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Optimization of 2-phenyl-pyrimidine-4-carboxamides towards potent, orally bioavailable and selective $P2Y_{12}$ antagonists for inhibition of platelet aggregation

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ABSTRACT

2-Phenyl-pyrimidine-4-carboxamide analogs were identified as $P2Y_{12}$ antagonists. Optimization of the carbon-linked or nitrogen-linked substituent at the 6-position of the pyrimidine ring provided compounds with excellent ex vivo potency in the platelet aggregation assay in human plasma. Compound **23u** met the objectives for activity, selectivity and ADMET properties.

When vascular integrity is compromised, signals are generated that cause circulating platelets to adhere to the vessel wall, become activated, and aggregate, forming a plug that seals off the site of injury to prevent blood loss. Autopsy studies demonstrated that the same processes occur upon rupture of an atherosclerotic plaque, and can lead to uncontrolled platelet thrombus formation and vessel occlusion.[1] Inhibition of platelet activation and aggregation is recognized as an effective strategy for prevention of atherothrombotic events in patients with atherosclerotic disease in the coronary, peripheral, and cerebrovascular circulation.[2]

The ADP receptor $P2Y_{12}$ plays a critical role in platelet activation and aggregation.[3] It is required to amplify and sustain the initial response to a vascular damage thereby leading to stable thrombus formation. The $P2Y_{12}$ receptor is a validated target for prevention of major adverse vascular events in patients with acute coronary syndromes (ACS, comprising unstable angina [UA], non-ST elevation myocardial infarction [MI] and ST elevation MI), as demonstrated by the thienopyridine class of drugs, including clopidogrel and prasugrel.[4] Thienopyridines are converted to active metabolites that irreversibly bind and inactivate the $P2Y_{12}$ receptor for the life of the platelet.

In patients with ACS, dual antiplatelet therapy with acetylsalicylic acid and clopidogrel is recommended today as the preferred treatment option.[4] Clopidogrel is an irreversible $P2Y_{12}$ receptor antagonist and its efficacy is limited by slow and variable conversion of the prodrug to the active metabolite, modest and variable inhibition of platelet aggregation,[5] and increased risk of bleeding at higher doses.[6] Prasugrel, achieving more pronounced inhibition of platelet aggregation, showed superior efficacy versus clopidogrel in moderate- to high-risk patients with ACS undergoing percutaneous coronary intervention (PCI), but the improved efficacy was associated with an increased bleeding risk.[7]

Novel principles of $P2Y_{12}$ antagonism may have the potential to improve efficacy without adversely affecting safety. Firstly, antagonists that bind reversibly to the receptor are less likely to cause bleeding than those that cause irreversible blockade, possibly because receptors remain responsive to high local concentrations of ADP.[8] Secondly, comparison of clopidogrel and prasugrel with genetic ablation of the $P2Y_{12}$ receptor in mice suggests that some of the excess bleeding associated with thienopyridines may be due to off-target effects.[9] Therefore, a reversible and selective $P2Y_{12}$ antagonist has the potential to provide an improved therapeutic window as compared with the thienopyridines.

Berlex,[10] Portola (Elinogrel[11]) and Astra Zeneca (Ticagrelor,[12] Brilinta®) were major players in the field of reversible $P2Y_{12}$ antagonists when we started our research. Ticagrelor and the Berlex molecules (**I**) were considered as starting points for knowledge-based programs (Fig. 1). One approach was to simplify the structure of Ticagrelor by cutting the triazolo-pyrimidine scaffold down to pyrimidine and by removing the sugar-like moiety. A set of

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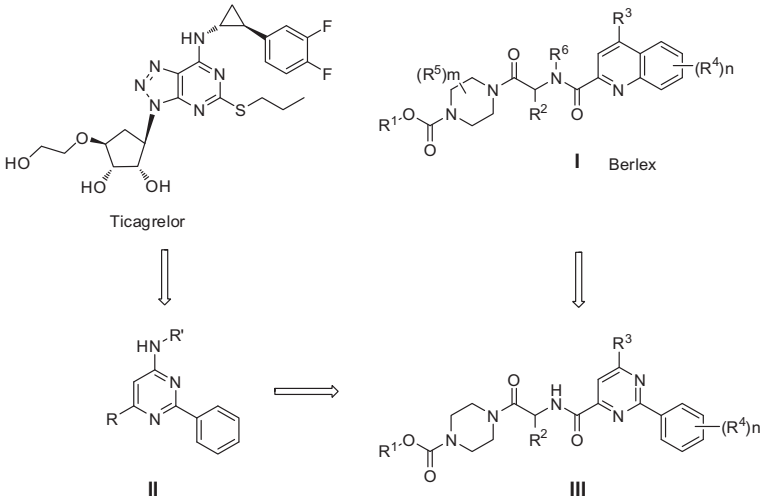


