Bioorganic & Medicinal Chemistry Letters 19 (2009) 4657–4663

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

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

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ARTICLE INFO

Article history:
Received 9 April 2009
Revised 19 June 2009
Accepted 22 June 2009
Available online 25 June 2009

Keywords:
P2Y12 receptor
GPCR antagonist
Antiplatelet
Antithrombotic
Cardiovascular disease
Polymer-assisted solution-phase

ABSTRACT

Polymer-assisted solution-phase (PASP) parallel library synthesis was used to discover a piperazinyl-glutamate-pyridine as a $P2Y_{12}$ antagonist. Exploitation of this lead provided compounds with excellent inhibition of platelet aggregation as measured in a human platelet rich plasma (PRP) assay. Pharmacokinetic and physiochemical properties were optimized leading to compound (4*S*)-4-[({4-[4-(methoxymethyl)piperidin-1-yl]-6-phenylpyridin-2-yl}carbonyl)amino]-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid **22J** with good human PRP potency, selectivity, in vivo efficacy and oral bioavailability.

Cardiovascular and cerebrovascular diseases remain the most common cause of mortality in the western world. Current therapy for these diseases includes antiplatelet agents such as aspirin, dipyridamole, glycoprotein IIb/IIIa antagonists, and thienopyridines.[1] Plavix® (clopidogrel), a thienopyridine approved for the reduction of atherosclerotic events (stroke, myocardial infarction (MI), and death) in patients with atherosclerosis documented by a recent event (stroke, MI or established peripheral vascular disease), acts by blocking adenosine diphosphate (ADP)-stimulated platelet aggregation. ADP is an important platelet agonist that induces a primary aggregation response and contributes to secondary aggregation following release from platelet dense granules following activation by other agonists. ADP-induced platelet aggregation is mediated by a dual receptor system involving activation of $P2Y_1$ and $P2Y_{12}$ receptors, both members of the G-protein coupled receptor (GPCR) family.[2] 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 primarily a platelet specific receptor and thus represents a more attractive therapeutic target for selective modulation of ADP-induced platelet activation. Several groups have research efforts aimed towards identifying inhibitors of the $P2Y_{12}$ receptor[3] including some recent patents[4] issued by Actelion which inspired us to describe our effort.

The P2Y12 receptor has been identified as the molecular target of clopidogrel.[5] Clopidogrel is a prodrug, the active metabolite of which irreversibly and selectively inhibits the $P2Y_{12}$ receptor; thus antiplatelet efficacy requires a loading dose and several days of treatment to achieve its full effect.[6] 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. This makes it less effective in acute settings and difficult to manage if a patient bleeds, experiences a trauma, or requires emergency 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).[7] 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. Therefore, we sought to discover and develop direct acting, reversible $P2Y_{12}$ antagonists through parallel medicinal chemistry to address the unmet medical need for safe and effective oral antiplatelet agents.

Quinoline derivatives **I** have been reported as antagonists of the platelet $P2Y_{12}$ receptor (Fig. 1).[8] The lead compound from this class is a pro-drug (R^4 side-chain possesses ethyl ester). Metabolism (systemic esterases) of the ester pro-drug to its corresponding acid provides the active metabolite, which contains two carboxylic acid

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doi:10.1016/j.bmcl.2009.06.075

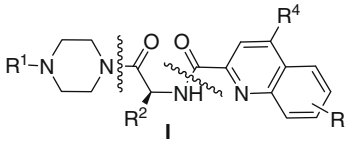
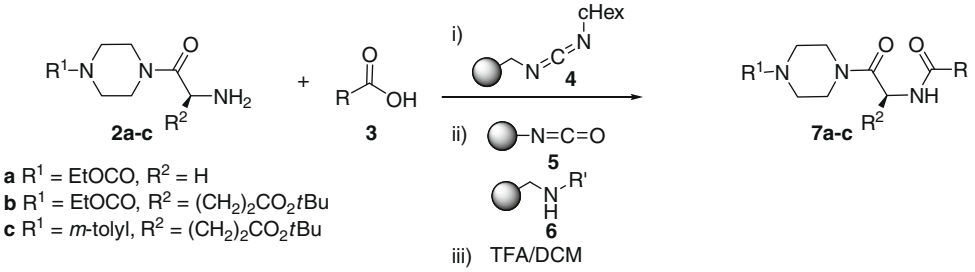


Figure 1. Berlex quinoline core structures.

groups. It would be desirable to eliminate the need for a pro-drug and improve upon the physical chemical properties as well as potency. Interestingly, only quinolines and naphthalenes were reported as the right-hand aryl moieties. We approached analog synthesis by considering the molecule as being composed of three parts: the piperazine, the amino acid, and the quinoline. Parallel syntheses allowing for variations of all three parts of the molecule were then developed.

