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Part II: Piperazinyl-glutamate-pyridines as potent orally bioavailable $P2Y_{12}$ antagonists for inhibition of platelet aggregation

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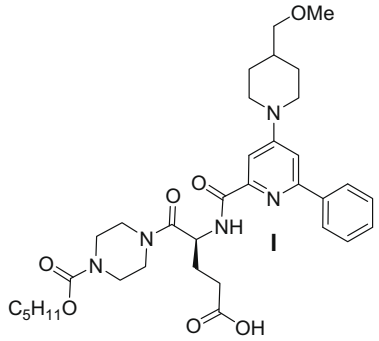
ABSTRACT

Efforts to refine the SAR of the piperazinyl-glutamate-pyridines for more potent analogs with improved pharmacokinetic profiles are described. Exploring substituted piperidines and other ring systems at the 4-pyridyl position led to compounds with improved potency and pharmacokinetic properties over candidate **I**. In particular, compounds **4t** and **5t** were discovered with a 10-fold improvement over potency and improved pharmacokinetic profiles in both the rat and dog.

The $P2Y_{12}$ receptor is a G-protein coupled receptor, primarily a platelet specific receptor, which is an attractive therapeutic target for selective modulation of adenosine diphosphate (ADP)-induced platelet activation.[1] Plavix® (clopidogrel) is an irreversible $P2Y_{12}$ antagonist that acts by means of platelet ADP inhibition. Clopidogrel is an orally prescribed antiplatelet agent used for the treatment of peripheral artery disease and acute coronary syndrome. Clopidogrel is a pro-drug, the active metabolite of which irreversibly and selectively inhibits the $P2Y_{12}$ receptor. Once it is activated, the drug becomes irreversibly bound to platelets. As a result, clopidogrel has a slow onset and slow offset of pharmacological action.[2] This makes it less effective in acute settings and difficult to manage if a patient bleeds, experiences a trauma, or requires emergency surgery. It is anticipated that a direct acting $P2Y_{12}$ inhibitor will not be associated with such difficulties and will therefore exhibit a significant improvement in efficacy while maintaining a better safety profile.

As part of our ongoing effort to identify reversible, potent, orally bioavailable $P2Y_{12}$ antagonists for inhibition of platelet aggregation, we recently described the synthesis and evaluation of piperazinyl-glutamate-pyridines.[3] These pyridyl compounds are antagonists for the $P2Y_{12}$ receptor exhibiting sub-nanomolar $P2Y_{12}$ binding K_i's and sub-micromolar platelet rich plasma (PRP) IC_{50}'s with excellent selectivity over $P2Y_1$ and $P2Y_{13}$. Candidate **I** (Fig. 1) was discovered from this series and showed good potency in a human platelet rich plasma assay with a good pharmacokinetic profile demonstrating efficacy in the rat ferric chloride thrombosis model. Efforts from our backup program were focused on refining the SAR for more potent analogs with improved pharmacokinetic properties. It was previously found that substitution at the 4-position of the pyridine ring greatly influenced the SAR. In particular, substituted piperidines at the 4-pyridyl position provided compounds with good potency. It was established that modulation of the potency and pharmacokinetic properties could be achieved through various substitutions at



I

Figure 1. Piperazinyl-glutamate-pyridine candidate **I**.

