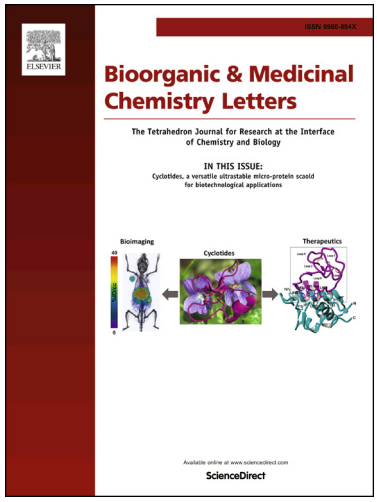
Accepted Manuscript



Reversible, Orally Available ADP Receptor ($P2Y_{12}$) Antagonists Part I: Hit to Lead Process

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Reversible, Orally Available ADP Receptor ($P2Y_{12}$) Antagonists Part I: Hit to Lead Process

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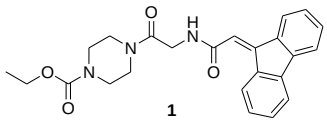
Abstract: A hit to lead process to identify reversible, orally available ADP receptor ($P2Y_{12}$) antagonists lead compounds is described. High throughput screening afforded **1**. Optimization of **1**, using parallel synthesis methods, a methyl scan to identify promising regions for optimization, and exploratory SAR on these regions, provided **22** and **23**. Compound **23** is an orally available, competitive reversible antagonist (K_B = 94 nM for inhibition of ADP-induced platelet aggregation). It exhibits high metabolic stability in human, rat and dog liver microsomes and is orally absorbed. Although plasma level after oral dosing of **22** and **23** to rats is low, reasonable levels were achieved to merit extensive lead optimization of this structural class.

Antiplatelet therapy reduces mortality and non-fatal events in patients with atherothrombotic

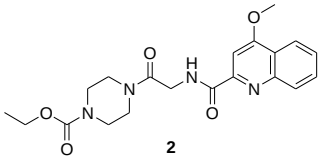
disease. Aspirin, which is modestly efficacious and relatively safe, remains the standard reference compound for antiplatelet treatment.[1] Clopidogrel (Plavix™) is slightly more effective than aspirin (8.7% added benefit).[2] When taken with aspirin, clopidogrel further reduces coronary events by 19%; however, the combination therapy results in a 30% increase in major bleeding compared to aspirin treatment alone.[3] Besides bleeding complications, there are other limitations and concerns about clopidogrel's use. It undergoes hepatic metabolism to produce active metabolites[4,5,6] that irreversibly inhibit the platelet ADP receptor $P2Y_{12}$.[7] At the therapeutic dose of 75 mg/day, 3 to 5 days are necessary to reach steady state inhibition of ADP-induced platelet aggregation, with inhibition levels between 40-60%.[8] Additionally, clopidogrel has a slow offset of action (about 5 days)[8], and there is a potential for drug resistance,[9,10,11,12], and drug-drug interaction[13,14]. While the beneficial effects of oral antiplatelet therapy are well established, the limitations of aspirin and clopidogrel, both irreversible inhibitors of platelet aggregation, underscore the need for new oral agents with improved efficacy and safety profiles.[15] Cangrelor (approved 2015) and ticagrelor (approved 2011) are reversible $P2Y_{12}$ inhibitors which are administered intravenously, thus have disadvantage over oral reversible $P2Y_{12}$ inhibitors. [16,17] Therefore, search for new $P2Y_{12}$ inhibitors having strong inhibition of platelet aggregation and low risk of bleeding are still being perused.[18] To achieve this goal we have already published in vitro effect, pharmacokinetic characterization and inhibition of platelet aggregation and thrombus formation in rat and dog model of novel and preclinical $P2Y_{12}$ antagonists BX 667 and metabolite BX 048.[19,20,21] Here in, we report hit to lead process to discover reversible, orally available ADP receptor ($P2Y_{12}$) Antagonists.

