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Exploration of a new series of PAR1 antagonists

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ABSTRACT

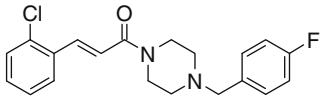
Two series of new PAR1 antagonists have been identified. The first incorporates a cinnamoylpiperidine motif and the second a cinnamoylpyridine pattern. The synthesis, biological activity and structure–activity relationship of these compounds are presented. In each series, one analog showed potent in vivo antithrombotic activity in a rat AV shunt model, with up to 53% inhibition at 1.25 mpk iv for compound **30**.

Protease activated receptors (PARs) or thrombin receptors constitute a class of G-protein coupled receptors (GPCRs) implicated in many activation mechanisms. Thus, thrombin activates several cell types such as vascular smooth muscle cells, leukocytes, endothelial cells and platelets via activation of these receptors.[1] In humans, thrombin-induced platelet aggregation is mediated by one subtype of these receptors, termed PAR1.[2] Therefore, antagonism of this receptor should lead to a new class of antiplatelet drugs. The main advantage of this approach is that by inhibiting thrombin-mediated platelet activation without affecting thrombin's role in the coagulation cascade, PAR1 antagonists are expected to have a limited impact on bleeding.

A new inhibitor of PAR1 incorporating a cinnamoylpiperazine moiety, F16357 (**1**, Fig. 1), and SARs of simple analogs, has been recently published by our group.[3] In this Letter, more diverse chemistry projects concerning more profound structural modifications of **1** will be described. This work has led to the discovery of two new antagonists of PAR1 showing good in vitro antiplatelet potency, and antithrombotic activity in an in vivo rat model.[4]

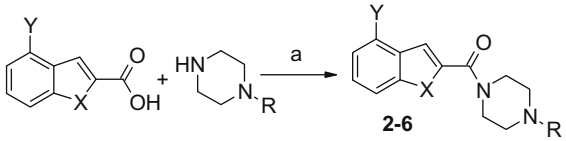
Starting from the left-hand part of the molecule, we first focused on the preparation of bicyclic analogs that incorporate a fused phenyl-alkene system. These compounds were easily obtained in one step from commercially available materials (Scheme 1).

Replacement of the central carbonyl moiety by the known cyclobut-3-ene-1,2-dione bioisostere,[5] as in compounds **7**–**12**, was less straightforward (Scheme 2). Starting from 3,4-dibutoxycyclobut-3-ene-1,2-dione, good conditions were found to prepare the requisite phosphonate precursor for building the alkene. The synthesis started with the 1,2-addition of the α-lithium-phosphonate salt to one carbonyl function, purification of the alcohol intermediate and then hydrolysis to promote the rearrangement.[6] One-pot procedures proved to be less efficient. The subsequent Wittig



1

Figure 1. 3-(2-Chloro-phenyl)-1-[4-(4-fluoro-benzyl)-piperazin-1-yl]-propenone (F16357).



2-6

X = NMe, NH, O, S, OCH_2
Y = H, F
R = Benzyl, 4-fluorobenzyl

Scheme 1. Reagents and conditions: (a) EDC, 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one, DIEA, DMF, rt, 50–92%.

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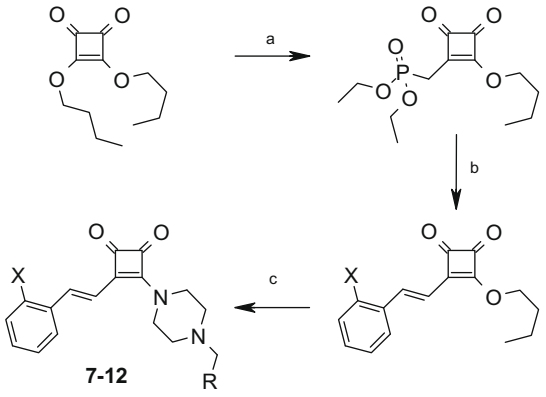
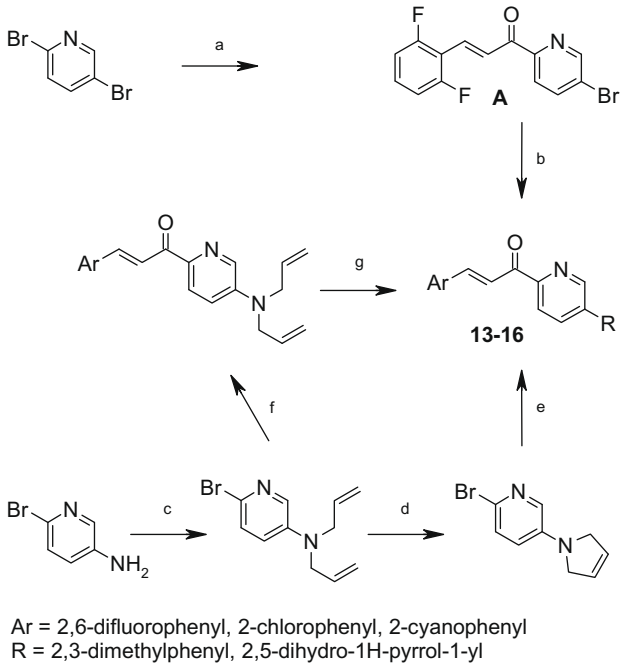
reaction was more difficult to control and gave poor yields even after extensive exploration of reaction conditions. Finally, introduction of the substituted piperazine in the last step of this approach was easy and therefore amenable to small library synthesis.[7]

