Bioorganic & Medicinal Chemistry Letters 19 (2009) 6148–6156

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Piperazinyl-glutamate-pyrimidines as potent $P2Y_{12}$ antagonists for inhibition of platelet aggregation

John J. Parlow [a,*], Mary W. Burney [b], Brenda L. Case [a], Thomas J. Girard [b], Kerri A. Hall [b], Ronald R. Hiebsch [b], Rita M. Huff [b], Rhonda M. Lachance [b], Deborah A. Mischke [a], Stephen R. Rapp [b], Rhonda S. Woerndle [a], Michael D. Ennis [a]

[a] Department of Medicinal Chemistry, Pfizer Global Research and Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017, USA
[b] Department of Biology, Pfizer Global Research and Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017, USA

ARTICLE INFO

Article history:
Received 23 June 2009
Revised 2 September 2009
Accepted 4 September 2009
Available online 10 September 2009

Keywords:
P2Y12 receptor
P2Y12 antagonist
GPCR antagonist
Antiplatelet
Antithrombotic
Cardiovascular disease

ABSTRACT

Piperazinyl-glutamate-pyrimidines were prepared with oxygen, nitrogen, and sulfur substitution at the 4-position of the pyrimidine leading to highly potent $P2Y_{12}$ antagonists. In particular, 4-substituted piperidine-4-pyrimidines provided compounds with exceptional potency. Pharmacokinetic and physicochemical properties were fine-tuned through modifications at the 4-position of the piperidine ring leading to compounds with good human PRP potency, selectivity, clearance and oral bioavailability.

Cardiovascular and cerebrovascular diseases are the first and third most common causes of morbidity and mortality in the western world. One current therapy for these diseases includes antiplatelet agents. There are many antiplatelet agents based on different mechanisms of action, such as aspirin, which irreversibly inhibits cyclooxygenase-1; dipyridamole and cilostazol, which are phosphodiesterase inhibitors; abciximab, eptifibatide, and tirofiban, which are intravenous, potent, glycoprotein IIb/IIIa antagonists; and thienopyridines, which are antagonists of the platelet adenosine diphosphate (ADP) receptor.[1] Plavix® (clopidogrel), belonging to the thienopyridine class, acts by means of platelet ADP inhibition and has been approved for the reduction of thrombotic events (stroke, myocardial infarction (MI), and death) for patients with acute coronary syndrome, a recent history of MI, stroke, or event-established peripheral vascular disease. $P2Y_{12}$ is an ADP G-protein coupled receptor (GPCR) found primarily on platelets.[2] ADP is an important platelet agonist that can induce a primary aggregation response and also contributes to secondary aggregation via release from platelet dense granules. ADP-induced platelet aggregation is mediated by a dual receptor system involving activation of $P2Y_1$ (activation of phospholipase C) and $P2Y_{12}$ (inhibition of adenylyl cyclase). Experimental studies have demonstrated that selective blockade of either receptor is sufficient to inhibit platelet activation. However, $P2Y_1$ has ubiquitous expression whereas $P2Y_{12}$ is a platelet-specific receptor and thus represents a more attractive therapeutic target for selective attenuation of ADP-induced platelet activation.

The $P2Y_{12}$ receptor has been identified as the molecular target of the commercial antiplatelet agent clopidogrel.[3] Clopidogrel is the only oral prescription antiplatelet that is marketed in most of the world. However, clopidogrel is a prodrug, the active metabolite of which irreversibly and selectively inhibits the $P2Y_{12}$ receptor, requiring a loading dose and several days of treatment to achieve its full effect.[4] Once it is activated, the drug becomes irreversibly bound to platelets. As a result, clopidogrel not only has a slow onset, but also a slow offset of pharmacological action. This makes it less effective in acute settings and difficult to manage if a patient bleeds, experiences a trauma, or requires surgery. In addition, there are subsets of individuals who either do not metabolize the prodrug adequately or who are resistant to the effects of clopidogrel (~12–25% of patients).[5] It is anticipated that a direct-acting, reversible $P2Y_{12}$ inhibitor will achieve a similar or significant improvement in efficacy while exhibiting an improved safety profile. Accordingly, a need still exists for new drug therapies for the treatment of subjects suffering from, or susceptible to, platelet aggregation-mediated conditions. A new $P2Y_{12}$ antagonist having one or more improved properties, such as safety

* Corresponding author. Tel.: +1 636 247 3494.
E-mail address: john.j.parlow@pfizer.com (J.J. Parlow).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.017

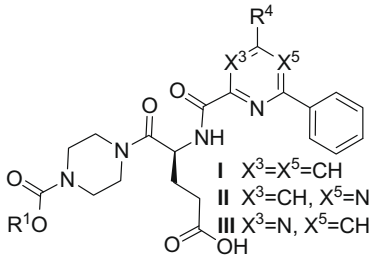
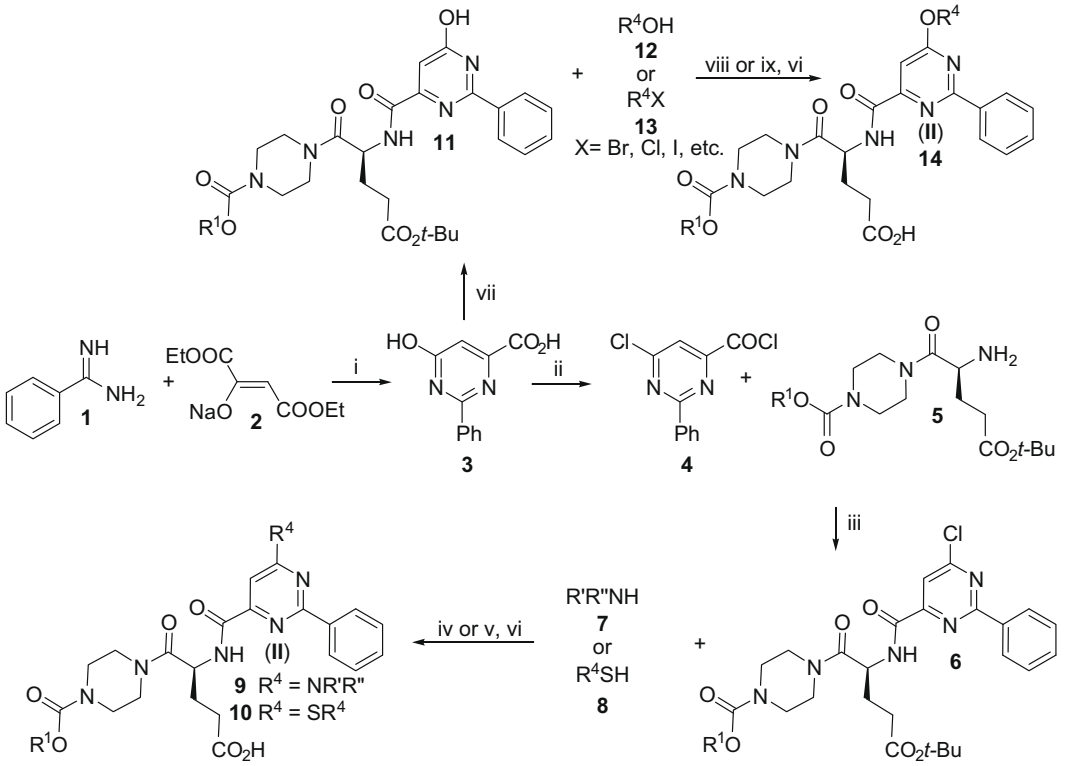