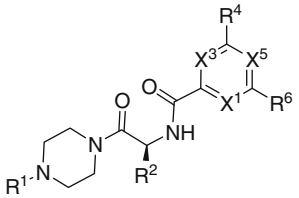
Initial efforts were focused at replacing the quinoline ring with other heteroaryl and aryl ring systems. It was envisioned that a polymer-assisted solution-phase (PASP) synthesis approach for the aryl carboxamides could be accomplished through a simple amide bond-forming reaction (Scheme 1). Thus, reacting aryl carboxylic acids with the amine would afford analogs with alternate aryl ring systems. Three amine templates **2a–c** were selected for initial library synthesis in which an ethyl carbamate and a *m*-tolyl ring system were substituted on the terminal piperazine nitrogen with either a glycine or *S*-glutamic acid as the amino acid. The aryl acid inputs **3** were initially biased towards fused ring systems mimicking the quinoline and naphthalene. The amines **2** were coupled to the aryl acids **3** using polymer-bound carbodiimide **4** as the coupling agent with hydroxybenzotriazole to afford the crude amide products. Upon completion of the reaction, a mixed-bed containing polymer-bound amine **6** and polymer-bound isocyanate **5** was added to the reaction mixture to sequester hydroxybenzotriazole and any unreacted acid **3** or amine starting material **2** followed by filtration and evaporation to afford purified products. Deprotection of the *t*-butyl ester using TFA in DCM provided the desired products **7**.

Several hundred compounds were prepared by this synthesis and screened in a human $P2Y_{12}$ receptor binding assay at 5–10 μM concentration, and K_i's were determined for those compounds with >50% inhibition. 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. The compounds were initially assayed at 100 or 50 μM and IC_{50}'s were determined for compounds with >50% inhibition of platelet aggregation. Emphasis, in regard to the SAR, was placed on the PRP potency



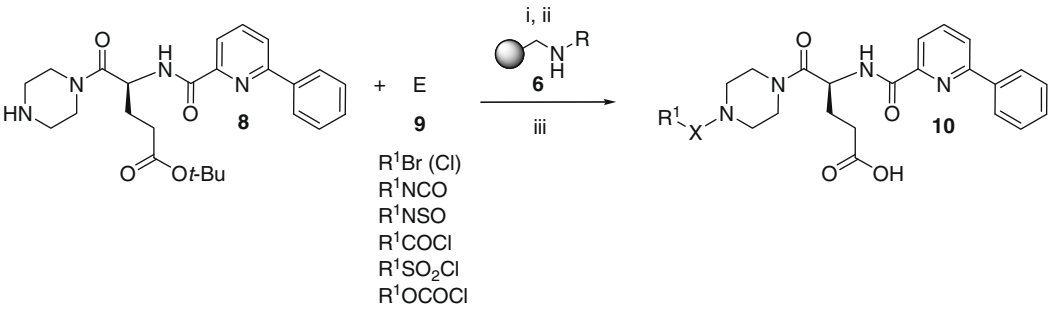
Scheme 1. Polymer-assisted solution-phase (PASP) library synthesis. Reagents and conditions: (i) 1.5 equiv **4**, NMM, HOBt, DCM/DMF; (ii) excess **5** and **6**, additional DCM; (iii) 10% TFA/DCM.

Table 1
Binding and PRP activity data for selected library compounds[9]



Compound	R^4	R^6	X^1	X^3	X^5	R^1	R^2	K_i[a] (nM)	IC_{50}[b] (μM)
7A	Ph	Ph	N	CH	CH	EtOCO	$(CH_2)_2CO_2H$	15	76
7B	H	H	N	CH	CH	EtOCO	$(CH_2)_2CO_2H$	>5400	>100
7C	Ph	H	N	CH	CH	EtOCO	$(CH_2)_2CO_2H$	>5700	>100
7D	H	Ph	N	CH	CH	EtOCO	$(CH_2)_2CO_2H$	209	71
7E	Ph	Ph	CH	CH	CH	EtOCO	$(CH_2)_2CO_2H$	373	>100
7F	H	Ph	CH	CH	CH	EtOCO	$(CH_2)_2CO_2H$	3640	>100
7G	Ph	Ph	CH	CH	N	EtOCO	$(CH_2)_2CO_2H$	484	>50
7H	H	Ph	N	CH	N	EtOCO	$(CH_2)_2CO_2H$	356	>100
7I	H	Ph	N	N	CH	EtOCO	$(CH_2)_2CO_2H$	1190	74
7J	Ph	Ph	N	CH	CH	EtOCO	H	733	>50
7K	H	Ph	N	CH	CH	EtOCO	H	1610	>100
7L	Ph	Ph	N	CH	CH	*m*-Tolyl	$(CH_2)_2CO_2H$	5.7	>100
7M	H	Ph	N	CH	CH	*m*-Tolyl	$(CH_2)_2CO_2H$	>5700	>100

[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.