Figure 1. Implementation of a pyrimidine core structure (**II**) into Berlex molecules (**I**) leading to the 2-phenyl-pyrimidine-4-carboxamide series (**III**).

derivatives was prepared and few compounds of structure (**II**) caught our attention: they showed weak activity in the binding assay (5–10 μM, data not shown) and the analogy to the Berlex quinoline structure (**I**) was compelling. Gratifyingly, the Berlex quinoline was successfully replaced by scaffold (**II**) resulting in the 2-phenyl-pyrimidine-4-carboxamide series (**III**).[13]

All residues R^1 to R^4 were thoroughly explored. We investigated the carbamate moiety of (**III**), R^1–O–CO–N–, and came to similar conclusions as in previously published work by Pfizer.[14] Attempts to modify the piperazine core led to substantial or complete loss of biological activity. Substitution of the phenyl ring by various R^4 groups did not lead to improved biological activities. Therefore in the present paper R^1 represents ethyl or *n*-butyl and R^4 represents hydrogen. For R^2, the side chain of L-glutamic acid was kept constant throughout the study. The SAR of the R^3 group whereby R^3 is linked to the pyrimidine core by a nitrogen or a carbon atom is reported in this Letter.

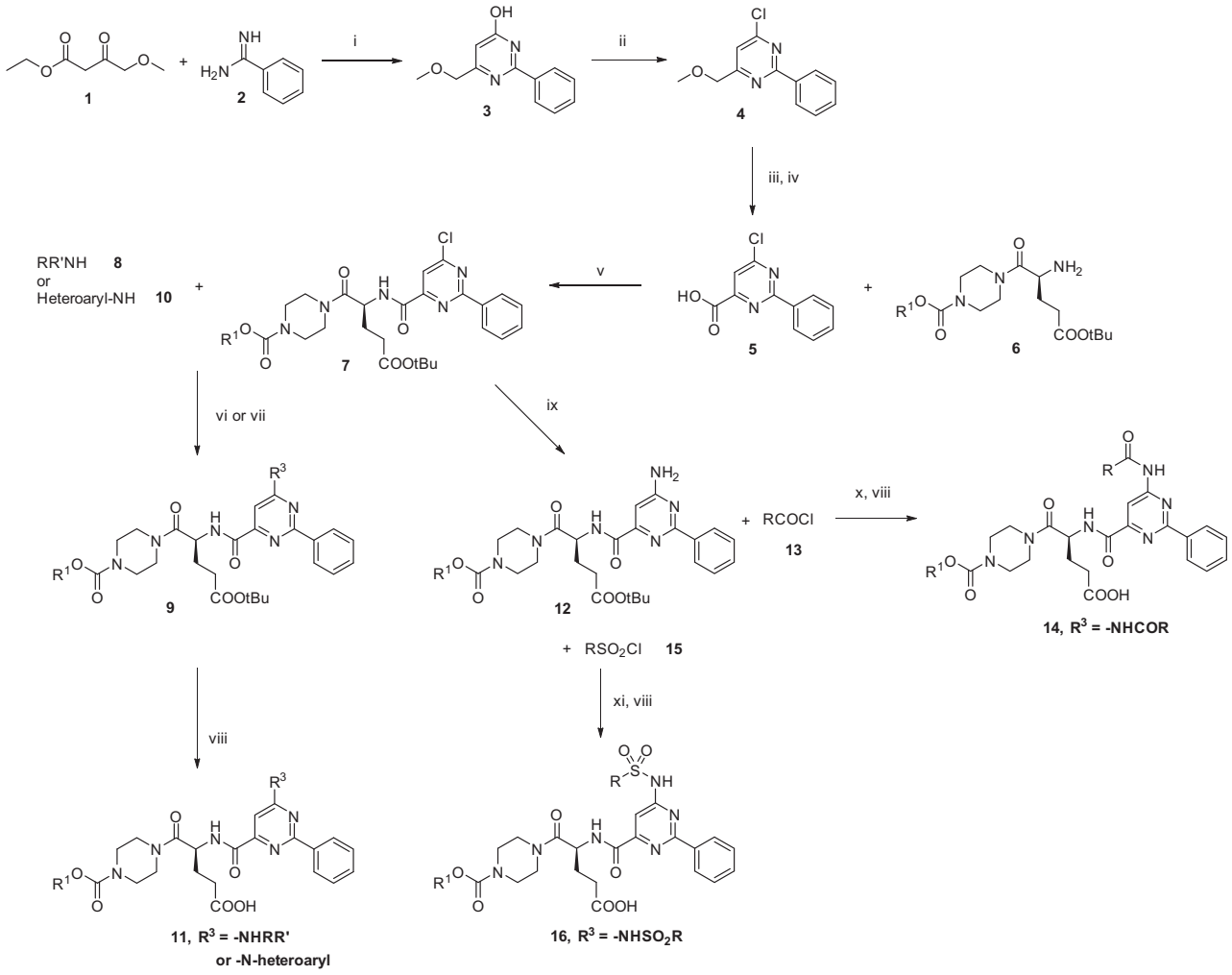
The synthesis of the molecules was designed and carried out as shown hereafter. The synthetic pathway to access 6-(N-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **11**, **14** and **16** is described in Scheme 1. The pyrimidine ring was built by condensation of ethyl 4-methoxy-3-oxobutanoate **1** with benzamidine **2** to provide hydroxypyrimidine **3**.[15] Subsequent chlorination in refluxing $POCl_3$ afforded the desired chloro derivative **4**. The methoxy group was cleaved using BBr_3 in DCM at 0 °C and the resulting primary alcohol was oxidized to the acid with $KMnO_4$/NaOH in water/dioxane at room temperature.[16] The resulting intermediate **5** was coupled to the amine **6**[17] to give intermediate **7**, ready for derivatization. Primary and secondary amines **8** were reacted with **7** in THF at 40 °C to give intermediate **9**. Heteroaromatic amines **10**, such as imidazole, were coupled to **7** using NaH in THF at room temperature. Final treatment of **9** with TFA in DCM provided the first set of final analogs **11**. The intermediate **12** was obtained by reacting **7** with NH_3 in MeOH and heating in a sealed tube at 90 °C in a microwave oven. Compound **12** was further treated with an acyl chloride **13** in pyridine heating at 70 °C. Final cleavage of the *tert*-butyl ester with TFA provided the second set of final compounds **14**. Reaction of **12** with sulfonyl chloride derivatives **15** using NaH in THF at 70 °C followed by deprotection with TFA afforded the third set of final molecules **16**.