Figure 1. Piperazinyl-glutamate-pyridine/pyrimidine core structures.

profile, efficacy, or physical properties, relative to currently available $P2Y_{12}$ antagonists could fulfill this unmet need. Several groups have research efforts aimed toward discovering ADP receptor antagonists, including AR-C69931MX (Cangrelor) and AZD-6140, both of which are in clinical studies.[6] We also sought to discover and develop potent, selective $P2Y_{12}$ antagonists to address the unmet medical need for safe and effective oral antiplatelet agents.

We and others have previously reported the preparation of piperazinyl-glutamate-pyridines as potent, orally bioavailable, $P2Y_{12}$ antagonists for inhibition of platelet aggregation.[7,8] These pyridyl compounds are antagonists for the $P2Y_{12}$ receptor exhibiting subnanomolar $P2Y_{12}$ binding K_i's and sub-micromolar platelet rich plasma (PRP) IC_{50}'s with excellent selectivity over $P2Y_1$ and $P2Y_{13}$. In an effort to increase the potency and influence the pharmacokinetic properties, other core ring systems were evaluated. Depicted in Figure 1 is the general piperazinyl-glutamate-pyridine structure **I**. Focusing on the pyridine ring, one of the studies was to add an additional nitrogen into the pyridine ring system, resulting in pyrimidine ring systems **II** and **III**.

Synthetic routes to 4-substituted pyrimidine analogs (**II**) are shown in Scheme 1. It was found in the pyridine series that an *S*-glutamic acid, a butyl or pentyl carbamate on the piperazine nitrogen (R^1), and the 6-phenylpyridine were optimal for potency (Fig. 1).[7] Thus, these groups were maintained and efforts were focused on derivatization at the 4-position of the pyrimidine ring. Assembly of the pyrimidine ring was achieved by condensation of benzamidine **1** with diethyl oxalacetate sodium salt **2** to provide the hydroxypyrimidine **3** with the phenyl ring in place. The hydroxypyrimidine intermediate **3** was reacted with oxalyl chloride to afford the chloropyrimidine acid chloride **4**. Addition of the piperazinyl-glutamate amine **5** to the pyrimidine acid chloride **4** afforded the versatile 4-chloropyrimidine intermediate **6**. The 4-chloropyrimidine intermediate **6** allows for substitution with nitrogen, oxygen, and sulfur nucleophiles at the 4-position. The 4-chloro was displaced by heating intermediate **6** with an excess of amine **7** and triethylamine in DMSO at 100 °C followed by deprotection of the *t*-butyl ester with TFA to yield the desired 4-aminopyrimidine analogs **9**. Similarly, using an excess of thiol **8** with triethylamine in DMSO at 100 °C followed by deprotection of the *t*-butyl ester with TFA afforded the desired 4-thiopyrimidine analogs **10**. Displacement of the 4-chloro with oxygen nucleophiles could be accomplished, but with limited success in a parallel fashion. As a result, the 4-hydroxypyrimidine intermediate **11** was prepared by reacting the piperazinyl-glutamate amine **5** with the hydroxypyrimidine acid **3** using EDC as the coupling agent with hydroxybenzotriazole and NMM as the base. Mitsunobu conditions or direct alkylation of the hydroxypyrimidine **11** provided 4-oxygen analogs **14** as shown in Scheme 1. Direct alkylation of the 4-hydroxypyrimidine **11** was accomplished using 2 equiv of the electrophile **13** with cesium carbonate as the base and a catalytic amount of potassium iodide in DMF followed by deprotection of the *t*-butyl ester with TFA to provide the desired 4-ether pyrimidine analogs **14**. Alternatively, employing Mitsunobu conditions with 2 equiv of the alcohol **12** using DEAD and triphenylphosphine