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0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
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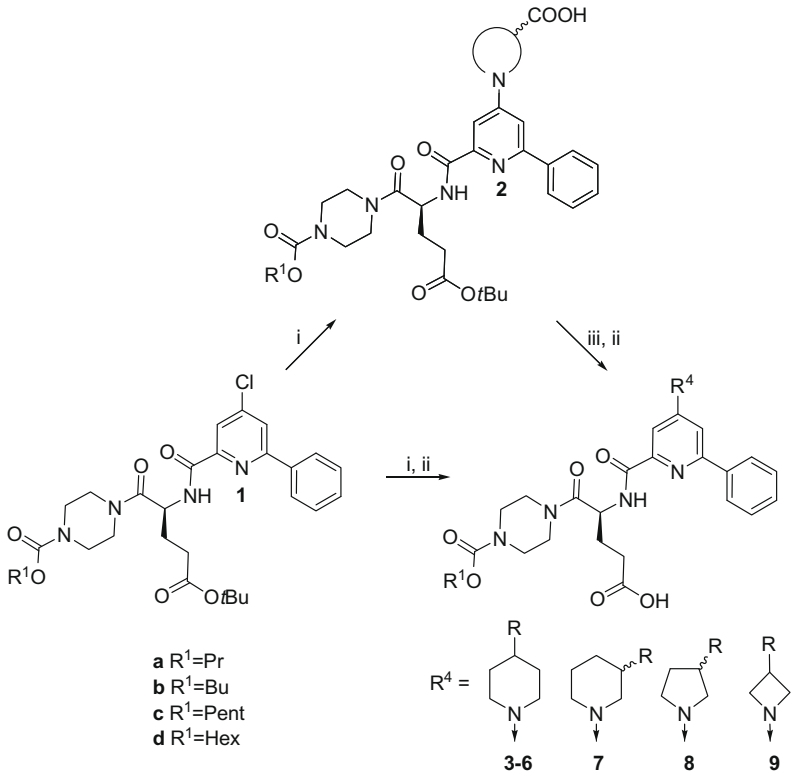
the 4-position of the piperidine ring. Described in this report are efforts at exploring substituted piperidines and other ring systems at the 4-pyridyl position to identify compounds with improved potency and pharmacokinetic properties over candidate **I**.

The synthesis of 4-substituted pyridines with cyclic amines of varying ring sizes is shown in Scheme 1. The 4-chloropyridine intermediates **1a–d** were reacted with substituted piperidines, pyrrolidines, and azetidines in DMSO at 100 °C to afford the cyclic amino-4-pyridine analogs. Deprotection of the *t*-butyl ester using TFA afforded the desired final products **3–9**. Cyclic amines, substituted with a free carboxylic acid, reacted cleanly with the 4-chloropyridine intermediates to afford the carboxylic acid intermediates **2**. The carboxylic acid intermediates **2** were coupled with amines using polymer-bound carbodiimide as the coupling agent with hydroxybenzotriazole to afford the amides, followed by deprotection of the *t*-butyl ester to afford the desired final amide products.

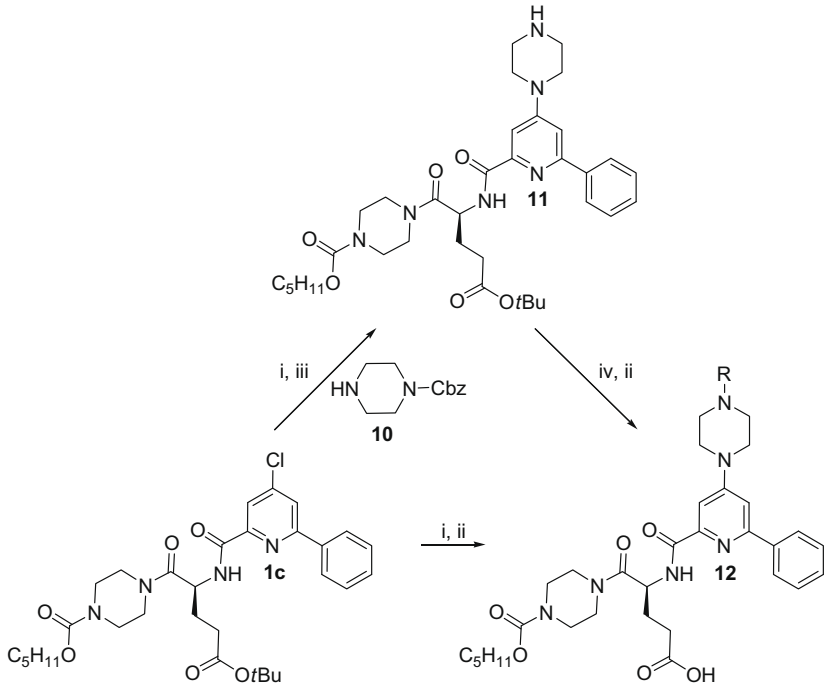
The synthesis of 4-substituted piperazine-4-pyridine analogs **12** is depicted in Scheme 2. The 4-chloropyridine intermediate **1c** was reacted with commercially available mono-substituted piperazines in DMSO at 100 °C to afford the substituted piperazine analogs. Deprotection of the *t*-butyl ester using TFA afforded the desired final 4-substituted piperazine-4-pyridine analogs **12**. Likewise, Cbz protected piperazine **10** was reacted with 4-chloropyridine intermediate **1c** using the same conditions followed by Cbz deprotection with hydrogen and palladium on carbon to afford the unsubstituted piperazine intermediate **11**. The unsubstituted piperazine **11** was reacted with various electrophiles including aliphatic halides, acid chlorides, and carbamoyl chlorides, followed by deprotection of the *t*-butyl ester to afford the desired final substituted piperazine-4-pyridine analogs **12**.