Chart 1: HTS and Parallel Synthetic Hits



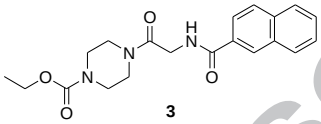
1

IC_{50} = 860 nM
C_{max} (2 mpk) = 2700 nM



2

IC_{50} = 690 nM
C_{max} (2 mpk) = 1200 nM
F(rat) = 42%

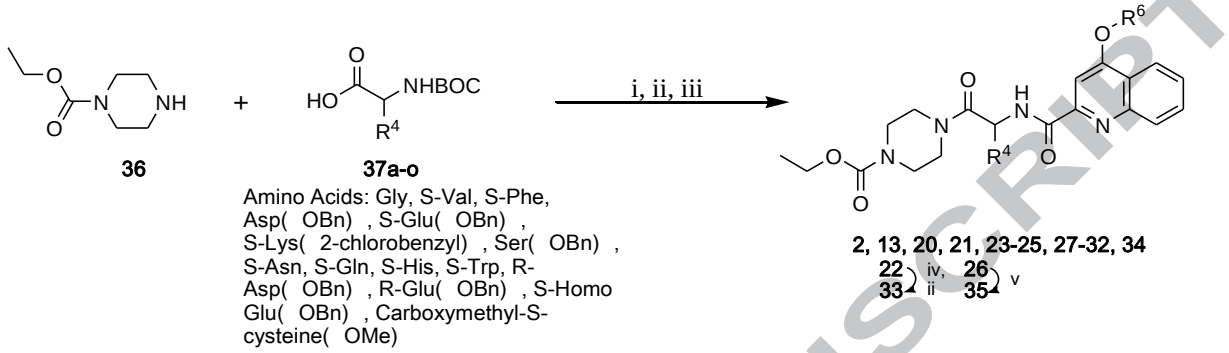


3

IC_{50} = 1800 nM
C_{max} (2 mpk) = 1500 nM

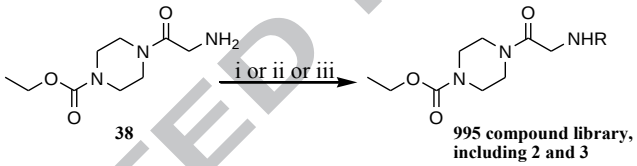
High Throughput Screening (HTS) of the Berlex proprietary library in a radioligand binding assay, which measured [^{33}P]2-MeSADP displacement from intact washed human platelets[19], provided the validated hit **1** (Chart 1). Although **1** exhibited sufficient potency and exposure levels after oral administration to rats to be considered a lead,[22] it contained an undesirable fluorenylmethyl group. The presence of two amide bonds in **1** made it a good candidate for parallel synthesis methods to find a replacement for the fluorenylmethyl group and to enable rapid SAR development. Utilizing a fragment of **1**, libraries of amides, carbamates, ureas and sulfonamides were synthesized; in total, 995 library compounds were prepared and tested (Schemes 1 and 2). Despite the relatively large number of compounds synthesized, only two library compounds, **2** and **3**, had reasonable potency in the binding assay (IC_{50} < 3000 nM) to merit further evaluation. Compound **2**, with similar potency to **1** and reasonable exposure levels after oral dosing,[22] was selected for further optimization.

Scheme 1.



Reagents and conditions. (i) EDC, HOBt, DIEA, amino acids 37 a-o, CH_2Cl_2; 67-91% (ii) TFA, CH_2Cl_2; 95-100% (iii) EDC, HOBt, DIEA, CH_2Cl_2/DMF, 4-methoxy-2-carboxyquinoline or 4-hydroxy-2-carboxyquinoline; 70-90% (iv) glycine(OtBu), EDC, HOBt, DIEA, THF; 85% (v) ethyl bromoacetic acid, KHMDS, THF; 65%

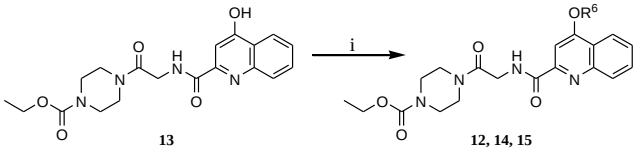
Scheme 2.