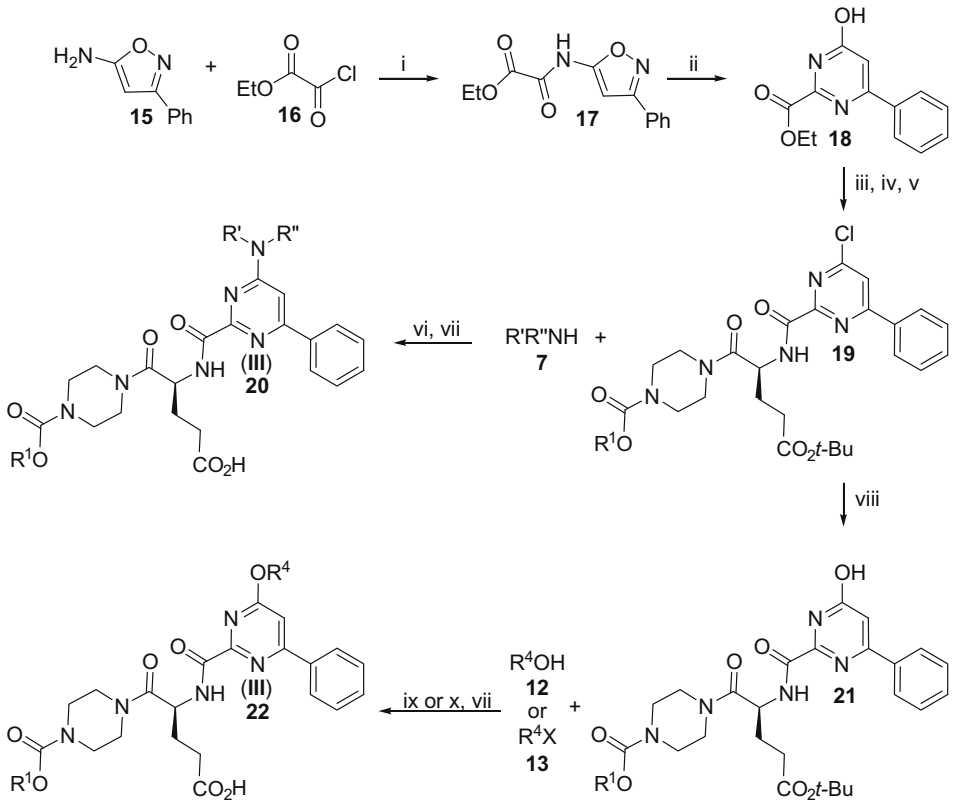
Scheme 1. Synthesis of 4-substituted pyrimidine analogs **II**. Reagents and conditions: (i) NaOH, H_2O; (ii) $Cl(CO)_2Cl$, DCM, DMF, 0 °C; (iii) 1.2 equiv **5**, DIEA, DCM; (iv) excess **7**, TEA, DMSO, 100 °C; (v) excess **8**, TEA, DMSO, 100 °C; (vi) 10% TFA/DCM; (vii) 1 equiv **5**, EDC, NMM, HOBt, DCM/DMF; (viii) 2 equiv **12**, DEAD, PPh_3, THF; (ix) 2 equiv **13**, Cs_2CO_3, KI, DMF, rt–100 °C.

in THF followed by deprotection of the *t*-butyl ester with TFA provided the 4-ether pyrimidine analogs **14**. Using both procedures allowed for a greater diversity of monomer inputs **12** and **13**, ultimately providing a wide array of 4-ether pyrimidine analogs **14**. Comparison of 4-ether analogs, prepared by both Mitsunobu conditions and direct alkylations of intermediate **11**, with analogs prepared by displacement of the 4-chloro intermediate **6** with alcohols showed the analogs were identical, demonstrating that alkylation was occurring on the oxygen and not the nitrogen.

The synthesis of 4-substituted pyrimidine analogs (**III**) is shown in Scheme 2. The pyrimidine ring was constructed by reacting 5-amino-3-phenylisoxazole **15** with ethyl oxalyl chloride **16** to afford intermediate **17**, followed by hydrogenation with platinum dioxide to provide the 4-hydroxypyrimidine intermediate **18**.[9] Hydrolysis of the ethyl ester **18** with sodium hydroxide gave the carboxylic acid, and subsequent treatment of the acid with oxalyl chloride generated the acid chloride as well as concomitant conversion of the 4-hydroxy to 4-chloro. Addition of the amine **5** to the acid chloride provided the versatile 4-chloropyrimidine intermediate **19**. The 4-chloro was displaced by heating intermediate **19** with an excess of amine **7** followed by deprotection of the *t*-butyl ester with TFA to yield the desired 4-aminopyrimidine analogs **20**. Displacement of the 4-chloropyrimidine intermediate **19** with oxygen nucleophiles was met with limited success. As a result, the 4-chloropyrimidine intermediate **19** was displaced using sodium hydroxide in THF to provide the 4-hydroxypyrimidine intermediate **21**, which could then be directly alkylated or used under Mitsunobu conditions to yield the desired 4-ether pyrimidine analogs **22**.

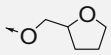
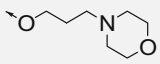
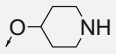
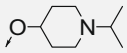
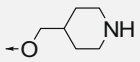
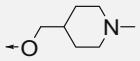
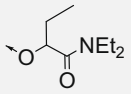
Several 100 compounds were prepared by the above syntheses and screened in a $P2Y_{12}$ receptor binding assay at 5–10 μM concentration, and K_i's were determined for those compounds with >50% inhibition.[10] These compounds were also tested as antiplatelet agents by measuring their inhibitory action on the in vitro aggregation of human platelet rich plasma (PRP) stimulated by 20 μM ADP using a turbidimetric method.[11] The compounds were initially assayed at 10 μM and IC_{50}'s were determined for compounds with >50% inhibition of platelet aggregation. Historical analysis of human PRP potency data for $P2Y_{12}$ antagonists revealed variations due to donor-to-donor variability. To reduce the influence of human donor-to-donor variability, the potency data for two reference standards (thienopyrimidine[12] and AZD-6140[6]) were determined on each 96-well plate and the data for the test compounds were normalized to the standards from that same plate. In regards to the SAR, emphasis was placed on the PRP potency as this was an indication of the functional activity taking into account the effect of protein binding.[13]

In general, the SAR trends for the pyrimidine series were very similar to the pyridine series.[7] Substituting the 4-position of either pyrimidine ring (**II** or **III**) with an amine or oxygen substituent was preferred for good $P2Y_{12}$ binding and PRP potency. Also, aliphatic straight chain carbamates of four- to five-carbon length (R^1) were preferred, and the *S*-glutamic acid piece was optimal for potency. Table 1 shows the data for a representative set of 4-oxygen analogs for both pyrimidine regioisomers **14** and **22**. While the SAR trends for both pyrimidine templates were consistent, the pyrimidine compounds **14** (**II**) were typically more potent than the corresponding regioisomers **22** (**III**). Extending a heteroatom such as an oxygen or nitrogen two to four carbon atoms away from the 4-oxygen atom tended to increase the PRP activity. In particular, the 4-oxypiperidine and



Scheme 2. Synthesis of 4-substituted pyrimidine analogs **III**. Reagents and conditions: (i) Pyridine, THF, 0 °C–rt; (ii) PtO_2, H_2, EtOH, 50 °C; (iii) NaOH, H_2O, THF; (iv) $Cl(CO)_2Cl$, DCM, DMF, 0 °C; (v) 1 equiv **5**, DIEA, DCM; (vi) excess **7**, TEA, DMSO, rt–100 °C; (vii) 10% TFA/DCM; (viii) excess NaOH, H_2O, THF, 80 °C; (ix) 2 equiv **12**, DEAD, PPh_3, THF; (x) 2 equiv **13**, Cs_2CO_3, KI, DMF, rt–100 °C.