Scheme 2. Polymer-assisted solution-phase (PASP) synthesis of substituted piperazine analogs **10**. Reagents and conditions: (i) 4 equiv electrophile **9**, TEA, DCM; (ii) excess **6**, DCM; (iii) 20% TFA/DCM.

as this was an indication of the functional activity taking into account the effect of protein binding. Many different heterocyclic ring systems were evaluated, and an apparent trend was observed. Compounds with a heteroatom, preferably a nitrogen, *ortho* to the amide position and a phenyl ring *ortho* to the heteroatom exhibited $P2Y_{12}$ binding activity. A second iteration with the heteroaryl acids possessing this type of substitution pattern led to the discovery of compound **7A** possessing a 4,6-diphenylpyridine ring system with a $P2Y_{12}$ binding K_i of 15 nM and a PRP IC_{50} of 76 μM. In an effort to follow-up on the lead compound **7A**, a focused library was prepared which explored elementary structural changes around the pyridine ring system. A representative set of compounds from this library is shown in Table 1. The unsusbstituted pyridine ring, compound **7B**, was devoid of any type of activity. Replacing the 6-phenyl ring with a hydrogen, compound **7C**, also resulted in a loss of activity. However, when replacing the 4-phenyl ring with a hydrogen, compound **7D**, PRP activity was maintained. Removing the nitrogen from the pyridine ring system, regardless of the phenyl substitution, resulted in loss of binding activity with no PRP activity (**7E,F**). An additional nitrogen at the 3-position with a 6-phenyl substitution, compound **7I**, maintained PRP activity, where as the additional nitrogen at the 5-position, compound **7H**, resulted in no PRP activity.

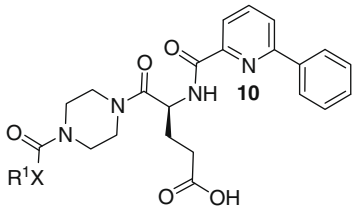
The SAR trends across the different amine templates were consistent. The glycine analogs (**7J,K**, $R^2 = H$) had reduced binding activity and no PRP activity while the *S*-glutamic acid piece ($R^2 = (CH_2)_2CO_2H$) was required for PRP activity. The ethyl carbamate group on the piperazine nitrogen was preferred over the *m*-tolyl for PRP activity. While the *m*-tolyl substitution provided compounds with excellent binding affinity, **7L**, no PRP activity was observed with any of these analogs, presumably due to high protein binding.[9]

Upon discovery of the pyridine ring system, modifications to other parts of the molecule were explored. Efforts were focused on derivatization of the piperazine nitrogen. With no difference in PRP activity of the 4,6-diphenyl (**7A**) versus 6-phenylpyridine (**7D**) ring system, the 6-phenylpyridine ring was used as the starting template for piperazine derivatization. Scheme 2 shows the synthesis of substituted piperazine analogs. The amine **8** was reacted with an excess of electrophile **9** in the presence of triethylamine. The electrophiles used (alkyl halides, chloroformates, acid halides, isocyanates, thioisocyanates, and sulfonyl halides) were selected to represent a diverse set, affording products **10** containing alkyl, carbamate, amide, urea, thiourea, or sulfonamide functionality. Upon completion of the reaction, polymer-bound amine **6** was added to the reaction mixture to sequester any unreacted electrophile **9** followed by filtration and evaporation to afford purified products. Deprotection of the *t*-butyl ester using TFA in DCM provided the desired substituted piperazine products **10**.

Many substituted piperazine compounds were prepared to provide a diverse set of analogs. In general the aliphatics, sulfonamides, and amides were inactive. Small aliphatic ureas and thioureas exhibited good binding activity and moderate PRP potency, while the aliphatic carbamates were optimal with respect to both binding and PRP activity. A representative set of carbamate analogs is shown in Table 2. The SAR clearly shows that carbamates with aliphatic straight chains of four to six carbon length are preferred. When unsaturation, branching, or a heteroatom is introduced into the carbamate side-chain, PRP activity is lost.