The first synthetic pathway to access 6-(C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **23** is described in Scheme 2. Intermediates **22** were prepared using various protocols for the formation of a C—C bond.[18] Intermediate **7** was reacted under Suzuki conditions with the boronic acid **17**, mostly using $Pd(PPh_3)_4$ as catalyst and K_3PO_4 as base in refluxing dioxane. In case an alkene boronic ester **20** was used, such as for compound **23m**, the coupling was performed in presence of $Pd_2(dba)_3/PPh_3$ in a mixture of EtOH, toluene and Na_2CO_3 at 100 °C. An iron-catalyzed cross-coupling reaction[19] provided intermediate **22** using alkyl-magnesium bromide **19** in presence of $Fe(acac)_3$ in THF at room temperature. The Stille protocol was performed using stannane derivative **20**[20] and $Pd(PPh_3)_4$ as catalyst in toluene at 110 °C. Finally, a Sonogashira reaction was carried out using alkyne **21** in presence of $Pd(PPh_3)_2(OAc)_2$, CuI and NEt_3 in DMF at room temperature. Treatment of intermediates **22** with TFA provided the set of final compounds **23**. In case of analogs **23g** and **23l**–**n**, the intermediates **22** were submitted to additional chemical transformations before TFA treatment.[21]

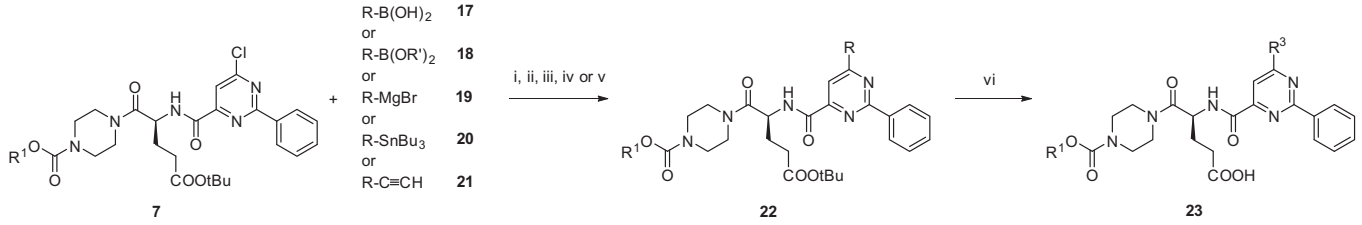
Another synthetic access to 6-(C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **23** is described in Scheme 3. The acid chloride **24** was reacted with acetylene **25** in presence of catalytic amounts of $Pd(PPh_3)_2(OAc)_2$ and CuI in NEt_3 at room temperature.[22] The resulting keto-alkyne **26** was coupled to benzamidine **2** with Na_2CO_3 and a catalytic amount of water in refluxing MeCN.[23] The acid derivative **28** was obtained after cleavage of the acetyl group of **27** in basic conditions (K_2CO_3 in MeOH/water) and oxidation of the resulting alcohol with $KMnO_4$ as previously described in Scheme 1. Finally intermediate **28** was coupled with compound **6** using EDCI and HOBt in DCM and the *tert*-butyl group was cleaved (TFA in DCM) to provide the final compounds **23d**–**e**.