Table 1
Binding and PRP activity data for a representative set of 4-ether pyrimidine analogs **14** (**II**) and **22** (**III**)[13]

Cmpd	X^3	X^5	R^1	R^4	K_i[a] (nM)	IC_{50}[b] (μM)	Norm ratio[c]	Norm ratio[d]
14A	CH	N	Bu		12	2.6	1.0	3.7
14a	CH	N	Pent	$-O(CH_2)_2OMe$	1.2	7.9	1.2	8.8
22a	N	CH	Pent		1.2	>10	—	—
14B	CH	N	Bu		55	4.4	1.4	3.8
14C	CH	N	Bu		72	3.0	1.1	4.3
14c	CH	N	Pent	$-O(CH_2)_2NEt_2$	27	15	2.4	17
22c	N	CH	Pent		184	>10	—	—
14d	CH	N	Pent		2.8	8.7	1.4	8.3
22d	N	CH	Pent		11	7.0	1.2	6.6
14E	CH	N	Bu		11	1.3	0.3	1.7
14F	CH	N	Bu		19	1.8	0.8	2.8
14G	CH	N	Bu		16	1.2	0.3	1.0
14g	CH	N	Pent		2.2	1.7	0.3	1.7
22g	N	CH	Pent		31	9.4	1.7	8.5
14H	CH	N	Bu		15	3.0	0.9	3.7
14h	CH	N	Pent		5.7	3.6	0.5	3.7
22h	N	CH	Pent		12	3.8	0.8	4.4
14I	CH	N	Bu		123	49	—	—

[a] Membranes from CHO cells expressing recombinant human $P2Y_{12}$ receptors incubated with ^{33}P ADP and compound. K_i values are corrected from IC_{50} using Cheng and Prusoff equation and are the geometric mean of $n = 2$ or greater.
[b] IC_{50} values are from human PRP incubated with 20 μM ADP.
[c] IC_{50} value of compound/IC_{50} value of thienopyrimidine control from same plate = normalized IC_{50} ratio.
[d] IC_{50} value of compound/IC_{50} value of AZD-6140 control from same plate = normalized IC_{50} ratio.

4-oxymethyl piperidine analogs (**E–H**) were the most potent compounds of the series. In general, the butyl carbamates were slightly more active in the PRP assay than the corresponding pentyl carbamates for the 4-oxygen substituted pyrimidine series.

Table 2 shows the data for a representative set of 4-thiopyrimidine analogs **10**. While the 4-thio substitution provided compounds with binding activity, no PRP activity was observed with these analogs, presumably due to high protein binding.[13]

Table 2
Binding and PRP activity data for a representative set of 4-thiopyrimidine analogs **10**[13]

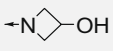
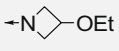
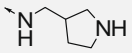
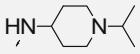
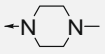
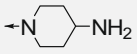
Cmpd	R^1	R^4	K_i[a] (nM)	IC_{50}[b] (μM)
10a	Pent	S*t*Bu	42	>10
10b	Pent	$S(CH_2)_2OH$	4.5	>10
10c	Pent	SCH_2CF_3	22	>10
10d	Pent		17	>10
10e	Pent		70	>10

[a] Membranes from CHO cells expressing recombinant human $P2Y_{12}$ receptors incubated with ^{33}P ADP and compound. K_i values are corrected from IC_{50} using Cheng and Prusoff equation and are the geometric mean of $n = 2$ or greater.
[b] IC_{50} values are from human PRP incubated with 20 μM ADP.

A large number of 4-aminopyrimidine analogs **9** (**II**) and **20** (**III**) was synthesized, and the data for a representative set are shown in Table 3. The SAR trends across the two pyrimidine templates **9** and **20** were consistent, and, as was observed with the corresponding 4-oxygen pyrimidines **14** and **22**, the pyrimidine compounds **9** were typically more potent with respect to both binding and PRP activity than their corresponding regioisomers **20**. In general, amino substitution at the 4-position provided pyrimidine compounds with even greater potency than the 4-oxygen analogs. The 4-amine analogs substituted with alkyl groups were less active than compounds with a heteroatom present in the side-chain (data not shown). Compounds **9a**–**d** provide examples of analogs with an oxygen heteroatom, as a hydroxy or ether, as part of the amine group with good binding and PRP activity. Extending an amine group, regardless of substitution, two to three carbon atoms away from the 4-nitrogen atom tended to increase PRP activity (**9e**–**i**). This trend was most notable with 4-substituted piperazines (**9h**) and piperidines (**9i**). As a result, efforts focused on the synthesis of additional analogs with various substitutions on the 4-nitrogen of the piperazine (**23**, **24**) and various substitutions on the 4-position of the piperidine (**25**, **26**) (Fig. 2).