The compounds were screened in a human $P2Y_{12}$ receptor binding assay with K_i's determined. 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 turbidity method. The compounds were assayed with IC_{50}'s reported. Historical analysis of human PRP potency data for $P2Y_{12}$ antagonists revealed significant donor-to-donor variability. To reduce this effect, the potency data for a reference standard (AZD-6140[4]) was determined on each 96-well plate and the data for the test compounds were normalized to the standard from the same plate. Emphasis, in regard to the SAR, was placed on the PRP potency as this was an indication of the functional activity taking into account the effect of protein binding.[5]

A representative set of 4-substituted piperidine-4-pyridine analogs is depicted in Table 1. Replacing the methoxy of candidate **I** with amines provided compounds with improved binding and PRP activity (**4b–f, 5a–f**). However, these amino compounds had high in vivo rat clearance values (**5a**, Table 6). Extending the methoxy of compound **I** by an additional methylene (**5g**) resulted in similar potency. Extending the amino compounds by an additional methylene (**5h–j**) provided compounds that were slightly more potent in the PRP assay than their corresponding aminomethyl analogs. Not unexpectedly, these compounds were highly cleared as well (Table 6, **5i**). Compounds with decreased basicity were explored by preparing the corresponding amide analogs of select potent amines. For example, acylating the primary amine of **5a** provided compound **5k**, which resulted in a significant loss of PRP activity. The amide of **4f** was prepared such that the carbonyl was at the 2-position of the pyrrolidine ring system to provide compound **4l**, which resulted in a slight decrease in activity. However, the presence of a carbonyl on the methylene linking the piperidine and R group provided compounds with exceptional potency (**4o–t, 5m–t**) with the tertiary amides being slightly more potent in the PRP assay. As has been the trend, the butyl and pentyl carbamates of the piperazine tail (R^1) were superior to the propyl and hexyl carbamates in terms of both binding and PRP potency as illustrated by the representative examples **3r** and **6r**.

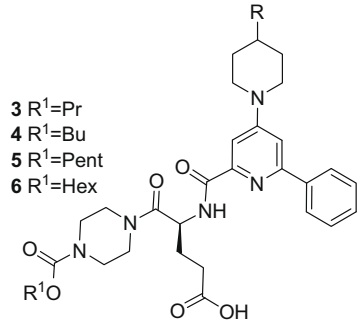


Scheme 1. Synthesis of substituted cyclic amino-4-pyridine analogs **3**–**9**. Reagents and conditions: (i) excess cyclic amine, TEA, DMSO, 100 °C; (ii) 10% TFA/DCM; (iii) 2 equiv PS-carbodiimide resin, 3 equiv amine, NMM, HOBt, DCM/DMF.



