. Biol. Med.* **2010**, *49* (3), 416–426.

(136) Tan, C.; Salehi, A.; Svensson, S.; Olde, B.; Erlinge, D. ADP Receptor $P2Y_{13}$ Induce Apoptosis in Pancreatic β-Cells. *Cell. Mol. Life Sci.* **2010**, *67* (3), 445–453.

(137) Tan, C.; Salehi, A.; Svensson, S.; Olde, B.; Erlinge, D. ADP Receptor $P2Y_{13}$ Induce Apoptosis in Pancreatic β-Cells. *Cell. Mol. Life Sci.* **2010**, *67* (3), 445–453.

(138) Zeng, J.; Wang, G.; Liu, X.; Wang, C.; Tian, H.; Liu, A.; Jin, H.; Luo, X.; Chen, Y. $P2Y_{13}$ Receptor-Mediated Rapid Increase in Intracellular Calcium Induced by ADP in Cultured Dorsal Spinal Cord Microglia. *Neurochem. Res.* **2014**, *39* (11), 2240–2250.

(139) Ortega, F.; Pérez-Sen, R.; Delicado, E. G.; Teresa Miras-Portugal, M. ERK1/2 Activation Is Involved in the Neuroprotective Action of $P2Y_{13}$ and $P2X_7$ Receptors against Glutamate Excitotoxicity in Cerebellar Granule Neurons. *Neuropharmacology* **2011**, *61* (8), 1210–1221.

(140) Amisten, S.; Meidute-Abaraviciene, S.; Tan, C.; Olde, B.; Lundquist, I.; Salehi, A.; Erlinge, D. ADP Mediates Inhibition of Insulin Secretion by Activation of $P2Y_{13}$ Receptors in Mice. *Diabetologia* **2010**, *53* (9), 1927–1934.

(141) Gao, Z.-G.; Ding, Y.; Jacobson, K. A. $P2Y_{13}$ Receptor Is Responsible for ADP-Mediated Degranulation in RBL-2H3 Rat Mast Cells. *Pharmacol. Res.* **2010**, *62* (6), 500–505.

(142) Lyubchenko, T.; Woodward, H.; Veo, K. D.; Burns, N.; Nijmeh, H.; Liubchenko, G. A.; Stenmark, K. R.; Gerasimovskaya, E. V. $P2Y_1$ and $P2Y_{13}$ Purinergic Receptors Mediate Ca^{2+} Signaling and Proliferative Responses in Pulmonary Artery Vasa Vasorum Endothelial Cells. *AJP Cell Physiol.* **2011**, *300* (2), C266–C275.

(143) Voss, U.; Turesson, M. F.; Robaye, B.; Boeynaems, J.-M.; Olde, B.; Erlinge, D.; Ekblad, E. The Enteric Nervous System of $P2Y_{13}$ Receptor Null Mice Is Resistant against High-Fat-Diet- and Palmitic-Acid-Induced Neuronal Loss. *Purinergic Signal.* **2014**, *10* (3), 455–464.

(144) Yano, S.; Tsukimoto, M.; Harada, H.; Kojima, S. Involvement of $P2Y_{13}$ Receptor in Suppression of Neuronal Differentiation. *Neurosci. Lett.* **2012**, *518* (1), 5–9.

(145) Chambers, J. K.; Macdonald, L. E.; Sarau, H. M.; Ames, R. S.; Freeman, K.; Foley, J. J.; Zhu, Y.; McLaughlin, M. M.; Murdock, P.; McMillan, L.; Trill, J.; Swift, A.; Aiyar, N.; Taylor, P.; Vawter, L.; Naheed, S.; Szekeres, P.; Hervieu, G.; Scott, C.; Watson, J. M.; Murphy, A. J.; Duzic, E.; Klein, C.; Bergsma, D. J.; Wilson, S.; Livi, G. P. A G Protein-Coupled Receptor for UDP-Glucose. *J. Biol. Chem.* **2000**, *275* (15), 10767–10771.

(146) Scrivens, M.; Dickenson, J. M. Functional Expression of the $P2Y_{14}$ Receptor in Human Neutrophils. *Eur. J. Pharmacol.* **2006**, *543* (1-3), 166–173.

(147) Skelton, L.; Cooper, M.; Murphy, M.; Platt, A. Human Immature Monocyte-Derived Dendritic Cells Express the G Protein-Coupled Receptor GPR105 (KIAA0001, P2Y14) and Increase Intracellular Calcium in Response to Its Agonist, Uridine

Diphosphoglucose. *J. Immunol.* **2003**, *171* (4), 1941–1949.

(148) Xu, J.; Morinaga, H.; Oh, D.; Li, P.; Chen, A.; Talukdar, S.; Lazarowski, E.; Olefsky, J. M.; Kim, J. J. GPR105 Ablation Prevents Inflammation and Improves Insulin Sensitivity in Mice with Diet-Induced Obesity. *J. Immunol.* **2012**, *189* (4), 1992–1999.

(149) Ault, A. D.; Broach, J. R. Creation of GPCR-Based Chemical Sensors by Directed Evolution in Yeast. *Protein Eng. Des. Sel.* **2005**, *19* (1), 1–8.