A representative set of 4-substituted piperazines (**23**, **24**) is depicted in Table 4. Again, the trend was observed where the pyrimidine regioisomer **II** was the preferred isomer in terms of potency, as the pyrimidine compounds **23** (**II**) were more potent than the corresponding regioisomeric analogs **24** (**III**). These compounds had good potency with relatively flat SAR across various function-

Table 3
Binding and PRP activity data for a representative set of 4-aminopyrimidine analogs **9** (**II**) and **20** (**III**)[13]

Cmpd	X^3	X^5	R^1	R^4	K_i[a] (nM)	IC_{50}[b] (μM)	Norm ratio[c]	Norm ratio[d]
9a	H	N	Pent	$NH(CH_2)_2OMe$	1.2	2.6	0.5	2.2
20a	N	H	Pent		5.2	>10	—	—
9b	H	N	Pent		1.0	6.3	2.0	7.9
20b	N	H	Pent		11	>10	—	—
9c	H	N	Pent		2.5	3.5	0.8	4.6
9d	H	N	Pent		1.2	4.1	1.0	3.4
9e	H	N	Pent	$NH(CH_2)_3NMe_2$	4.8	2.6	0.6	1.7
20e	N	H	Pent		38	>10	—	—
9f	H	N	Pent		4.1	2.6	0.5	2.3
20f	N	H	Pent		19	>10	—	—
9g	H	N	Pent		9.4	3.7	0.9	3.5
20g	N	H	Pent		35	>10	—	—
9h	H	N	Pent		2.7	1.4	0.3	1.3
20h	N	H	Pent		11	8.1	1.8	8.1
9i	H	N	Pent		0.69	0.72	0.2	0.5
20i	N	H	Pent		8.4	2.7	0.6	3.1

[a] Membranes from CHO cells expressing recombinant human $P2Y_{12}$ receptors incubated with ^{33}P ADP and compound. K_i values are corrected from IC_{50} using Cheng and Prusoff equation and are the geometric mean of $n = 2$ or greater.
[b] IC_{50} values are from human PRP incubated with 20 μM ADP.
[c] IC_{50} value of compound/IC_{50} value of thienopyrimidine control from same plate = normalized IC_{50} ratio.
[d] IC_{50} value of compound/IC_{50} value of AZD-6140 control from same plate = normalized IC_{50} ratio.

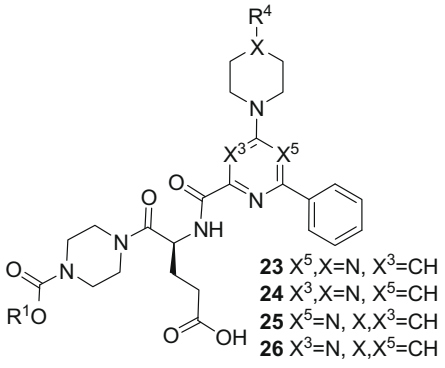


Figure 2. 4-Substituted piperazine (**23**, **24**) and piperidine (**25**, **26**) 4-pyrimidine core structures.

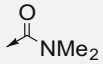
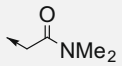
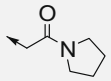
alities at the 4-position, including the unsubstituted nitrogen analog **23a**, amide **23b**, ureas **23c**–**d**, and other various groups **23e**–**g**.

Table 5 shows the data for a representative set of 4-substituted piperidine-4-pyrimidines (**25**, **26**). Overall, these compounds were optimal with respect to both binding and PRP potency. The SAR trends across the two pyrimidine templates (**25** and **26**) were consistent, and again the pyrimidine compounds **26** (**III**) were less potent than the corresponding regioisomer pyrimidine compounds **25** (**II**). Substitution of the piperidine ring system with alkyl groups (**25a**) resulted in loss of PRP activity, while substitution with amines at the 4-position of the piperidine provided compounds with excellent PRP activity (**25b–c**). Extending the amine group (primary, secondary, or tertiary) by one or two carbon atoms maintained both binding and PRP activity (**25d**–**k**). Replacing the amine with an ether link to the piperidine ring (**25m**–**o**) resulted in a three- to fourfold loss in PRP activity. Extending an oxygen atom, as a hydroxy or ether, from the 4-position of the piperidine ring also resulted in a loss of PRP activity (**25p**–**r**).

Compounds with decreased basicity were explored by preparing the corresponding amide analogs of select amines. For example, the amide of compound **25h** was prepared such that the carbonyl was at the 2-position of the pyrrolidine ring system to provide compound **25s**, which resulted in a threefold decrease in PRP activity. However, the presence of a carbonyl on the 4-piperidine methylene carbon provided compounds with exceptional potency (**25y**). In general, the 4-piperidinecarboxamides with small alkyl amides were the most potent compounds of the series (**25t–y**). Extending the amide group by an additional carbon atom away from the piperidine ring generally resulted in a loss of activity (**25aa–ee**). Unlike the 4-ether pyrimidine series (**14**, **22**), there was no consistent trend in PRP activity between the butyl carbamates and the corresponding pentyl carbamates for the 4-substituted piperidine-4-pyrimidine series (**25**, **26**).

Having identified many compounds with exceptional potency, we sought to evaluate the in vivo pharmacokinetic properties of selected analogs. In general, this class of compounds had good solubility and was chemically stable. These inhibitors also showed excellent metabolic stability in both the rat and human microsomal assays.[14] The CYP inhibition profiles were satisfactory[15] and none showed hERG activity.[16] The in vivo pharmacoki-

Table 4
Binding and PRP activity data for a representative set of 4-substituted piperazine-4-pyrimidine analogs **23** (**II**) and **24** (**III**)[13]

Cmpd	X^3	X^5	R^1	R^4	K_i[a] (nM)	IC_{50}[b] (μM)	Norm ratio[c]	Norm ratio[d]
23a	CH	N	Pent	H	2.0	1.3	0.3	1.4
24a	N	CH	Pent		22	5.2	1.1	6.0
23b	CH	N	Pent		3.7	1.5	0.3	1.0
24b	N	CH	Pent		29	6.5	1.8	8.9
23c	CH	N	Pent		1.2	0.94	0.2	0.8
24c	N	CH	Pent		5.8	3.6	1.0	4.7
23d	CH	N	Pent		2.9	1.6	0.3	1.5
24d	N	CH	Pent		14	>10	—	—
23e	CH	N	Pent		2.6	0.53	0.1	0.5
24e	N	CH	Pent		6.8	3.0	0.8	3.5
23f	CH	N	Pent		2.1	1.1	0.2	1.5
23g	CH	N	Pent	$(CH_2)_2OMe$	2.3	2.2	0.3	1.5
24g	N	CH	Pent		12	5.1	1.6	8.3