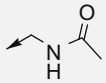
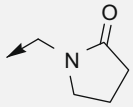
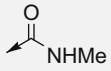
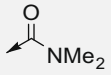
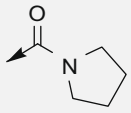
Scheme 2. Synthesis of substituted piperazine-4-pyridine analogs **12**. Reagents and conditions: (i) excess mono-substituted piperazine, TEA, DMSO, 100 °C; (ii) 10% TFA/DCM; (iii) H_2, Pd/C, methanol; (iv) 2 equiv electrophile, TEA, DCM.

Table 1
Binding and PRP activity data for a representative set of 4-substituted piperidine-4-pyridine analogs **3**–**6**[5]



Compd	R^1	R	K_i[a] (nM)	IC_{50}[b] (μM)	Relative potency to AZD6140[c]
I	Pent	OMe	15	3.9	3.3
5a	Pent	NH_2	15	0.78	0.62
4b	Bu	NHMe	4.0	0.91	1.0
5b	Pent		1.5	0.71	0.99
4c	Bu	NHEt	3.8	1.3	1.4
5c	Pent		1.5	0.95	1.2
4d	Bu	NMe_2	3.2	0.89	1.3
5d	Pent		1.9	1.0	1.3
4e	Bu	NEt_2	7.5	2.4	2.1
5e	Pent		4.0	2.2	2.7
4f	Bu	N	1.5	0.80	1.0
5f	Pent		1.1	0.74	0.93
5g	Pent	OMe	11	2.7	3.5
5h	Pent	NH_2	2.8	0.86	0.66
5i	Pent	NMe_2	12	0.39	0.54
5j	Pent	N	8.0	0.75	0.59

Table 1 (*continued*)

Compd	R^1	R	K_i[a] (nM)	IC_{50}[b] (μM)	Relative potency to AZD6140[c]
5k	Pent		25	6.8	3.9
4l	Bu		0.79	1.1	1.5
5m	Pent		6.5	2.4	0.90
5n	Pent		7.9	1.6	0.86
4o	Bu		0.68	1.2	0.90
5o	Pent		0.58	0.65	1.0
4p	Bu		4.3	0.62	0.82
5p	Pent		6.0	0.75	0.64
4q	Bu		2.6	0.63	0.53
5q	Pent		0.98	0.60	0.72
3r	Pr		12	2.0	2.3
4r	Bu		1.1	0.43	0.71
5r	Pent		4.0	0.52	0.65
6r	Hex		4.8	0.82	1.7
5s	Pent		1.2	0.41	0.64
4t	Bu		2.1	0.36	0.54
5t	Pent		2.8	0.40	0.65

[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.

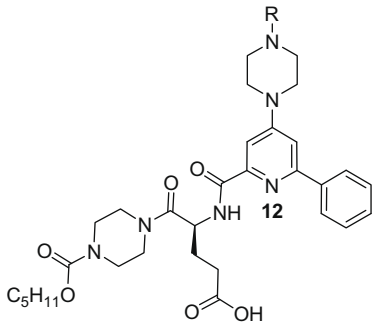
[c] IC_{50} value of compound/IC_{50} value of AZD-6140 control from same plate = normalized IC_{50} ratio.

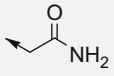
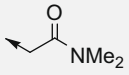
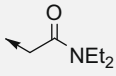
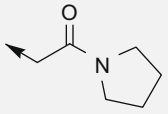
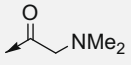
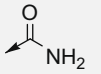
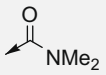
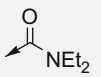
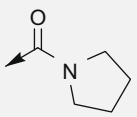
Replacing the piperidine with a piperazine ring at the 4-position of the pyridine was explored and a representative set of analogs is shown in Table 2. Only the pentyl carbamates of the piperazine tail (R^1) are listed, as there was no significant difference in PRP activity between the butyl carbamates and the corresponding pentyl carbamates. The unsubstituted piperazine analog **12a** had good binding and PRP potency while substitution of the nitrogen with alkyl chains of length beyond a methyl tended to decrease the potency as demonstrated with the propyl analog **12c**. Substitution with a hydroxyethyl **12d** had slightly improved potency relative to the unsubstituted analog **12a**, but capping the hydroxy with an alkyl group **12e,f** tended to slightly decrease the PRP potency. Extending a tertiary amine two to three carbons away from the 4-position of the piperidine **12h,i** increased the PRP potency. The small acetamides, both primary **12k** and the dimethyl tertiary **12l**, were some of the most potent 4-substituted piperazines. The amides **12o–q** and the ureas **12r–u** were slightly less active than the unsubstituted analog **12a**. Overall, these compounds had good potency compared to candidate **I** and relatively flat SAR across a variety of functionalities. Substituted homopiperazines at the 4-position of the pyridine were also investigated and similar SAR was observed with no advantage in terms of potency (data not shown).