Reagents and conditions. (i) acid chloride, sulfonyl chloride or carbamoyl chloride, DIEA, DMAC/DME; 80-95% (ii) isocyanates, DIEA, DMAC;62-70% (iii) carboxylic acids, DIEA, isobutyl chloroformate in THF, DMAC;55-83%

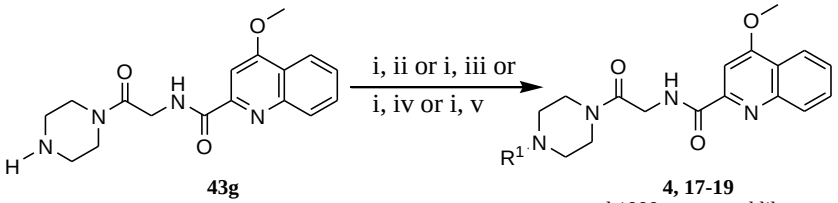
To prioritize areas on **2** to optimize, a "methyl scan" was performed by systematically adding or deleting a single methyl group at different sites on the molecule (Schemes 1, 3, 4, and 5).

Scheme 3.



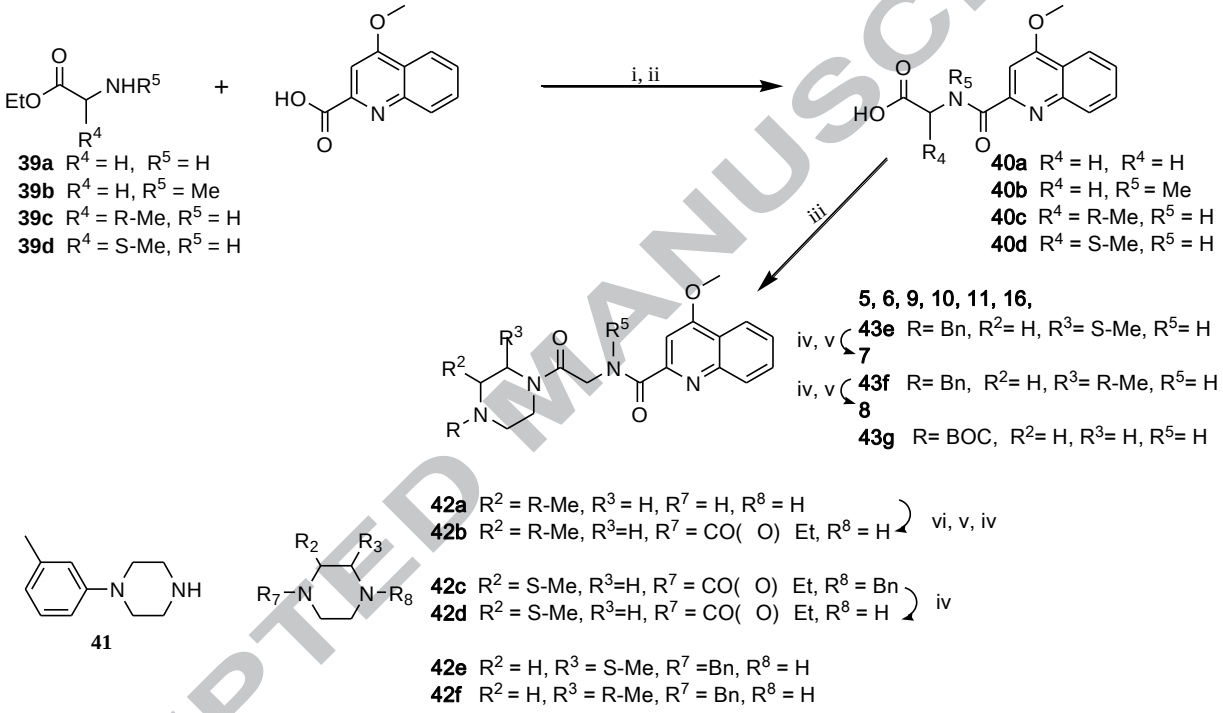
Reagents and conditions. (i) Cs_2CO_3, DMF; 55-73% ethyliodide for **12**, propyliodide for **14**, benzylbromide for **15**

Scheme 4.