The synthetic pathway to access 6-(C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **33** wherein R^3 is CH_2–NRR′ is described in Scheme 4. Compound **29** was submitted to the Suzuki conditions described previously in Scheme 2 using phenylboronic acid. The resulting intermediate was oxidized to the aldehyde in presence of SeO_2 in refluxing dioxane. After reduction of the aldehyde function with $NaBH_4$ in MeOH/DCM at 0 °C, the resulting alcohol was converted to the chloro derivative by refluxing in $POCl_3$. After saponification, intermediate **30** was further coupled with compound **6** using EDCI and HOBt. The chlorine atom of intermediate **31** was displaced by the amine **8** in THF at a temperature from 100 °C to 150 °C. Cleavage of the *tert*-butyl group provided the set of final compounds **33**.

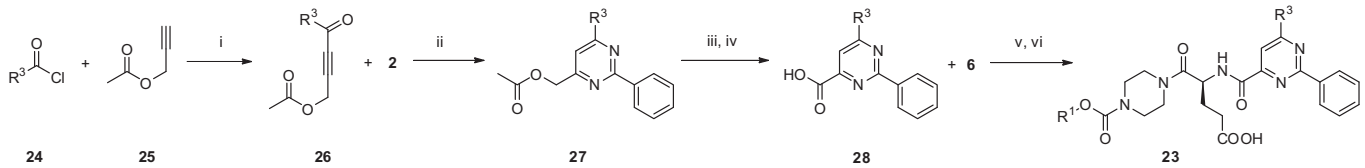
The project aimed at the discovery of potent $P2Y_{12}$ antagonists in vivo, that is, active at the site of action (platelets) in human blood. The first goal was to get affinity in the $P2Y_{12}$ receptor binding assay.[24] Since the inhibition of platelet aggregation in human



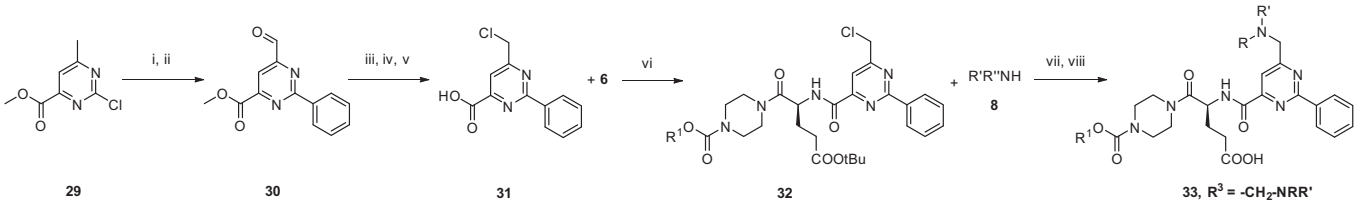
Scheme 1. Synthesis of 6-(N-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **11**, **14** and **16**. Reagents and conditions: (i) NaOMe, EtOH, 80 °C; (ii) $POCl_3$, 110 °C; (iii) BBr_3, DCM, 0 °C; (iv) $KMnO_4$, NaOH, H_2O, dioxane, rt; (v) $(COCl)_2$, MeCN, 80 °C, then 1 equiv **6**, NEt_3, MeCN, 0 °C–rt; (vi) excess **8** or 1 equiv **8** and Et_3N, THF, 40 °C; (vii) NaH, 1 equiv **10**, THF, rt; (viii) TFA/DCM, rt; (ix) excess NH_3, MeOH, 90 °C microwave; (x) 1 equiv **13**, pyridine, 70 °C; (xi) 1 equiv **15**, NaH, THF, 70 °C.



Scheme 2. Synthesis of 6-(C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **23**. Reagents and conditions: (i) 1 equiv **17**, $Pd(PPh_3)_4$, K_3PO_4, dioxane, 100 °C or $Pd(PPh_3)_4$, 2 M K_2CO_3, EtOH/DME, 90 °C; (ii) 1 equiv **18**, $Pd_2(dba)_3$, PPh_3, 1 M Na_2CO_3, EtOH/toluene, 100 °C; (iii) 2 equiv **19**, $Fe(Acac)_3$, THF, rt; (iv) 1.2 equiv **20**, $Pd(PPh_3)_4$, toluene, 110 °C; (v) 2 equiv **21**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, rt; (vi) TFA/DCM, rt.