[a] Membranes from CHO cells expressing recombinant human $P2Y_{12}$ receptors incubated with ^{33}P ADP and compound. K_i values are corrected from IC_{50} using Cheng and Prusoff equation and are the geometric mean of $n = 2$ or greater.
[b] IC_{50} values are from human PRP incubated with 20 μM ADP.
[c] IC_{50} value of compound/IC_{50} value of thienopyrimidine control from same plate = normalized IC_{50} ratio.
[d] IC_{50} value of compound/IC_{50} value of AZD-6140 control from same plate = normalized IC_{50} ratio.

netic characteristics in rat of a representative set of inhibitors are profiled in Table 6. Most of the initial compounds evaluated had high clearance values despite good in vitro metabolic stability. As expected for high molecular weight carboxylic acids, these compounds generally had low volumes of distribution. The 4-substituted piperidine-4-pyrimidine series (**25**) provided compounds with exceptional potency and provided a handle to modulate the pharmacokinetic properties by varying the substituent at the 4-position of the piperidine ring. It was found that compounds with the 4-piperidine group containing a basic amine usually had high clearance values and no bioavailability, such as compound **25b** with a pyrrolidine at the 4-position, or 4-aminomethyl substituted analogs containing a primary **25d**, secondary **25e**, or tertiary amine **25h**. Despite the inferior potency with the pyrimidine series (**III**), several were profiled, including **26d**. This compound showed slight improvement with respect to clearance over the corresponding regioisomer (**25d**) but also lacked bioavailability.

Making the amine less basic by converting it into a secondary or tertiary amide had a profound effect on the clearance values. The primary amide **25t** still had a high clearance value, but the secondary and tertiary amides had acceptable clearance values as observed with the *N*-ethylamide **25v**, diethylamide **25w**, and the pyrrolidine amide **25y**, all with single-digit clearance values. Compound **25w** also displayed moderate bioavailability. Another strategy to reduce the basicity was to replace the basic amine on the piperidine with an alkoxy group (**25m–o**). This resulted in compounds with a slight loss in potency, but improved clearance values (relative to the amines) and good oral bioavailability, as seen with compound **25p**. The corresponding regioisomer pyrimidine **26p** had a slightly better clearance value, but poor bioavailability. Despite high molecular weight and, in numerous cases, poor Caco-2 permeability values, several compounds had good to acceptable bioavailability and probably have transporter-mediated absorption, as exemplified by **25p** (molecular weight 639, P_{app} 0.4×10^{-6}).

In vitro receptor binding, signaling, and functional studies have shown that these pyrimidine analogs are high-affinity, selective, and competitive antagonists at $P2Y_{12}$ receptors. All of the compounds tested showed more than 340-fold selectivity for $P2Y_{12}$ over the other purinergic receptors tested, including the closest homologue, $P2Y_{13}$ (48% homology to $P2Y_{12}$), and a second platelet purinergic GPCR, $P2Y_1$ (19% homology to $P2Y_{12}$).

In summary, we have shown that changing the core ring system of the piperazinyl-glutamate-pyridines from pyridine (**I**) to pyrimidine (**II** and **III**) provides highly potent $P2Y_{12}$ antagonists. In general, the pyrimidine template (**II**) was more potent than the regioisomer pyrimidine template (**III**). The 4-nitrogen substituted pyrimidine series proved more potent than the 4-oxygen and 4-thio substituted pyrimidines. In particular, the 4-substituted piperidine-4-pyrimidine series (**25**) provided compounds with exceptional potency with sub-nanomolar $P2Y_{12}$ binding K_i's and sub-micromolar PRP IC_{50}'s (vs 20 μM ADP). With sufficient levels of potency attained, pharmacokinetic and physicochemical properties were modulated by altering the substituent at the 4-position of the piperidine ring (**25**, **26**). Compounds with basic groups as the 4-substituent of the piperidine ring generally had high in vivo clearance. However, when the amine was replaced with a non-basic group, acceptable clearance values could be achieved. Non-basic groups, such as ethers, improved the clearance and provided compounds with good bioavailability (**25p**, $F = 61\%$). Other non-basic groups, such as 4-piperidinecarboxamides with small alkyl amides, provided low clearance compounds, with compound **25w** having a clearance of 6 mL/min/kg and bioavailability of 38%. The potency, selectivity, safety, and pharmacokinetic profiles for these piperazinyl-glutamate-pyrimidines $P2Y_{12}$ antagonists support further evaluation.

Table 5
Binding and PRP activity data for a representative set of 4-substituted piperidine-4-pyrimidine analogs **25** (**II**) and **26** (**III**)[13]

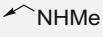
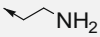
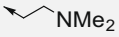
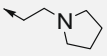
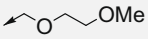
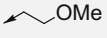
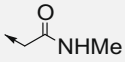
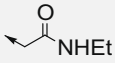
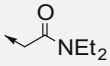
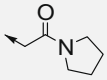
Cmpd	X^3	X^5	R^1	R^4	K_i[a] (nM)	IC_{50}[b] (μM)	Norm ratio[c]	Norm ratio[d]
25a	CH	N	Pent	Me	7.5	>10	—	—
25B	CH	N	Bu		1.7	0.66	0.2	0.8
25b	CH	N	Pent		1.5	0.51	0.1	0.4
26b	N	CH	Pent		7.4	2.1	0.4	2.4
25C	CH	N	Bu		1.3	0.88	0.2	1.1
25c	CH	N	Pent		2.9	0.78	0.2	0.7
26c	N	CH	Pent		5.9	3.0	0.8	3.9
25d	CH	N	Pent		3.0	0.49	0.1	0.6
26d	N	CH	Pent		9.4	0.77	0.2	1.2
25e	CH	N	Pent		1.2	0.74	0.1	0.7
26e	N	CH	Pent		11	1.3	0.4	1.0
25f	CH	N	Pent		2.2	0.77	0.1	0.7
26f	N	CH	Pent		11	1.2	0.4	1.6
25g	CH	N	Pent		0.61	0.86	0.2	0.8
25h	CH	N	Pent		0.40	0.56	0.1	0.7
25i	CH	N	Pent		2.7	0.62	0.1	0.4
26i	N	CH	Pent		3.0	1.4	0.4	1.9
25j	CH	N	Pent		2.8	0.40	0.1	0.4
25k	CH	N	Pent		2.1	0.81	0.1	0.5
26k	N	CH	Pent		4.2	1.7	0.4	1.7
25M	CH	N	Bu		1.4	1.8	0.4	2.7
25m	CH	N	Pent	OMe	5.0	2.7	0.5	1.6
26m	N	CH	Pent		7.4	>10	—	—
25n	CH	N	Pent	$O(CH_2)_2OMe$	5.3	1.4	0.2	0.8
26n	N	CH	Pent		7.1	5.3	1.2	5.3
25o	CH	N	Pent	$O(CH_2)_2OH$	0.71	1.3	0.4	0.9
26o	N	CH	Pent		10	>10	—	—
25p	CH	N	Pent		8.4	2.0	0.4	2.8
26p	N	CH	Pent		12	6.8	1.4	9.5
25q	CH	N	Pent		4.3	2.0	0.3	1.2
26q	N	CH	Pent		8.1	>10	—	—
25R	CH	N	Bu		2.0	1.2	0.5	2.1
25r	CH	N	Pent		10.2	6.3	1.3	5.4
26r	N	CH	Pent		8.4	>10	—	—
25s	CH	N	Pent		0.99	1.6	0.4	2.0
25t	CH	N	Pent		2.0	0.69	0.1	0.7
26t	N	CH	Pent		3.5	2.3	0.4	1.9
25U	CH	N	Bu		1.3	1.5	0.2	1.1
25u	CH	N	Pent		2.5	0.37	0.1	0.3