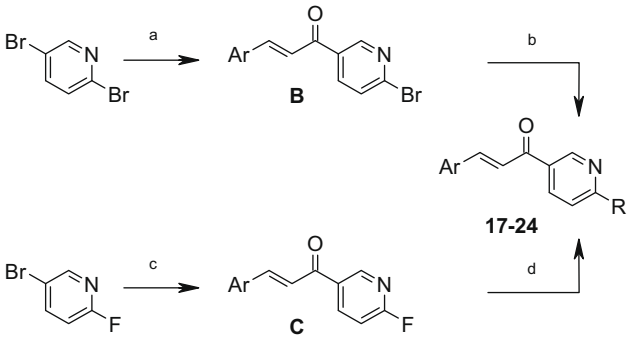
Exploration of the piperazine portion of **1**, gave rise to several chemistry projects. Pyridine analog syntheses are depicted in Schemes 3 and 4. When R is an aromatic, the synthesis proceeded smoothly and started with a regioselective Br–Li exchange of one of the bromides in 2,5-dibromopyridine as already reported,[8] followed by quenching with the appropriate Weinreb amide. Finally, a Suzuki reaction with the remaining aromatic bromide allowed the introduction of a large range of aromatics. When R is an heterocycle such as pyrrolidine or 2,5-dihydro-1*H*-pyrrole, direct amination of intermediates A (Scheme 3) and B (Scheme 4) was not successful, and a different route had to be designed. In pyridine analogs bearing the nitrogen atom in the *ortho* position to the carbonyl function (Scheme 3), the syntheses started with bis allylation of 2-bromo-5-aminopyridine, which was followed by a metathesis reaction to create the 2,5-dihydro-1*H*-pyrrole. The resulting compounds were then subjected to the same organolithium chemistry as before in order to introduce the phenylbutenone left-hand por-



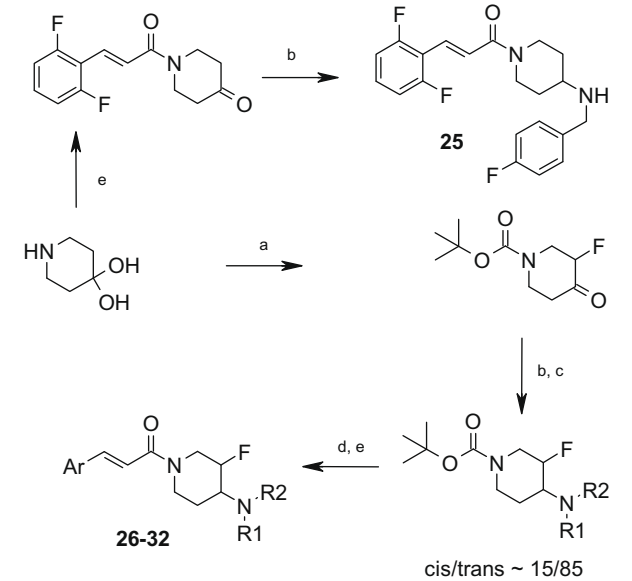
Scheme 2. Reagents and conditions: (a) (1) diethylmethylphosphonate, *n*BuLi, THF, −78 °C, 2 h, 75%; (2) DCM, HCl concd, rt, 1 h, 88%; (b) 2-chlorobenzaldehyde or 2-cyanobenzaldehyde, 0.5 equiv Na_2CO_3, THF, rt, 18 h, 10–33%; (c) alkylpiperazine, DIEA, DCM, EtOH, rt, 4 h, 33–75%.



Scheme 3. Reagents and conditions: (a) (*E*)-3-(2,6-difluoro-phenyl)-*N*-methoxy-*N*-methyl-acrylamide, *n*BuLi, toluene, −78 °C, 74%; (b) 1-bromo-2,3-dimethylbenzene, Na_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, 90 °C, 18 h, 55%; (c) allylbromide, toluene, NaH, 90 °C, 88%; (d) 0.2 equiv Grubbs II, DCM, rt, 18 h, 76%; (e) (*E*)-3-aryl-*N*-methoxy-*N*-methyl-acrylamide, *n*BuLi, Et_2O, THF, −100 °C, 15–29%; (f) (*E*)-3-(2,6-difluoro-phenyl)-*N*-methoxy-*N*-methyl-acrylamide, *n*BuLi, THF, −78 °C, 76%; (g) 0.2 equiv Grubbs II, DCM, rt, 18 h, 42%.