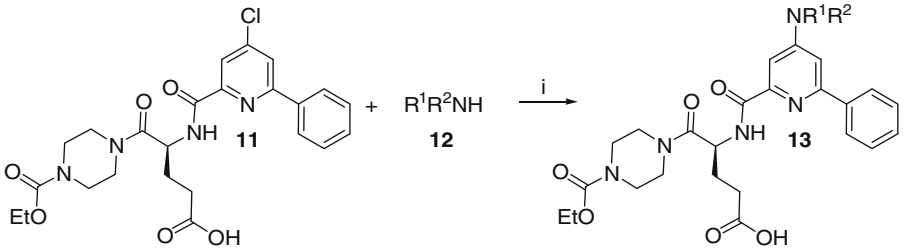
Additional chemistry efforts explored replacing the 4-phenyl substituent on the pyridine ring of lead **7A** with other groups such as substituted amines and ethers. The 4-chloropyridine intermediate **11** allows for substitution at the 4-position with nitrogen nucleophiles as shown in Scheme 3. The 4-chloro was displaced by heating intermediate **11** with an excess of amine **12** and triethylamine in DMSO at 100 °C to afford the desired 4-aminopyridine analogs **13**. Displacement of the chlorine at the 4-position afforded clean products with either the free carboxylic acid or the *t*-butyl ester group present. Attempts to displace the 4-chloro with oxygen nucleophiles provided limited success. As a result, 4-hydroxypyridine intermediate **14** was prepared and under Mitsunobu conditions or direct alkylation provided 4-oxygen analogs **17** as shown in Scheme 4. Direct alkylation of the 4-hydroxypyridine **14** was accomplished using 2 equiv of the electrophile **16** with cesium carbonate as the base and a catalytic amount of potassium iodide in DMF to afford the desired 4-ether analogs **17**. Alternatively, employing Mitsunobu conditions with 2 equiv of the alcohol **15** using DEAD and triphenylphosphine in THF provided the 4-ether

Table 2
Binding and PRP activity data for selected straight chain carbamate analogs **10**[9]

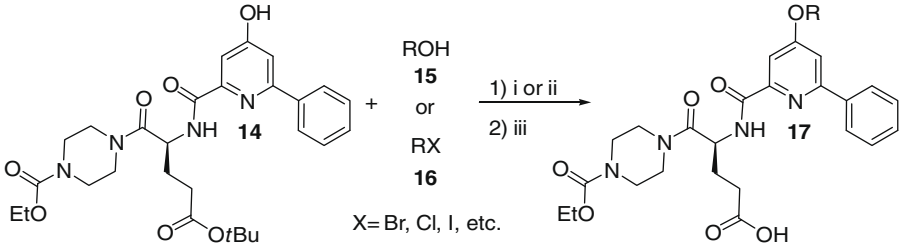


Compound	R_1	K_i[a] (nM)	IC_{50}[b] (μM)
10A	OMe	2440	>100
7D	OEt	209	71
10C	OPr	27	62
10D	OBu	29	28
10E	OPent	11	15
10F	OHex	43	19
10G	OHept	7.0	56
10H	OOct	14	>100

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



Scheme 3. Synthesis of 4-aminopyridine analogs **13**. Reagents and conditions: (i) 1.5–20 equiv **12**, Et_3N, DMSO, 100 °C.



Scheme 4. Synthesis of 4-ether pyridine analogs **17**. Reagents and conditions: (i) 2 equiv **16**, Cs_2CO_3, KI, DMF, rt to 100 °C; (ii) 2 equiv **15**, DEAD, PPh_3, THF; (iii) 10% TFA/DCM.

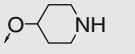
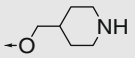
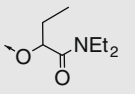
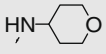
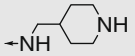
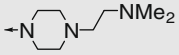
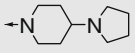
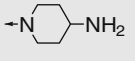
analogs **17**. Using both procedures allowed for a greater diversity of monomer inputs **15** and **16**, ultimately providing a wide array of 4-ether analogs. Deprotection of the *t*-butyl ester using TFA in DCM afforded the desired final ether products **17**.

Table 3 shows the data for a representative set of compounds of both the 4-ether and 4-aminopyridine libraries of **17** and **13** respectively. Substituting the 4-position with either an amine or oxygen substituent led to a favorable enhancement in both the $P2Y_{12}$ binding and PRP activity. In the ether series, substituting the oxygen with alkyl chains of varying length attenuated the binding activity, but the PRP activity remained equivalent to that of the methoxy analog **17A** (data not shown). Comparable PRP activity was observed with the benzyl ether analog **17B,** and relatively flat SAR was observed across various substituted benzyl ether analogs with PRP activity in the range of 9–39 μM (data not shown). Extending a hydroxy or alkyl ether two to four carbon atoms away from the 4-oxygen atom increased the PRP activity, as seen with compounds **17C** and **17D**. A similar trend was observed by extending a primary or secondary amine two to four carbon atoms away from the 4-oxygen atom. In particular, the 4-piperidine ether analogs, **17G** and **17H**, were two of the most potent compounds of the series. The racemic mixture of **17I** also exhibited good PRP activity.