The 3-substituted piperidine regioisomers were explored and a representative set of analogs is shown in Table 3. In most cases, the diastereomeric mixtures were tested and if the mixture warranted, then the corresponding enantiomers were prepared. Overall, the majority of these compounds were slightly less potent in the PRP assay than their corresponding 4-substituted piperidine regioisomers. Substitution with a hydroxy had similar activity to candidate **I** with no significant difference in activity between the two enantiomers **7a** and **7b**. The methoxy analog **7c** was less active than the hydroxy. One of the most dramatic differences in activity was observed with **7d**, the regioisomer of candidate **I**, which was considerably less active in the PRP assay. A trend similar to that seen with the 4-substituted analogs was observed with the 3-substituted analogs, where replacing the hydroxy or methoxy with an amine tended to increase both the binding and PRP activity, as illustrated with compounds **7e,f**. Extending the amino group by an additional methylene provided compounds that were slightly more potent in the PRP assay (**7g**). The primary **7j** and tertiary **7k** amides were considerably less active in both the binding and PRP assays as compared to the corresponding 4-substituted piperidines **5m** and **5r**, respectively (Table 1).

Replacing the piperazine ring of candidate **I** with other ring sizes was investigated. A representative set of pyrrolidine analogs is shown in Table 4. The majority of these compounds were slightly less potent in the PRP assay than their corresponding 4-substituted piperidine analogs. Several pure diastereomers of the amide analogs were prepared and none showed any significant difference in activity. The glycine amide ethers **8b** and **8c** showed exceptional PRP potency but unfortunately had high in vivo rat clearance values (Table 6). Reducing the ring size by an additional methylene provided the 3-substituted azetidine analogs (Table 5). These ana-

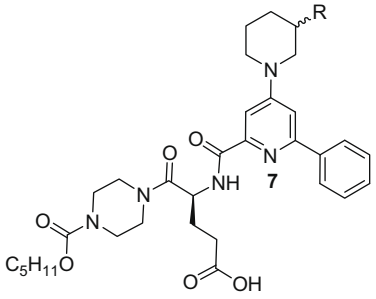
Table 2
Binding and PRP activity data for a representative set of 4-substituted piperazine-4-pyridine analogs **12**[5]

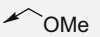
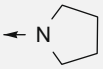
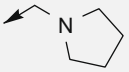
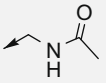
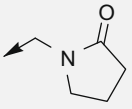
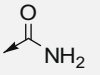
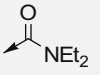


Compd	R	K_i[a] (nM)	IC_{50}[b] (μM)	Relative potency to AZD6140[c]
12a	H	6.5	1.9	2.3
12b	Me	15	2.3	2.5
12c	Pr	29	5.0	6.3
12d	$(CH_2)_2OH$	13	1.8	1.1
12e	$(CH_2)_2OMe$	8.3	1.1	2.1
12f	$(CH_2)_2OPr$	6.3	1.5	3.1
12g	$(CH_2)_2NH_2$	4.5	1.1	1.6
12h	$(CH_2)_2NMe_2$	8.5	0.51	0.70
12i	$(CH_2)_3NMe_2$	8.4	0.52	0.72
12j	$(CH_2)_2SO_2Me$	8.9	0.68	0.81
12k		1.7	0.51	1.8
12l		4.9	0.69	1.1
12m		5.9	2.9	3.2
12n		5.2	1.6	2.2
12o		22	2.3	2.7
12p		16	5.0	6.6
12q		2.8	0.71	2.5
12r		6.3	1.9	1.8
12s		5.9	3.0	3.6
12t		12	3.2	4.6
12u		10	6.1	14

[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 AZD-6140 control from same plate = normalized IC_{50} ratio.