(150) Fricks, I. P.; Maddileti, S.; Carter, R. L.; Lazarowski, E. R.; Nicholas, R. A.; Jacobson, K. A.; Harden, T. K. UDP Is a Competitive Antagonist at the Human $P2Y_{14}$ Receptor. *J. Pharmacol. Exp. Ther.* **2008**, *325* (2), 588–594.

(151) Carter, R. L.; Fricks, I. P.; Barrett, M. O.; Burianek, L. E.; Zhou, Y.; Ko, H.; Das, A.; Jacobson, K. A.; Lazarowski, E. R.; Harden, T. K. Quantification of Gi-Mediated Inhibition of Adenylyl Cyclase Activity Reveals That UDP Is a Potent Agonist of the Human $P2Y_{14}$ Receptor. *Mol. Pharmacol.* **2009**, *76* (6), 1341–1348.

(152) Guay, D.; Beaulieu, C.; Belley, M.; Crane, S. N.; DeLuca, J.; Gareau, Y.; Hamel, M.; Henault, M.; Hyjazie, H.; Kargman, S.; Chan, C. C.; Xu, L.; Gordon, R.; Li, L.; Mamane, Y.; Morin, N.; Mancini, J.; Thérien, M.; Tranmer, G.; Truong, V. L.; Wang, Z.; Black, W. C. Synthesis and SAR of Pyrimidine-Based, Non-Nucleotide $P2Y_{14}$ Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2011**, *21* (10), 2832–2835.

(153) Hamel, M.; Henault, M.; Hyjazie, H.; Morin, N.; Bayly, C.; Skorey, K.; Therien, A. G.; Mancini, J.; Brideau, C.; Kargman, S. Discovery of Novel $P2Y_{14}$ Agonist and Antagonist Using Conventional and Nonconventional Methods. *J. Biomol. Screen.* **2011**, *16* (9), 1098–1105.

(154) Gauthier, J. Y.; Belley, M.; Deschênes, D.; Fournier, J.-F.; Gagné, S.; Gareau, Y.; Hamel, M.; Hénault, M.; Hyjazie, H.; Kargman, S.; Lavallée, G.; Levesque, J.-F.; Li, L.; Mamane, Y.; Mancini, J.; Morin, N.; Mulrooney, E.; Robichaud, J.; Thérien, M.; Tranmer, G.; Wang, Z.; Wu, J.; Black, W. C. The Identification of 4,7-Disubstituted

Naphthoic Acid Derivatives as UDP-Competitive Antagonists of $P2Y_{14}$. *Bioorg. Med. Chem. Lett.* **2011**, *21* (10), 2836–2839.

(155) Robichaud, J.; Fournier, J.-F.; Gagné, S.; Gauthier, J. Y.; Hamel, M.; Han, Y.; Hénault, M.; Kargman, S.; Levesque, J.-F.; Mamane, Y.; Mancini, J.; Morin, N.; Mulrooney, E.; Wu, J.; Black, W. C. Applying the pro-Drug Approach to Afford Highly Bioavailable Antagonists of $P2Y_{14}$. *Bioorg. Med. Chem. Lett.* **2011**, *21* (14), 4366–4368.

(156) Barrett, M. O.; Sesma, J. I.; Ball, C. B.; Jayasekara, P. S.; Jacobson, K. A.; Lazarowski, E. R.; Harden, T. K. A Selective High-Affinity Antagonist of the $P2Y_{14}$ Receptor Inhibits UDP-Glucose-Stimulated Chemotaxis of Human Neutrophils. *Mol. Pharmacol.* **2013**, *84* (1), 41–49.

(157) Kiselev, E.; Barrett, M. O.; Katritch, V.; Paoletta, S.; Weitzer, C. D.; Brown, K. A.; Hammes, E.; Yin, A. L.; Zhao, Q.; Stevens, R. C.; Harden, T. K.; Jacobson, K. A. Exploring a 2-Naphthoic Acid Template for the Structure-Based Design of $P2Y_{14}$ Receptor Antagonist Molecular Probes. *ACS Chem. Biol.* **2014**, *9* (12), 2833–2842.

(158) Baker, J. G.; Middleton, R.; Adams, L.; May, L. T.; Briddon, S. J.; Kellam, B.; Hill, S. J. Influence of Fluorophore and Linker Composition on the Pharmacology of Fluorescent Adenosine A1 Receptor Ligands. *Br. J. Pharmacol.* **2010**, *159* (4), 772–786.

(159) Jayasekara, P. S.; Barrett, M. O.; Ball, C. B.; Brown, K. A.; Hammes, E.; Balasubramanian, R.; Harden, T. K.; Jacobson, K. A. 4-Alkyloxyimino Derivatives of Uridine-5′-Triphosphate: Distal Modification of Potent Agonists as a Strategy for Molecular Probes of $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors. *J. Med. Chem.* **2014**, *57* (9), 3874–3883.

(160) Kiselev, E.; Balasubramanian, R.; Uliassi, E.; Brown, K. A.; Trujillo, K.; Katritch, V.; Hammes, E.; Stevens, R. C.; Harden, T. K.; Jacobson, K. A. Design, Synthesis, Pharmacological Characterization of a Fluorescent Agonist of the $P2Y_{14}$ Receptor.

Bioorg. Med. Chem. Lett. **2015**, *25* (21), 4733–4739.

Table of contents graphic