Reagents and conditions. (i) TFA, CH_2Cl_2; (ii) Et_3N, CH_2Cl_2methyl chloroforamte$_2$; 64-89% (iii) DIEA, DMAC, isocyanates;56-85% (iii) DIEA, DMAC, carboxylic acids, isobutyl chloroformate in THF; 70-89%(iv) DIEA, DMAC/DME.acid chlorides, sulfonyl chlorides or carbamoyl chlorides;67-89%

Scheme 5.



Reagents and conditions. (i) EDC, HOBt, CH_2Cl_2, Et_3N;65-90% (ii) LIOH, MeOH/H_2O;84-90% (iii) EDC, HOBt, CH_2Cl_2, Et_3N;67-90% **36** or **41** or **42b** or **42d** or **42e** of **42f** or Boc piperazine (**42g**); (iv) Pd/C, H_2, MeOH; 89% (v) ethyl chloroformate, Et_3N, CH_2Cl_2;82% (vi) benzyl chloride, Et_3N, CH_2Cl_2 0 °C; 67%

Data from the "methyl scan" are presented in Table 1 and a representation of methyl group tolerance is shown in Chart 2. Incorporation of a methyl group throughout the molecule is generally tolerated (**5** - **9**, **12**), with the exception of replacing glycine with either D-alanine (**10**) or N-methyl glycine (**11**). However, the removal of a methyl group at two different sites (**4** and **13**) was not tolerated. Based on these methyl scan data (Table 1) and an analysis of the synthetic complexity for making additional analogs at each sites, three areas were prioritized

for initial optimization efforts: 1) phenol analogs, 2) ethyl carbamate analogs, 3) amino acid scan at the glycine site **(Chart 2).**

Chart 2. Areas Tolerant of Methyl Group Substitution.

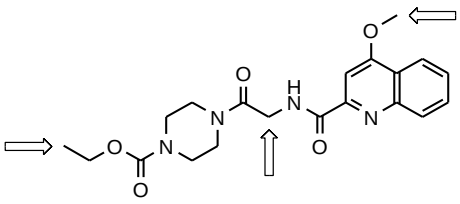
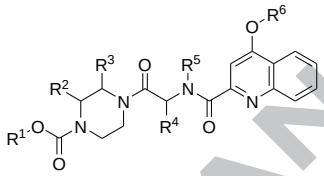


Table 1. Methyl Scan assay results for compounds **4-13**



Cmpd	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} (nM)[a]
2	Et	H	H	H	H	Me	690
4	Me	H	H	H	H	Me	>3000[b]
5	Et	S-Me	H	H	H	Me	1700
6	Et	R-Me	H	H	H	Me	4300
7	Et	H	R-Me	H	H	Me	4500
8	Et	H	S-Me	H	H	Me	1100
9	Et	H	H	S-Me	H	Me	1900
10	Et	H	H	R-Me	H	Me	14000
11	Et	H	H	H	Me	Me	21000
12	Et	H	H	H	H	Et	840
13	Et	H	H	H	H	H	>2000[b]

[a]Values represent the average of at least two experiments. [b]Highest concentration tested.

Alkylation of the phenolic oxygen of **13** provided a simple means to generate exploratory SAR at this site (Scheme 3). Although only a small number of phenolic ether analogs were synthesized, small alkyl ethers, **2**, **12**, **14**, are preferred over the larger benzyl ether analog **15**. Ethyl carbamate analogs were synthesized from carboxylic acid **40a** (Scheme 5) or through parallel synthesis methods on amine **43g** (Scheme 4). Of the 1000 library analogs synthesized, few active compounds were identified (examples include **16–19**), and none were more potent than **2** (Table 2). Conversely, an amino acid scan at the glycine site of **2**, employing naturally occurring amino acids (Scheme 1), provided a range of acceptable functionality: alkyl, **9** and **20**; amides, **26** and **27**; alcohol, **25**; imidazole, **28**; and carboxylic acids, **22** and **23**, but the lysine

analog **24** and the aromatic amino acids, **21** and **29,** were not tolerated (Tables 1 and 2). There was a clear preference for carboxylic acid-containing amino acids, suggesting that this could be a fruitful area for optimization. To further explore SAR at this site a number of acid-containing unnatural amino acids were incorporated into **2** (Scheme 1 and 5). Several analogs had reasonable potency, three of which gave IC_{50} values less than 500 nM, **32**, **34**, **35**. In addition, S-amino acids **22** and **23** are preferred over the corresponding R-amino acids **30** and **31**. With some SAR established for this potential lead series, and a 6-fold increase in potency for **23** over the high throughput screening lead **1**, compound **23** was selected for further characterization.