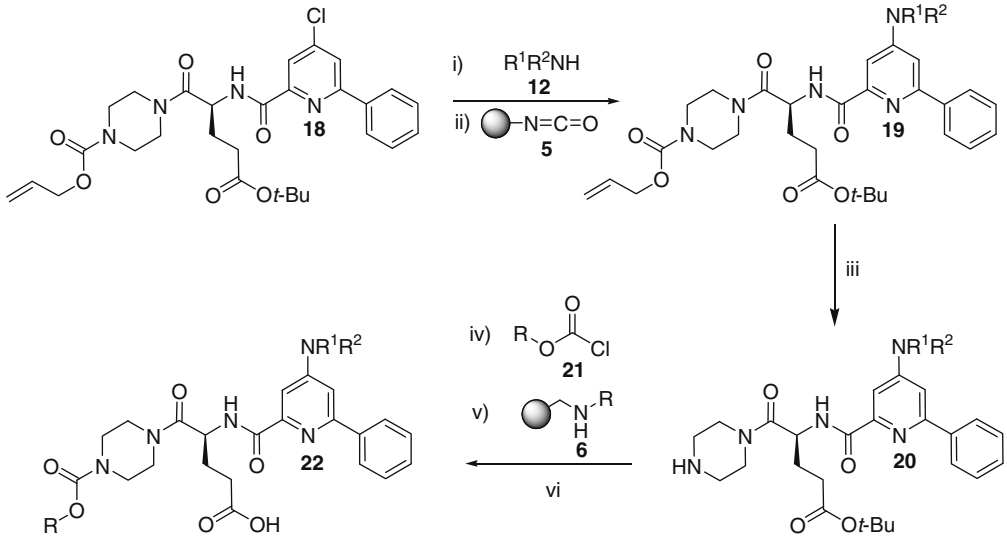
Similar trends were observed in the 4-aminopyridine series. Small secondary alkyl amines (**13A, B**) were preferred over tertiary amines (**13C**). As the alkyl chain was lengthened (>4 carbons) or branched, activity was lost (data not shown). Compounds with an oxygen heteroatom **13D–F**, as a hydroxy or ether, as part of the secondary amine group (several atoms removed from the nitrogen) increased PRP activity relative to the corresponding alkyl group. Extending an amine group two to four carbon atoms away from the 4-nitrogen atom increased PRP activity. This trend was most notable with substituted piperazines **13J** and piperidines **13K–L**, as they are the most potent compounds of the 4-aminopyridine series.

The initial efforts described above at optimizing both the 4-position of the pyridine ring and the piperazine carbamate occurred simultaneously. Each alteration led to compounds with good PRP potency. The next obvious step was to make both changes within the same molecule to determine whether an additive effect could be achieved. A multi-step PASP synthesis was devised allowing

Table 3
Binding and PRP activity data for a representative set of 4-ether and 4-aminopyridine analogs[9]

Compound	OR/NR¹R²	K_i[a] (nM)	IC_{50}[b] (μM)
17A	OMe	96	39
17B	OCH_2Ph	28	31
17C	$O(CH_2)_2OMe$	92	5.8
17D	$O(CH_2)_3OH$	68	9.0
17E	$O(CH_2)_2NH_2$	328	7.5
17F	$O(CH_2)_3NH_2$	214	3.8
17G		68	1.9
17H		76	4.0
17I		81	3.0
13A	NHMe	35	45
13B	NHPr	64	28
13C	NEt_2	23	>100
13D	$NH(CH_2)_2OMe$	99	14
13E	$NH(CH_2)_2OH$	114	8.1
13F		455	21
13G	$NHCH_2Ph$	26	81
13H	$NH(CH_2)_2NMe_2$	270	36
13I		190	8.5
13J		47	2.3
13K		34	2.0
13L		27	2.8

[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.



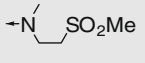
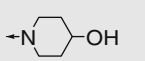
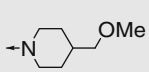
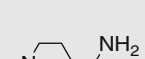
Scheme 5. PASP synthesis of 4-aminopyridine-piperazine carbamate analogs **22**. Reagents and conditions: (i) 1.5 equiv **12**, Et_3N, DMSO, 100 °C; (ii) excess **5**, DCM; (iii) $(Ph_3P)_4Pd$, morpholine, MeCN; (iv) **21**, Et_3N, DCM; (v) excess **6**, DCM; (vi) 10% TFA/DCM.