Scheme 3. Alternative synthesis of 6-(C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **23**. Reagents and conditions: (i) $Pd(PPh_3)_2(OAc)_2$, CuI, NEt_3, rt; (ii) 2 equiv **2**, Na_2CO_3, MeCN, 1 drop H_2O, 90 °C; (iii) K_2CO_3, $MeOH/H_2O$, rt; (iv) $KMnO_4$, NaOH, H_2O, dioxane, rt; (v) 1 equiv **6**, HOBt, EDCI, DCM, rt; (vi) TFA/DCM, rt.



Scheme 4. Synthesis of 6-(C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **33**. Reagents and conditions: (i) 1 equiv phenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, dioxane, 100 °C; (ii) SeO_2, dioxane, 100 °C; (iii) $NaBH_4$, MeOH, DCM, 0 °C; (iv) $POCl_3$, 100 °C; (v) 1 M NaOH, MeOH, rt; (vi) 1 equiv **6**, HOBt, EDCI, DCM, rt; (vii) excess **8**, THF, 100–150 °C; (viii) TFA/DCM, rt.

platelet-rich plasma (PRP) was shown to correlate with antithrombotic efficacy in human clinical trials, the second key step was to optimize the potency in PRP. A first set of 6-(N/C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **11**, **14**, **16**, **23** and **33** were prepared by the above synthetic pathways. The most active compounds in the binding assay (generally IC_{50} value below 200 nM) were further profiled by measuring their inhibitory action in the ex vivo light transmission aggregometry (LTA) assay performed in human PRP.[25] Remaining activity (RA) was measured at 1000 nM, 500 nM or 100 nM concentration of the compounds, followed by determination of the IC_{50} for the most promising molecules. A panel of examples shown in Table 1 illustrates the first optimization round. Regarding 6-(N-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **11**, alkyl groups on the 6-nitrogen atom generally provided antagonists in the binding assay that translated into low to moderate potency in the LTA assay (see examples **11a**–**b**). Aryl and benzylic groups showed low ex vivo activities (see examples **11c**–**d**). The introduction of a hydroxyl group onto the alkyl chain (**11e**) provided for the first time promising activities with no plasma shift between in vitro and ex vivo assays. Replacing the hydroxyl of **11e** by an acid (**11f**) retained the in vitro activity but the ex vivo activity was lost. Replacement of the hydroxyl of **11e** by a dimethylamino function (**11g**) led to a significant loss in binding affinity. Finally, replacement of the hydroxyl of **11e** by the weakly basic morpholino group (**11h**) led only to moderate activities in both assays. Derivatives with the 6-nitrogen atom embedded into a cycloalkyl ring showed promises in the LTA assay, especially when a second heteroatom was included into the ring (**11j**, **11k**). Including the 6-nitrogen atom into a heteroaryl ring (see example **11l**) was found to increase the binding activity but the plasma shift remained high.

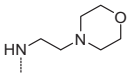
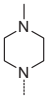
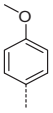
Finally, incorporation of the 6-nitrogen atom into an amide (see examples **14a**–**b**) or sulfonamide (see examples **16a**–**b**) function led to moderate in vitro biological activities. Therefore amides and sulfonamides **14** and **16** were not explored further.

Similar SAR trends were observed for 6-(C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **23**. Alkyl groups led to active compounds in the binding assay, but devoid of ex vivo activity as measured in the LTA assay (see examples **23a**–**d**). Introduction of a hydroxyl group two carbon atoms away from the pyrimidine core provided potency in both assays, as exemplified by **23e**. Aryl and heteroaryl groups (see examples **23f**, **23h**, **23i**–**k**) also showed good binding IC_{50} values, but moderate or low activity in the LTA assay. One exception was analog **23g** with an oxygen atom attached onto the aromatic ring as an N-oxide, displaying in the LTA assay an IC_{50} value of 126 nM and a plasma shift of 0.98. Finally it was also decided to investigate compounds with a nitrogen atom attached onto the carbon atom on position 6 of the pyrimidine ring. In particular, piperidine and morpholine rings were introduced to compare with analogs **11i** and **11k**. The corresponding analogs **33a** and **33b** were not potent enough in vitro to be further profiled.