Pharmacological characterization of **23** was carried out by the method of Schild[23]. Concentration-response curves of **23** on ADP-induced platelet aggregation in the absence and presence of three increasing concentrations of **23** is shown in **Figure 1**. From these data a reciprocal replot was constructed, which quantitatively resulted in a slope of 0.82 and K_B = 94 nM. The pharmacologic behavior of **23** is consistent with a competitive, reversible mechanism of action as indicated by the parallel shift to the right in the concentration-response curves with negligible attenuation of the maximal response.

Figure 1. Concentration response curve of **23** on ADP-induced platelet aggregation

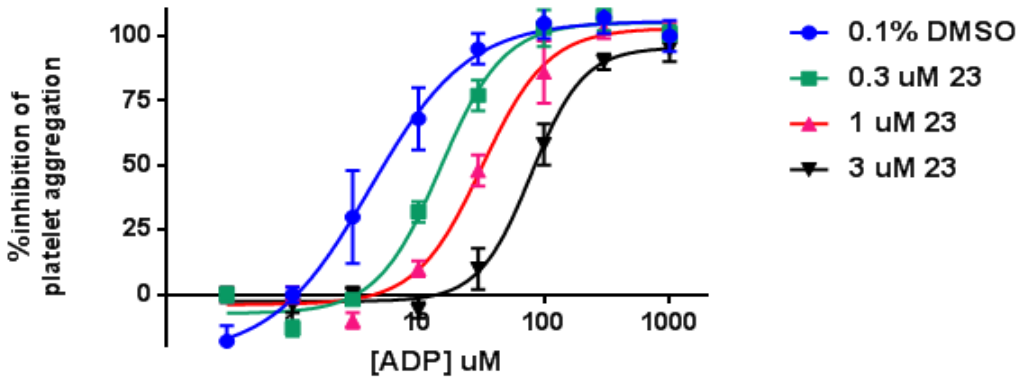
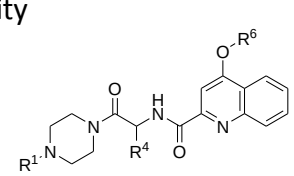


Table 2. ADP Receptor Binding Activity



Cmpd	R^1	R^4	R^6	IC_{50} (nM)[a]
2	CO_2Et	H	Me	690
14	CO_2Et	H	Pr	520
15	CO_2Et	H	Bn	>3000[b]
16	(3-Me)Ph	H	Me	890
17	CONHtBu	H	Me	910
18	$CO(CH_2)_4CH_3$	H	Me	1200
19	CO(4-Et)Ph	H	Me	1800
20	CO_2Et	S-iPr	Me	1500
21	CO_2Et	S-Bn	Me	8600
22	CO_2Et	$S\text{-}CH_2CO_2H$	Me	150
23	CO_2Et	$S\text{-}(CH_2)_2CO_2H$	Me	86
24	CO_2Et	$S\text{-}(CH_2)_4NH_2$	Me	26000
25	CO_2Et	$S\text{-}CH_2OH$	Me	1100
26	CO_2Et	$S\text{-}CH_2CONH_2$	Me	2100
27	CO_2Et	$S\text{-}(CH_2)_2CONH_2$	Me	2500
28	CO_2Et	$S\text{-}CH_2$(3H-imidazol-4yl)	Me	1500
29	CO_2Et	$S\text{-}CH_2$(1H-indol-3-yl)	Me	7900
30	CO_2Et	$R\text{-}CH_2CO_2H$	Me	18000
31	CO_2Et	$R\text{-}(CH_2)_2CO_2H$	Me	22000
32	CO_2Et	$S\text{-}(CH_2)_3CO_2H$	Me	270
33	CO_2Et	$S\text{-}CH_2CONHCH_2CO_2H$	Me	1100
34	CO_2Et	$S\text{-}CH_2SCH_2CO_2H$	Me	190
35	CO_2Et	$S\text{-}CH_2OCH_2CO_2H$	Me	200

[a]Values represent the average of at least two experiments. [b]Highest concentration tested.