for substitution at both positions as shown in Scheme 5. Compound **18** was selected as the starting template with the piperazine nitrogen protected as the Alloc and the acid group protected as the *t*-butyl ester. The protecting groups were chosen to allow for orthogonal deprotection in a parallel format. The first step of the synthesis involved reaction of the starting template **18** with excess amine **12** at 100 °C. After total consumption of the template, polymer-bound isocyanate **5** was added to sequester the unreacted amine **12**. Simple filtration and rinsing with dichloromethane yielded a filtrate whereupon evaporation of the solvents left highly purified product **19** from each parallel reaction. Selective deprotection of the Alloc group was accomplished using 1 equiv of morpholine with *tetrakis*(triphenylphosphine)palladium (0) followed by filtration through Celite® to afford the amine intermediate **20**. The morpholine derivative side-product was carried on to the next step and could serve as the base during acylation. Deprotection using polymer-bound piperazine and palladium on carbon was unsuccessful. The next step in the synthesis called for derivatization of the piperazine nitrogen. The amine intermediate **20** was reacted with an excess of chloroformate **21** in the presence of triethylamine. Upon completion of the reaction, polymer-bound amine **6** was added to the reaction mixture to sequester any unreacted chloroformate **21** followed by filtration and evaporation to afford purified products. Deprotection of the *t*-butyl ester using TFA in DCM provided the desired bi-substituted products **22**.

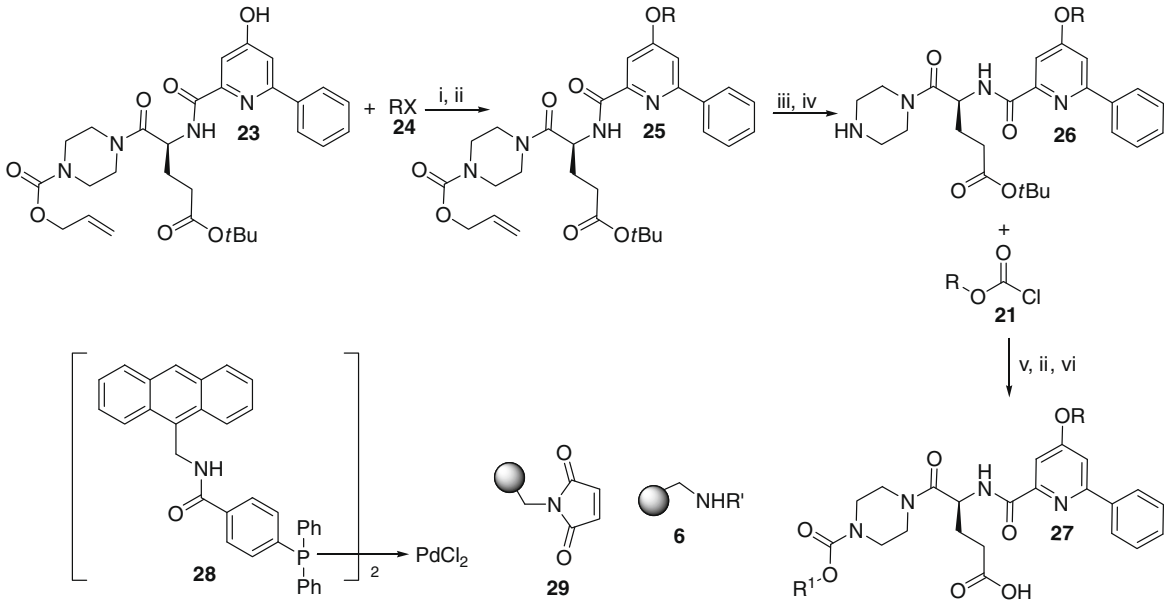
A focused library was designed in which ethyl, pentyl, 2-cyclopentylethyl, and hexyl chloroformates **21** were selected based on some of the most active carbamate analogs (Table 2). The amine inputs **12** included substituted piperidines and *N*-methyl-2-(methylsulfonyl)ethanamine. The data for this library is shown in Table 4 and demonstrates the additive effects of optimizing the piperazine carbamate and the 4-position of the pyridyl ring. Improved PRP potency was observed, with the pentyl and hexyl carbamates preferred in most cases. The 4-aminomethylpiperidine analog **22N** had exceptional potency with a PRP IC_{50} of 770 nM.