Table 3
Binding and PRP activity data for a representative set of 3-substituted piperazine-4-pyridine analogs **7**[5]



Compd	R	K_i[a] (nM)	IC_{50}[b] (μM)	Relative potency to AZD6140[c]
7a[d]	OH	18	2.4	3.7
7b[e]		16	4.4	4.9
7c	OMe	31	5.2	6.3
7d		22	11	—
7e	NH_2	5.5	1.8	2.2
7f		11	3.3	3.3
7g		7.3	1.7	1.5
7h		9.9	2.9	3.4
7i		10	2.6	3.8
7j		17	7.8	7.3
7k		26	7.7	4.2

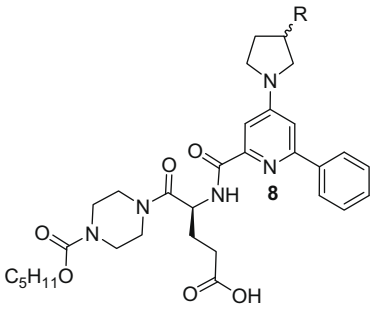
[a] Membranes from CHO cells expressing recombinant human $P2Y_{12}$ receptors incubated with ^{33}P ADP and compound. K_i values are corrected from IC_{50} using Cheng and Prusoff equation and are the geometric mean of $n = 2$ or greater.
[b] IC_{50} values are from human PRP incubated with 20 μM ADP.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

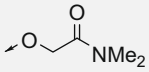
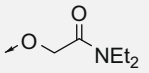
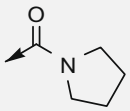
logs showed similar SAR as the 4-substituted piperidines, but were slightly less active in the PRP assay.

The potency optimization described above resulted in many analogs with improved potency over candidate **I**. The next goal was to identify a compound with similar or improved pharmacokinetic characteristics. 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. Many compounds were evaluated for their in vivo pharmacokinetic characteristics in the rat, and a representative set of pharmacokinetic profiles is shown in Table 6.[6]

Several compounds from the 4-substituted piperazine-4-pyridine series (**12**) were evaluated, which included acylated (**12o**) and aliphatic (**12b**) substitution at the 4-nitrogen of the piperazine ring. All of the compounds evaluated in this series had unacceptable clearance values. The most potent analogs from the 3-substi-

Table 4
Binding and PRP activity data for a representative set of 3-substituted pyrrolidine-4-pyridine analogs **8**[5]



Compd	R	R^4	K_i[a] (nM)	IC_{50}[b] (μM)	Relative potency to AZD6140[c]
8a[d]	OH	H	13	7.1	3.9
8b[e]		H	5.0	0.66	0.75
8c[f]		H	3.5	0.33	0.38
8d[d]		H	21	29	11
8e[d]		H	31	7.3	10
8g[e]	NMe_2	H	26	3.0	4.1
8f[f]			24	3.6	3.8
8h[e]		H	1.7	4.8	4.6
8i[f]			2.6	6.6	5.0
8j[e]		H	1.7	2.6	2.8
8k[f]			1.4	2.2	1.7

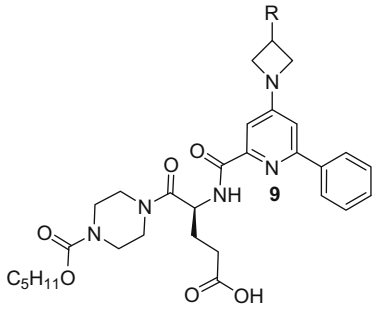
[a] Membranes from CHO cells expressing recombinant human $P2Y_{12}$ receptors incubated with ^{33}P ADP and compound. K_i values are corrected from IC_{50} using Cheng and Prusoff equation and are the geometric mean of $n = 2$ or greater.
[b] IC_{50} values are from human PRP incubated with 20 μM ADP.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