Preparation of active compounds in the binding assay was successfully achieved during the first round of optimization. Nevertheless, reaching potency in the LTA assay remained challenging, as experienced by other groups.[26] Several compounds such as **11e**, **11j**, **11k**, **23e** and **23g**, that combine high activities in vitro and ex vivo, showed us the path to move forward. We decided to put emphasis on compounds extending an oxygen or nitrogen atom one to five atoms away from the position 6 of the pyrimidine core. A second set of compounds **11** and **23** was subsequently prepared following synthetic procedures described previously. Table 2 displays representative analogs illustrating the findings discussed hereafter. Firstly, O/N heteroatoms introduced as alcohol, ether or amine were tolerated by the receptor. Secondly, three parameters seemed to play a role in modulating the binding affinity: (a) the rigidity of the R^3 substituent which could impact on the entropic loss upon antagonist binding, (b) the lipophilic volume close to the position 6 of the pyrimidine and finally (c) the specific position of the heteroatom for additional favorable interactions in the pocket. For example, analogs **11m** and **23l** show moderate binding IC_{50} values of 279 nM and 218 nM, respectively. Both possess linear linkers that place a hydroxyl function four and three atoms away from position 6 of the pyrimidine, respectively. The flexibility of the linkers may allow to reach an acceptable position for the hydroxyl group but comes at an entropic cost. The more constrained analogs **11n** and **11w** show medium binding activities as well. This may be due to an unfavorable presentation of the heteroatoms. One could also speculate that analog **11u** is more active than **11p** because, although being structurally very close, the cyclic ether function is more favorable than the acyclic ether with regard to entropy. Finally, **11t** and **23r** were the most potent compounds in the binding assay with IC_{50} values of 16 nM and 15 nM, respectively, closely followed by **11s**, **11u** and **23o** (IC_{50} values of 21, 25 and 23 nM, respectively). All five compounds combine the structural requirements observed in the second optimization round: indeed the R^3 substituents are relatively rigid, contain some lipophilic volume close to the position 6 of the pyrimidine and might orient the oxygen atom favorably within the receptor pocket. As a conclusion, the second optimization round successfully produced highly potent antagonists in the binding and LTA assays, all analogs of Table 2 with the exception of compound **23q** exhibiting plasma shifts below 6. In addition, the structural requirements of R^3 for efficient binding to the $P2Y_{12}$ receptor were refined.

We postulated that the heteroatom in R^3 as hydrogen bond donor or acceptor would decrease plasma protein binding (PPB) thereby improving the IC_{50} value in the LTA assay. Table 3 displays a selection of protein binding data in human plasma and plasma shifts between in vitro IC_{50} and ex vivo IC_{50} values measured in human PRP. The compounds are listed according to decreasing plasma shift. High plasma shifts (>20) were indeed associated with high PPB (>98.5%, analogs **23b**, **11a**, **23a**) and low shifts (<2) characterized less plasma protein bound compounds (PPB <91.5%, analogs **11u**, **23l**, **11e**). Analog **23r** showed a plasma shift and PPB in the medium range. Within this set of compounds **11** and **23**, R^1 being ethyl, our hypothesis that the heteroatom in R^3 is required to decrease PPB thereby decreasing plasma shift seems to hold true.

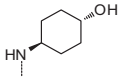
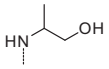
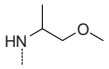
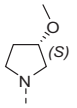
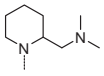
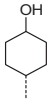
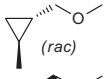
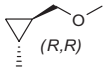
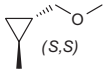
Table 1
First optimization round

Compound	R^3	Binding IC_{50}[a] (nM)	LTA activity[b]	LTA IC_{50}[c] (nM)	Plasma shift[d]
11a	–NH-*n*Pr	162		3820	24
11b	–NH-*i*Pr	95.5		724	7.6
11c	–NH-Ph	177	(–)		
11d	–NH-Bn	122	(–)		
11e	–NH–CH_2–CH_2–OH	169	(++)	114	0.67
11f	–NH–CH_2–CH_2–COOH	198	(–)		
11g	–NH–CH_2–CH_2–N(Me)$_2$	2780			
11h		257	(+)		
11i		201	(+)		
11j		46.0	(++)		
11k		100	(++)		
11l		27.0	(+)	318	12
14a	–NH–CO–cyclohexyl	219	(–)		
14b	–NH–CO–Ph	178	(–)		
16a	–NH–SO_2–Et	142	(–)		
16b	–NH–SO_2–Ph	266			
23a	–*n*Bu	98.0	(–)	1930	20
23b	–Cyclopropyl	59.5		3230	54
23c	–*i*Pr	52.0	(–)	1140	22
23d	–*t*Bu	36.0	(–)	2050	57
23e		21.0	(++)	121	5.8
23f		53.0	(–)		
23g		129	(++)	126	0.98
23h		64.0	(–)		
23i		31.0	(+)	523	17
23j		11.0	(–)		
23k		73.0	(–)		
33a		533			
33b		428			