A similar multi-step PASP synthesis was designed to allow for various carbamates at the piperazine nitrogen with oxygen substituents at the pyridine 4-position as shown in Scheme 6. Compound **23** was selected as the starting template with the hydroxy at the 4-position allowing for the preparation of ether analogs. The first step of the synthesis was alkylation of the 4-hydroxy using 2 equiv of electrophile **24** with cesium carbonate as the base. After consumption of the 4-hydroxy template **23**, polymer-bound amine **6** was added to sequester the unreacted alkyl halide **24**. It was observed that with some reactions, despite excess electrophile or prolonged reaction times, the reaction would not go to completion. In these cases the polymer-bound amine **6** was sufficiently basic enough to also sequester the unreacted 4-hydroxy template **23**. Simple filtration and rinsing with dichloromethane yielded a filtrate whereupon evaporation of the solvents left purified product **25**. Alloc deprotection was attempted with *tetrakis*(triphenylphosphine)palladium (0) as previously used, but with an oxygen at the 4-position, triphenylphosphine and triphenylphosphine oxide by-products tended to carry through the synthesis, resulting in impure final products even after chromatography. Thus, an anthracene-tagged palladium catalyst **28** was employed to allow for easy sequestration of any phosphine by-products.[10] The reaction was conducted using the anthracene-tagged palladium reagent **28** and excess DEA in dichloromethane. Upon complete deprotection of the Alloc group, evaporation removed the DEA and allyldiethyl-

Table 4
Binding and PRP activity data for focused 4-aminopyridine library[9]

Compound	R	NR^1R^2	K_i[a] (nM)	IC_{50}[b] (μM)
22A	Et		48	7.6
22B	Pent		15	4.1
22C	Hex		36	17.7
22D	2-Cyclopentylethyl		80	17.5
22E	Et		42	9.8
22F	Pent		11	2.4
22G	Hex		24	6.4
22H	2-Cyclopentylethyl		40	57.8
22I	Et		18	9.9
22J	Pent		15	3.9
22K	Hex		33	7.6
22L	2-Cyclopentylethyl		—	—
22M	Et		14	3.3
22N	Pent		7	0.77
22O	Hex		18	1.6
22P	2-Cyclopentylethyl		69	14.1

[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.



Scheme 6. PASP synthesis of 4-ether pyridine-piperazine carbamate analogs **27**. Reagents and conditions: (i) 2 equiv **24**, Cs_2CO_3, KI, DMF, rt to 100 °C; (ii) 10 equiv **6**; (iii) 0.1 equiv **28**, 20 equiv DEA, DCM; (iv) 10 equiv **29**, microwave, 80 °C, dioxane; (v) 1.5 equiv **21**, 5 equiv TEA, DCM; (vi) 10% TFA/DCM.

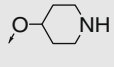
amine by-product. The resulting mixture was dissolved in dioxane and incubated with polymer-bound maleimide **29** under microwave conditions to effect complete sequestration of the catalyst **28** and any dissociated phosphine and phosphine oxide ligand. Simple filtration and evaporation of the solvent left purified products **26**. Derivatization of the piperazine nitrogen intermediate **26** was accomplished with excess chloroformate **21** followed by sequestration of the remaining chloroformate **21** with polymer-bound amine **6** to afford the *t*-butyl ester products. Deprotection of the *t*-butyl ester using TFA in DCM provided the desired bi-substituted products **27**.

A focused library was designed in which ethyl, isopropyl, butyl, and pentyl chloroformates **21** were selected with a variety of alkyl halides **24** (Table 5). Again, an additive effect was observed providing analogs with excellent PRP potency with the ethyl, butyl and pentyl carbamates preferred in most cases. This focused library provided 4-piperidine analog **27T** having exceptional potency with a PRP IC_{50} of 550 nM.

Several hundred compounds were prepared with nitrogen or oxygen substituents at the 4-position of the pyridine ring and varying carbamate chain lengths on the piperazine terminal nitrogen. The SAR clearly showed that butyl, pentyl, and hexyl carbamates were optimal and enhancement of potency and other properties could be obtained by variations at the 4-position of the pyridine, providing compounds with submicromolar PRP levels of activity.

The potency optimization described above was conducted with full consideration of maximizing reasonable drug-like properties. In general this class of compounds has good solubility and is chemically stable. These inhibitors also show 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. Compounds **7D**, **10E** and **10F**, each possessing a hydrogen at the 4-position with an ethyl, pentyl and hexyl carbamate, respectively, were selected to profile the general template and test if the carboxylic acid would be detrimental to oral absorption. Gratifyingly, all of the compounds were bioavailable with high bioavailabilities for the pentyl and hexyl carbamate analogs. However, all three of the compounds had high clearance values. Another concern with oral absorption was the molecular weight. The core templates **10E** and **10F** have molecular weights over 500. Substituting at the pyridine 4-position only adds to the molecular weight, potentially resulting in the loss of good bioavailability. Despite high molecular weight and, in numerous cases, poor Caco-2 permeability values, many compounds had good bioavailability [and probably have transporter mediated absorption, as exemplified by **27I** (molecular weight 570, P_{app} 1.1×10^{-6}).] Unfortunately, most of the initial compounds profiled had high clearance values despite good in vitro metabolic stability. The 4-piperidine analogs typically provided the more potent compounds and also allowed for optimizing the AMDE properties. It was found that compounds with the 4-piperidine substituted with a basic amine (**22N**) usually had high clearance values. Replacing the