Table 5 (*continued*)

Cmpd	X^3	X^5	R^1	R^4	K_i[a] (nM)	IC_{50}[b] (μM)	Norm ratio[c]	Norm ratio[d]
25V	CH	N	Bu		0.38	0.49	0.1	0.6
25v	CH	N	Pent		0.60	0.93	0.2	1.5
25W	CH	N	Bu		0.79	0.28	0.1	0.6
25w	CH	N	Pent		1.5	0.40	0.1	0.6
26w	N	CH	Pent		3.6	1.3	0.4	1.5
25x	CH	N	Pent		0.83	0.23	0.04	0.3
25Y	CH	N	Bu		0.70	0.39	0.08	0.4
25y	CH	N	Pent		0.46	0.27	0.06	0.4
26y	CH	N	Pent		3.6	1.5	0.5	1.8
25aa	CH	N	Pent		0.90	1.1	0.2	1.2
25bb	CH	N	Pent		0.67	1.6	0.3	1.6
25cc	CH	N	Pent		3.1	2.6	0.6	3.3
25dd	CH	N	Pent		0.92	1.0	0.2	1.1
25ee	CH	N	Pent		1.6	1.5	0.3	2.0

[a] Membranes from CHO cells expressing recombinant human $P2Y_{12}$ receptors incubated with ^{33}P ADP and compound. K_i values are corrected from IC_{50} using Cheng and Prusoff equation and are the geometric mean of $n = 2$ or greater.
[b] IC_{50} values are from human PRP incubated with 20 μM ADP.
[c] IC_{50} value of compound/IC_{50} value of thienopyrimidine control from same plate = normalized IC_{50} ratio.
[d] IC_{50} value of compound/IC_{50} value of AZD-6140 control from same plate = normalized IC_{50} ratio.

Table 6
Rat pharmacokinetic profiles of selected $P2Y_{12}$ antagonists

Cmpd	CL (mL/min/kg)	Vdss (L/kg)	$T_{1/2,\ eff}$ (h)	F_{oral} (%)
25b	75	3.9	0.6	—
25d	33	4.9	1.7	1
25e	31	2.0	0.7	2
25h	98	7.7	0.3	1
25o	18	0.8	0.5	—
25p	14	1.1	0.9	61
25t	68	2.4	0.4	—
25V	16	0.3	0.1	7
25v	8	0.2	0.3	8
25w	6	0.21	0.4	38
25y	2	0.2	0.8	13
26d	16	0.28	0.2	1
26p	6	0.15	0.3	6

Male Sprague-Dawley rats ($n = 2$–4 rats).
Dose: iv infusion at 2 mg/kg; po at 5 mg/kg ($n = 2$–4 rats).