R^1 is ethyl. Binding IC_{50}, LTA activity and IC_{50} and plasma shift of analogs **11**, **14**, **16**, **23** and **33** analogs.
[a] CHO cells expressing recombinant $P2Y_{12}$ receptors incubated with tritium-labeled 2-methyl-thio-adenosine 5′-diphosphate and compound.
[b] Incubation in human PRP with 3 μM ADP. (–): RA at 1000 nM >30%, or RA at 500 nM >60%, (+): 30% <RA at 500 nM <60%; (++): RA at 500 nM <30% or RA at 100 nM <70%.
[c] Incubation in human PRP with 3 μM ADP.
[d] Plasma shift = LTA IC_{50}/binding IC_{50}.

Finally, extending R^1 from ethyl to *n*-butyl improved the biological activity in the binding assay while slightly increasing the plasma shift, as seen with examples **11t**/**11x**, **23p**/**23s**, **23r**/**23u** (Table 4).

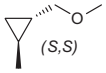
Table 2
Second optimization round

Compound	R^3	Binding IC_{50}[a] (nM)	LTA activity[b]	LTA IC_{50}[c] (nM)	Plasma shift[d]
11m	–NH–CH_2–CH_2–CH_2–OH	279	(+)		
11n		223	(+)		
11o		56	(++)	55	0.98
11p		97	(++)	139	1.4
11q		52	(++)	99	1.9
11r		46	(++)	68	1.5
11s		21	(++)	73	3.5
11t		16	(++)	32	2.0
11u		25	(++)	58	2.3
11v		76	(++)	86	1.1
11w		291	(+)		
23l		218	(++)	264	1.2
23m		40	(++)	171	4.3
23n		35	(++)	118	3.4
23o		23	(++)	71	3.1
23p		39	(++)	192	4.9
23q		43	(+)	589	14
23r		15	(++)	87	5.8

R^1 is ethyl. Binding IC_{50}, LTA activity and IC_{50} and plasma shift of **11** and **23** analogs.
[a] CHO cells expressing recombinant $P2Y_{12}$ receptors incubated with tritium-labeled 2-methyl-thio-adenosine 5′-diphosphate and compound.
[b] Incubation in human PRP with 3 μM ADP. (−): RA at 1000 nM >30%, or RA at 500 nM >60%, (+): 30% <RA at 500 nM <60%; (++): RA at 500 nM <30% or RA at 100 nM <70%.
[c] Incubation in human PRP with 3 μM ADP.
[d] Plasma shift = LTA IC_{50}/binding IC_{50}.

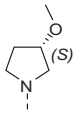
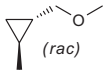
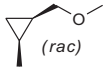
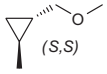
After the second optimization round, our criteria in terms of in vitro biological activity and ex vivo potency were met and further profiling was undertaken. Solubility was generally low at acidic pHs for analogs **23**, and increased for analogs **11**. At neutral and basic pHs, all compounds showed good solubility. No inhibition of cytochrome P450 enzymes (CYP3A4, CYP2D6, CYP2C9) was seen.[27] Overall, metabolic stability in human and rat liver microsomes was high[28] and none of the compounds tested on the hERG channel showed any inhibitory effect.[29] Selected potent $P2Y_{12}$ antagonists were therefore profiled in vivo in the rat. Oral administration was performed primarily, followed by intravenous experiments, if deemed necessary.

Table 3
Binding IC_{50}, LTA IC_{50}, plasma shift and PPB of selected analogs **11** and **23**

Compound	R^3	Heteroatom in R^3	Plasma shift[a]	PPB[b] (%)
23b	–cyclopropyl	No	54	99.7
11a	–NH-*n*Pr	No	24	98.5
23a	–*n*Bu	No	20	99.3
23r		Yes	5.8	96.6
11u		Yes	2.3	91.5
23l		Yes	1.2	87.2
11e	–NH–CH_2–CH_2–OH	Yes	0.67	86.5

R^1 is ethyl. The compounds are organized according to decreasing plasma shift.
[a] Plasma shift = LTA IC_{50}/binding IC_{50}.
[b] Protein binding in human plasma.