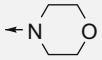
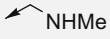
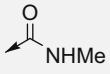
tuted pyrrolidine-4-pyridine series (**8**), which included the glycine amide ethers (**8b,c**), also had high clearance values. It was previously found that compounds with 4-piperidine substituted with a side-chain containing a basic amine usually had high clearance values, and this same trend was observed for the 3-substituted piperidines (**7g**). However, replacing the basic amine group with a methoxy resulted in compounds with acceptable clearance values, although a slight loss in potency was observed. These studies led to the previously reported discovery of candidate **I**.[3] Another strategy to reduce the basicity was converting the amine into an amide, in particular the amides with the carbonyl on the methylene linker which provided compounds with exceptional potency. This also had a profound effect on the clearance values. The primary amide **5m** still had a high clearance value, but the secondary (**5o**) and tertiary (**4r,5r,5s**) amides had acceptable to good clearance values. This led to the discovery of compounds **4t** and **5t**, both pyrrolidine amides, with single digit clearance values and good bioavailability. The dog pharmacokinetic profiles are shown in Table 7 and are consistent with the rat in regards to the low clearance with slightly lower bioavailability.[6]

Further evaluation of compounds **4t** and **5t** showed they had acceptable margins versus hERG activity (IC_{50}'s > 90 μM) and a satisfactory CYP inhibition profile. No safety issues were observed as both were inactive in the Ames and Micronucleus assays and both were evaluated in the rat safety studies (single dose and 7-day repeat dose) without significant toxicological findings. Both compounds had good solubility.[7] In vitro receptor binding, signaling, and functional studies have shown that these pyridine analogs are high affinity, selective, and competitive antagonists at $P2Y_{12}$ receptors. Schild analysis indicates that **4t** and **5t** are competitive inhibitors of ADP in the platelet aggregation assay. All of the compounds were inactive at 10 μM against 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 $P2Y_{12}$). The potency of compounds **4t** and **5t** was determined by repeated measurements of their IC_{50} in the PRP assay.[8] Further evaluation of the potency of compounds **4t** and **5t** in three human donors using 20 μM ADP-stimulated PRP aggregation with the 4-well Chronolog PRP aggregometry assay found the average IC_{50} to be 0.255 ± 0.156 μM and 0.325 ± 0.267 μM, respectively. The K_b for compounds **4t** and **5t** in the human PRP platelet aggregation assays is 49 nM and 68 nM, respectively. Both compounds **4t** and **5t** showed only a slightly reduced binding affinity when 0.4% human serum albumin was added to the binding assay, resulting in K_i's of 6.3 nM and 6.7 nM, respectively. This is consistent with their measured plasma protein binding data.[9]

In conclusion, we have described our efforts at refining the SAR for more potent analogs with improved pharmacokinetic properties.

Table 5
Binding and PRP activity data for a representative set of 3-substituted azetidine-4-pyridine analogs **9**[5]



Compd	R	K_i[a] (nM)	IC_{50}[b] (μM)	Relative potency to AZD6140[c]
9a	OH	2.5	8.6	9.2
9b	OMe	2.0	>10	—
9c	NMe_2	5.5	2.7	—
9d		3.3	1.4	—
9e		7.1	1.3	1.9
9f		0.64	1.9	—
9g		1.7	2.0	—
9h		2.0	1.8	1.5

[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 AZD-6140 control from same plate = normalized IC_{50} ratio.