References and notes

1. Horiuchi, H. *Ann. Med.* **2006**, *38*, 162.
2. Gachet, C. *Thromb. Haemostasis* **2001**, *86*, 222.
3. 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. *Nature* **2001**, *409*, 202.
4. Savi, P.; Pereillo, J. M.; Uzabiaga, M. F.; Combalbert, J.; Picard, C.; Maffrand, J. P.; Pascal, M.; Herbert, J. M. *Thromb. Haemostasis* **2000**, *84*, 891.
5. Gurbel, P. A.; Bliden, K. P.; Hiatt, B. L.; O'Connor, C. M. *Circulation* **2003**, *107*, 2908.
6. (A) For reviews see: (a) Angiolillo, D. J. *Am. J. Cardiovasc. Drugs* **2007**, *7*, 423; (b) Cattaneo, M. *Ex. Rev. Cardiovasc. Ther.* **2007**, *5*, 45; (c) Storey, R. F. *Curr. Pharm. Design* **2006**, *12*, 1255; (d) Boeynaems, J. M.; van Giezen, H.; Savi, P.; Herbert, J. M. *Curr. Opin. Invest. Drugs* **2005**, *6*, 275; (B) For papers see: (a) Bryant, J.; Post, J. M.; Alexander, S.; Wang, Y. X.; Kent, L.; Schirm, S.; Tseng, J. L.; Subramanyam, B.; Buckman, B.; Islam, I.; Yuan, S.; Sullivan, M. E.; Snider, M.; Morser, J. *Thromb. Res.* **2008**, *122*, 523; (b) Post, J. M.; Alexander, S.; Wang, Y. X.; Vincelette, J.; Vergona, R.; Kent, L.; Bryant, J.; Sullivan, M. E.; Dole, W. P.; Morser, J.; Subramanyam, B. *Thromb. Res.* **2008**, *122*, 533; (c) Wang, Y. X.; 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. *Thromb. Haemostasis* **2007**, *97*, 847; (d) 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. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6013; (e) Husted, S.; Emanuelsson, H.; Heptinstall, S.; Sandset, P. M.; Wickens, M.; Peters, G. *Eur. Heart J.* **2006**, *27*, 1038; (f) Van Giezen, J. J. J.; Humphries, R. G. *Sem. Thromb. Hemost.* **2005**, *31*, 195; (g) De Marco, A.; de Candia, M.; Carotti, A.; Cellamare, S.; De Candia, E.; Altomare, C. *Eur. J. Pharm. Sci.* **2004**, *22*, 153; (h) Yang, J.; Hua, W.; Wang, F.; Wang, Z.; Wang, X. *Bioorg. Med. Chem.* **2004**, *12*, 6547; (i) Bryant, J. A.; Buckman, B. O.; Islam, I.; Mohan, R.; Morrissey, M. M.; Wei, G. P.; Xu, W.; Yuang, S. WO 052366, 2004; (j) De Candia, M.; Summo, L.; Carrieri, A.; Altomare, C.; Nardecchia, A.; Cellamare, S.; Carotti, A. *Bioorg. Med. Chem.* **2003**, *11*, 1439; (k) Yang, S. W.; Buivich, A.; Chan, T. M.; Smith, M.; Lachowicz, J.; Pomponi, S. A.; Wright, A. E.; Mierzwa, R.; Patel, M.; Gullo, V.; Chu, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1791; (l) Xu, B.; Stephens, A.; Kirschenheuter, G.; Greslin, A. F.; Cheng, X.; Sennelo, J.; Cattaneo, M.; Zighetti, M. L.; Chen, A.; Kim, S. A.; Kim, H. S.; Bischofberger, N.; Cook, G.; Jacobson, K. A. *J. Med. Chem.* **2002**, *45*, 5694; (m) Bryant, J. A.; Buckman, B. O.; Islam, I.; Mohan, R.; Morrissey, M. M.; Wei, G. P.; Xu, W.; Yuang, S. WO 098856, 2002
7. Parlow, J. J.; 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. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4657.

8. (a) Caroff, E.; Hilpert, K.; Meyer, E. WO 050301, 2008; (b) Caroff, E.; Hilpert, K.; Meyer, E. WO 044217, 2008; (c) Caroff, E.; Fretz, H.; Hilpert, K.; Houille, O.; Hubler, F.; Meyer, E. WO 114774, 2006.
9. (a) Honma, Y.; Sekine, Y.; Hashiyama, T.; Takeda, M.; Ono, Y.; Tsuzurahara, K. *Chem. Pharm. Bull.* **1982**, *30*, 4314; (b) Shaw, G.; Sugowdz, G. *J. Chem. Soc.* **1954**, 665.
10. $P2Y_{12}$ Radioligand Binding Assay: CHO (chinese hamster ovary) cells transfected with a synthetic human $P2Y_{12}$-gene were cultured in Alpha-MEM containing 10% dialyzed FBS. Washed membranes prepared from near confluent cells were stored at −80 °C. Binding reactions were conducted in polypropylene assay plates in a volume of 150 mL of assay buffer (50 mM Tris, 100 mM NaCl, 1 mM EDTA) including 0.3 mg/well membrane protein, 0.2 nM ^{33}P-MeSADP, and serial dilutions of test compounds, vehicle or 300 nM 2-MeSADP for the definition of non-specific binding. Dry compounds were prepared as 10 mM DMSO stocks and were diluted in seven-point, threefold dilution series in assay buffer with 0.02% BSA. Each concentration was run in triplicate beginning at 10 mM, final concentration in the assay. Binding reactions were incubated at room temperature for 1 h and stopped by dilution and transfer/aspiration of the mixture onto GF/B UniFilter 96 Well Plates (Perkin–Elmer), and washed 3× with ice-cold 50 mM Tris, pH 7.4. The filter plates were counted on a Top Count (Perkin–Elmer) and data were analyzed using GraphPad Prism using a single site binding equation.
11. Diluted test compounds (10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 μM final concentrations) were pre-incubated with PRP for 5 min at 37 °C and aggregation was then initiated by addition of 20 μM ADP (final concentration). The progress of the platelet aggregation response to ADP was monitored by light transmission aggregometry at 626 nm using a SpectraMax Plus plate reader for 15 min at 37 °C. Duplicate concentration–response data (15 min end-point read) was analyzed using BioAssay Solver to derive IC_{50} values. The reference compounds (2S)-4-({4-[4-(1,1′-biphenyl-4-ylcarbonyl)piperazine-1-yl]-6-ethylthieno[2,3-d]pyrimidin-2-yl}oxy)butane-1,2-diol and AZD-6140 were run on every plate and the test compound IC_{50} data was normalized against these two standards to minimize donor-to-donor variability.
12. Ennis, M. D.; Kortum, S. W.; Rahman, H.; Schweitzer, B. A.; Tenbrink, R. E. WO 2006079916, 2006.
13. In most cases, the difference in K_i value from the binding assay to the IC_{50} value of the functional PRP assay was attributed to protein binding. These compounds showed significantly reduced binding affinity when 0.4% human serum albumin was added to the binding assay.
14. Compounds were incubated in human liver and rat liver microsomes for 30 min and the percent remaining was measured. These compounds can be categorized as having good metabolic stability with percent remaining values of >80%.
15. Compounds were examined at a single concentration (3 μM) and conducted in duplicate using a minimum of one positive control inhibitor per CYP-specific assay which included CYP1A2, CYP2C9, CYP2D6, and CYP3A4. Incubation time for the assays were 30 min at 37 °C and end-point measurements were used to assess the degree of inhibition. These compounds can be categorized as weak inhibitors with percent inhibition values of <25%.
16. Compounds were examined at a single concentration (10 μM) in a 384 well fluorescence polarization assay using Cy3B tagged *N*-desmethyl dofetilide to competitively bind to HEK-hERG membrane homogenates. Incubation time for the assay was 120 min and end-point measurements were used to assess the degree of inhibition. These compounds can be categorized as weak inhibitors with percent inhibition values of <25%.