Table 4
Binding IC_{50}, LTA IC_{50} and plasma shift of **11** and **23** compounds wherein R^1 is *n*-butyl, available corresponding ethyl analogs are shown in brackets for comparison

Compound	R^1	R^3	Binding IC_{50}[a] (nM)	LTA IC_{50}[b] (nM)	Plasma shift[c]
11x (**11t**)	Bu (Et)		3 (16)	21 (32)	7.0 (2.0)
23s (**23p**)	Bu (Et)		3 (39)	21 (192)	7.0 (4.9)
23t	Bu		9	287	32
23u (**23r**)	Bu (Et)		4.8 (15)	31 (87)	6.4 (5.8)

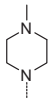
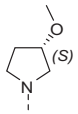
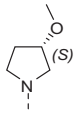
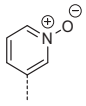
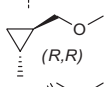
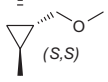
[a] CHO cells expressing recombinant $P2Y_{12}$ receptors incubated with tritium-labeled 2-methyl-thio-adenosine 5′-diphosphate and compound.
[b] Incubation in human PRP with 3 μM ADP.
[c] Plasma shift = LTA IC_{50}/binding IC_{50}.

Representative data are summarized in Table 5. Systemic plasma clearance in the rat spanned over a large range, from 14 up to 100 mL/min kg. The volume of distribution at steady-state was in the range of total body water, as expected for acidic compounds with extensive binding to plasma proteins. All molecules containing a hydroxyl group in R^3 (**11e**, **23m**, **23n**, **23p**) showed low oral exposures. A methoxy group seemed to be better tolerated (see examples **11t**, **11x**, **23q**, **23u**). The N-oxide derivative **23g** was not detected in rat plasma. The higher oral exposure of **23u** as compared to **23q** was likely a consequence of the lipophilicity increase resulting from the elongation of the ethyl chain to a *n*-butyl group. The lipophilicity increase was indeed reflected in the log*D* (pH 7.4) of the two compounds, being 0.8 for **23q** and 1.9 for **23u**. Overall, **23u** offered an acceptable profile with a low clearance and a medium bioavailability of 33%.

Further characterization of **23u** was undertaken. **23u** was found to be a reversible $P2Y_{12}$ antagonist (data not shown). No antagonistic activity on genetically related P2Y receptors was seen. Specifically, up to 10 μM of **23u** did not interfere with agonist binding to $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$ and $P2Y_{13}$ (data not shown). Furthermore, no antagonistic activity was detected on $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$ and $P2X_7$ receptors (data not shown). In addition, **23u** was tested at 10 μM for potential antagonistic or agonistic activity on a panel of 161 GPCRs using the PathHunter technology (DiscoveRx). In this assay, no significant agonistic or antagonistic activity, other than on $P2Y_{12}$, was detected (data not shown). In conclusion, **23u** is a selective and reversible antagonist of $P2Y_{12}$.

In summary, we have identified within the 2-phenyl-pyrimidine-4-carboxamide series (**III**) two sets of highly potent $P2Y_{12}$ antagonists, the 6-(N-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **11** and the 6-(C-linked-R^3)-2-phenyl-pyrimidine-4-carboxamide analogs **23**. The structural requirements for high affinity to the $P2Y_{12}$ receptor both in binding and in the LTA assay were described and a correlation between the plasma shift and protein binding could be established within this optimization round. Extensive fine-tuning led to the discovery of two R^3 groups (exemplified in **11t/11x** and **23q/23u**) which provided excellent potency in the low nanomolar range both in binding and in the ex vivo platelet aggregation assay performed in human PRP. Furthermore, moving from R^1 being ethyl to *n*-butyl increased the lipophilicity of the compounds thereby improving oral absorption in the rat. No inhibition of the main human cytochrome P450 enzymes nor of the hERG channel was observed. Compound **23u** showed the best profile amongst **11** and **23** analogs, being an orally bioavailable, selective and reversible $P2Y_{12}$ antagonist with an excellent inhibitory effect on platelet aggregation in human plasma. The cyclopropyl derivative **23u** was

Table 5
Rat pharmacokinetic profiles of selected $P2Y_{12}$ antagonists

Compound	R^1	R^3	CL (mL/min kg)	*Vss* (L/kg)	$T_{1/2}$ (h)	AUC (nM h)	C_{max} (nM)	*F* (%)
11e	Et	–NH–CH_2–CH_2–OH	100	1.0	0.20	8.44	17.5	3
11j	Et		49	0.8	0.30	164	192	3
11t	Et		36	1.8	0.63	638	683	8
11x	Bu		24	0.5	1.2	1170	1690	9
23g	Et					BLQ	BLQ	
23m	Et					78.2	46.3	
23n	Et					109	56.1	
23q	Et		14	0.3	0.55	2310	2820	11
23u	Bu		17	0.4	2.0	6000	7620	33

Male Wistar rats.
Dose: iv infusion at 1 mg/kg (n = 2 rats); po at 10 mg/kg (n = 3 rats).
BLQ: below limit of quantification (ca. 1 ng/mL).

selected for further in vivo antithrombotic and safety experiments in preclinical species. This work will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.070.

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29. For details of the hERG inhibition assay, see the Supplementary material.