Table 6
Rat pharmacokinetic profiles of selected $P2Y_{12}$ antagonists[a,b]

Compd	CL (mL/min/kg)	Vdss (L/kg)	$T_{1/2,\ eff}$ (h)	F_{oral} (%)
I	3	0.3	0.7	89
5a	70	2.6	0.4	0
5i	145	5.1	0.4	1
5m	44	2.2	0.6	2
5o	11	0.3	0.3	—
4r	24	0.9	0.4	24
5r	13	2.5	0.6	42
5s	9	0.2	0.3	6
4t	5	0.7	1.5	89
5t	5	0.5	1.1	100
7g	141	10	0.8	—
8b	70	2.4	0.4	—
8c	47	1.8	0.4	—
12b	41	0.8	0.2	—
12o	87	2.1	0.3	—

[a] Male Sprague–Dawley rats ($n = 2$–4 rats).
[b] Dose: iv infusion at 2 mg/kg; po at 5 mg/kg. Vehicle: 50% PEG400/40% PBS/10% ethanol.

Table 7
Dog pharmacokinetic profiles of selected $P2Y_{12}$ antagonists[a]

Compd	CL (mL/min/kg)	Vdss (L/kg)	$T_{1/2,\ eff}$ (h)	F_{oral} (%)
I[b]	1	0.14	1.5	93
5r[b]	2	0.12	0.7	51
4t[c]	3	0.22	0.8	31
5t[c]	2	0.18	1.0	36

[a] Male beagle dogs ($n = 2$–4 dogs).
[b] Dose: iv infusion at 0.2 mg/kg; po at 0.2 mg/kg. Vehicle: 50% PEG400/40% PBS/10% ethanol.
[c] Dose: iv infusion at 0.5 mg/kg; po at 1.0 mg/kg. Vehicle: 50% PEG400/40% PBS/10% ethanol.

Exploring cyclic amines of varying ring sizes at the 4-position of the pyridine ring determined that piperidines were the optimal ring size and that substitution at the 4-position was favored. It was found that modulation of the potency and pharmacokinetic properties could be achieved through various substitutions at the 4-position of the piperidine ring. 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 4-piperidinecarboxamides, provided low clearance compounds. In particular, the 4-pyrrolidineamide piperidine compounds **4t** and **5t** exhibited exceptional potency with single-digit nanomolar $P2Y_{12}$ binding K_i's and sub-micromolar PRP IC_{50}'s (vs 20 μM ADP) with low clearance in both rat and dog and good bioavailability in both species. The potency, selectivity, safety, and pharmacokinetic profiles for these two compounds support further evaluation.

References and notes

1. Gachet, C. *Thromb. Haemost.* **2001**, *86*, 222.
2. (a) Meadows, T. A.; Bhatt, D. L. *Circ. Res.* **2007**, *100*, 1261; (b) Savi, P.; Pereillo, J. M.; Uzabiaga, M. F.; Combalbert, J.; Picard, C.; Maffrand, J. P.; Pascal, M.; Herbert, J. M. *Thromb. Haemost.* **2000**, *84*, 891.
3. (a) 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. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4657; (b) 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. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6148; (c) 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. *J. Med. Chem.*, in press.
4. 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.
5. These compounds showed significantly reduced binding affinity when 0.4% human serum albumin was added to the binding assay. In most cases, the difference in K_i value from the binding assay to the IC_{50} value of the functional assay was attributed to protein binding.
6. The Pfizer Institutional Animal Care and Use Committee reviewed and approved the animal use in these studies. The animal care and use program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.
7. Compound **4t** solubility: 921 μM at pH 2.0; 195 μM at pH 6.5; 969 μM at pH 9.0. Compound **5t** solubility: 299 μM at pH 2.0; 48 μM at pH 6.5; 1291 μM at pH 9.0.
8. The IC_{50}'s for compounds **4t** and **5t** were determined from geometric mean of seven IC_{50}'s in the PRP assay.
9. Definitive human PPB data: **4t** Fu 3.4% at 0.2 μM, 4.4% at 2 μM, and 3.3% at 10 μM; **5t** Fu 5.3% at 0.2 μM, 5.7% at 2 μM, and 6.1% at 10 μM. Neither showed concentration-dependent PPB.