Table 5
Binding and PRP activity data for focused 4-ether pyridine library[9]

Compound	R^1	OR	K_i[a] (nM)	IC_{50}[b] (μM)
27A	Et		157	7.6
27B	Allyl		203	11.1
27C	*i*-Pr	$O(CH_2)_2OH$	145	25.6
27D	Bu		21	7.8
27E	Pent		32	11.5
27F	Et		92	5.8
27G	Allyl		95	13.3
27H	*i*-Pr	$O(CH_2)_2OMe$	189	20.1
27I	Bu		18	4.4
27J	Pent		10	4.3
27K	Et		39	33.9
27L	Allyl		99	61.7
27M	*i*-Pr	OBu	146	—
27N	Bu		36	26.9
27O	Pent		49	28.4
27P	Et		68	1.8
27Q	Allyl		93	9.4
27R	*i*-Pr		157	—
27S	Bu		12	1.1
27T	Pent		3.8	0.55

[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.

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

Compound	CL (mL/min/kg)	*V*dss (L/kg)	$T_{1/2,\ eff}$ (h)	F_{oral} (%)
7D	98	15.5	1.8	34
10E	89	10.3	1.3	70
10F	29	1.7	0.7	>95[c]
27I	49	1.5	0.4	77
27T	133	3.1	0.3	1
22B	69	1.3	0.2	—
22N	70	2.6	0.4	0
22J	3	0.3	0.7	89

[a] Male Sprague–Dawley rats (n = 2–4 rats).
[b] Dose: iv infusion at 2 mg/kg; po at 5 mg/kg (n = 2–4 rats).
[c] F_{oral} (n = 3) 92%, 95%, and 181%.

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 discovery of compound **22J** with a low clearance of 3 mL/min/kg (1 mL/min/kg, dog) and good bioavailability (>90%, dog). Clearance of **22J** in rats appears to be mediated through extensive biliary excretion, with roughly 70% of an iv dose (2 mg/kg) recovered in the bile within 4 h.

Further evaluation of compound **22J** showed good solubility and chemical stability with the crystalline form of the compound. Compound **22J** was found to have acceptable margins versus hERG activity and a satisfactory CYP inhibition profile. Further evaluation on the potency of compound **22J** was determined by measuring the IC_{50} in 13 human donors using 20 μM ADP-stimulated PRP aggregation with the 4-well Chronolog PRP aggregometry assay and found the average IC_{50} to be 1.8 μM.[11,12] Compound **22J** was orally efficacious in the rat ferric chloride thrombosis model in which it dose-dependently prevented thrombus formation in this model of injury induced thrombosis.[13]

In vitro receptor binding, signaling, and functional studies have shown that **22J** is a high affinity, selective, and competitive antagonist at $P2Y_{12}$ receptors. Compound **22J** is more than 340-fold selective for $P2Y_{12}$ over the other purinergic receptors tested including the closest homolog, $P2Y_{13}$ (48% homology to $P2Y_{12}$), and a second platelet purinergic GPCR, $P2Y_1$ (19% homology to $P2Y_{12}$). The K_b (8.5 nM) for functional antagonism in the $P2Y_{12}$ signaling assay is in good agreement with the K_i (15 nM) for the receptor. The K_b in human PRP platelet aggregation assays (300 nM) is higher, due to the presence of plasma proteins. Schild analysis indicates the interaction of **22J** with the receptor in the aggregation assays is competitive with ADP.

In summary, we have utilized PASP parallel library synthesis to identify a $P2Y_{12}$ lead. This lead was optimized with generation of structure–activity relationships leading to highly potent $P2Y_{12}$ antagonists. With sufficient levels of potency attained, pharmacokinetic and physiochemical properties were modulated through various substituents at the 4-pyridine position. Fine-tuning the PK properties led to **22J**, an orally bioavailable, direct acting, reversible $P2Y_{12}$ antagonist. This compound is a selective antagonist of the human $P2Y_{12}$ receptor and demonstrates oral antiplatelet and anti-thrombotic efficacy in preclinical species. No safety issues were observed with **22J** as it was inactive in both the Ames and Micronucleus assays, and has been evaluated in both rat and dog safety studies (single dose and 7-day repeat dose) without significant toxicological findings. The potency, selectivity, safety, and overall pharmacokinetic profile for **22J** support advancement for clinical evaluation. Reports from our backup program where efforts at refining the SAR for more potent analogs with improved pharmacokinetic properties will be forthcoming. In addition, alternate aryl ring systems in place of the pyridine ring have been synthesized and will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.075.

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