Stability of **23** in human, rat and dog liver microsomes was determined after incubation for 3h at 3000 nM. High metabolic stability was observed in all three species. (**Table 3**). Compounds **22** and **23** were simultaneously administered orally to rats (compound dosed at 2 mg/kg) and plasma samples from the portal and jugular veins were analyzed for **22** and **23** (**Table 3**). Although relatively low plasma levels were observed after oral dosing of **22** and **23**, both compounds were orally absorbed.[22]

Table 3. Stability of **23** in liver microsomes and plasma concentrations of **22** and **23**, after oral dosing to rats

Time	% Remaining[a]		
	Human	Rat	Dog
1 h	> 90	> 90	> 90

Cmpd	Plasma Concentration (μM)			
	Systemic		Portal	
	1 h	3 h	1 h	3 h
22	0.27	0.20	0.41	0.38
23	0.12	0.10	0.34	0.34

[a]Percent of **23** remaining after 3h incubation with liver microsomes at 3000 nM concentration.

A hit to lead process for the HTS hit **1** resulted in lead compounds **22** and **23**. Compound **23** is a potent, competitive antagonist with a K_B = 94 nM for inhibition of ADP-induced platelet aggregation. It exhibits high metabolic stability in human, rat and dog liver microsomes and is orally absorbed, although plasma levels after oral dosing to rats is low. The half-life ($t_{1/2}$), C_{max} and Caco-2 permeability for analog **23** was found to be 1.2 h, 0.12 μM, and 1.66 x10^{-7} cm/sec respectively [24] . These data demonstrate that the structural class presented here, which contains compounds **22** and **23**, is a promising starting point for extensive lead optimization. The optimization of **23** to lead compounds BX 667 and BX 048 will be reported in Part II of this series.

Acknowledgement. We thank the Berlex Compound Logisitc and Parallel Synthesis Groups for providing compound samples for testing, and the DMPK group for metabolism and pharmacokinetic studies.

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22. Oral bioavailability can be limited by several factors including low solubility, presystemic lability, low permeability, and first-pass metabolism. Established *in vitro* and *in vivo* model systems are useful to assess whether a compound has properties favorable for absorption/bioavailability in humans. Fasted male Sprague-Dawley rats were used in these studies. Compounds **22** and **23** (2 mg/kg) was administered in a PEG based vehicle (PEG 300/EtOH/Water: 94/4/2) by oral gavage (po). Following dosing, blood samples were collected through the sampling cannula (0-24 hours post -dose). Following collection, the blood samples were centrifuged and plasma was harvested. The plasma samples were kept frozen until analysis.

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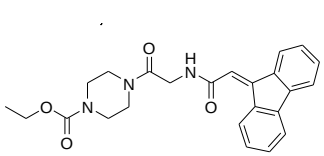
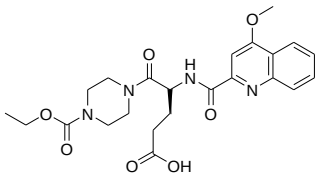
24. Bioavailability (F) was not determined



1

IC_{50} = 860 nM
F(rat) =45%
$t_{1/2}$ =1.8h



23

IC_{50} = 86 nM
$t_{1/2}$ =1.2h

Highlights:

- A hit to lead process to discover reversible, orally available ADP receptor ($P2Y_{12}$) antagonists has been described
- Parallel synthesis, methyl scan of the hit enhances the discovery of new analogs
- Exploratory SAR provided orally available competitive reversible antagonist of $P2Y_{12}$ receptor
- Two analogs show high metabolic stability in liver microsomes and found to be orally absorbed