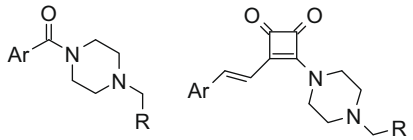


Scheme 4. Reagents and conditions: (a) (*E*)-3-aryl-*N*-methoxy-*N*-methyl-acrylamide, *n*BuLi, Et_2O, −78 °C, 29–79%; (b) 1-bromo-2,3-dimethylbenzene, Na_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, 90 °C, 18 h, 12–15%; (c) (*E*)-3-aryl-*N*-methoxy-*N*-methyl-acrylamide, *n*BuLi, Et_2O, −100 °C, 74–81%; (d) 1 equiv amine, 0.5 equiv K_2CO_3, DMF, 0 °C to rt, 21–67%.

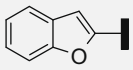
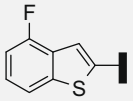
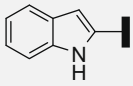
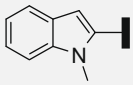
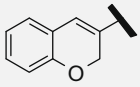


Scheme 5. Reagents and conditions: (a) (1) BOC_2O, $NaHCO_3$, H_2O, 50 °C, 3 h, 99%; (2) TMSCl, DMF, TEA, 80 °C, 24 h, 87%; (3) Selectfluor, CH_3CN, rt, 86%; (b) $R1NH_2$, $NaBH(OAc)_3$, AcOH, 1,2-DCE, rt, 24 h, 45–76%; (c) omitted or R2CHO, $NaBH(OAc)_3$, AcOH, 1,2-DCE, rt, 24 h, 66%; (d) TFA, toluene, 0 °C to rt, 62–98%; (e) (*E*)-3-aryl-acrylic acid, EDC, HOOBT, DIEA, DMF, rt, 49–89%.

Table 1
Antagonist activity results for analog series I and II of compound **1**

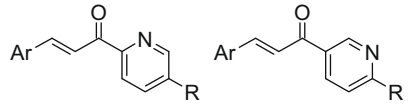


Series I Series II

Compds	Ser.	Ar	R	% Antag. at 10 μM*
1	—	—	—	93
2	I		Phenyl	1
3	I		Phenyl	4
4	I		Phenyl	2
5	I		Phenyl	11
6	I		4-Fluorophenyl	16
7	II	2-Chlorophenyl	Phenyl	85
8	II	2-Chlorophenyl	4-Fluorophenyl	23
9	II	2-Chlorophenyl	Thiophen-3-yl	27
10	II	2-Chlorophenyl	Cyclohexyl	41
11	II	2-Cyanophenyl	Thiophen-3-yl	97
12	II	2-Cyanophenyl	Cyclohexyl	84

* Inhibition of calcium release induced by 1 μM of SFLLR-NH_2. Data are expressed as mean SEM of at least two independent determinations performed in triplicate.

Table 2
Antagonist activity results for analog series III and IV of compound **1**



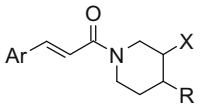
Series III Series IV

Compds	Ser.	Ar	R	% Antag. at 10 μM*
13	III	2,6-Difluorophenyl	2,3-Dimethylphenyl	40
14	III	2,6-Difluorophenyl		60
15	III	2-Chlorophenyl		13
16	III	2-Cyanophenyl		52
17	IV	2,6-Difluorophenyl	2,3-Dimethylphenyl	74
18	IV	2,6-Difluorophenyl	Pyrrolidin-1-yl	20
19	IV	2,6-Difluorophenyl	Cyclohexylamino	41
20	IV	2-Chlorophenyl	2,3-Dimethylphenyl	32
21	IV	2-Chlorophenyl	Cyclohexylamino	97
22	IV	2-Chlorophenyl	Cyclopentylamino	11
23	IV	2-Cyanophenyl	Cyclohexylamino	53
24	IV	2-Cyanophenyl	Cyclopentylamino	84

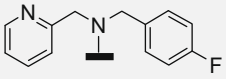
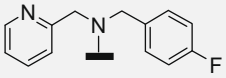
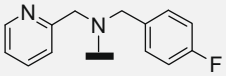
* Inhibition of calcium release induced by 1 μM of SFLLR-NH_2. Data are expressed as mean SEM of at least two independent determinations performed in triplicate.

tion of the molecule. In pyridine analogs bearing the nitrogen atom in the *meta* position to the carbonyl function (Scheme 4), the amino substituents were introduced by S_NAr of fluoro intermediate C, which was obtained in one step from 2-fluoro-5-bromopyridine. Finally, we decided also to explore the replacement of the piperazine ring of **1** by an aminopiperidine. The desired compounds were

Table 3
Antagonist activity results for analog series V of compound **1**



Series V

Compds	Ar	R	X	% Antag. at 10 μM*
25	2,6-Difluorophenyl	4-Fluorobenzylamino	H	0
26	2,6-Difluorophenyl	4-Fluorobenzylamino	F (*cis*)	25
27	2-Chlorophenyl	4-Fluorophenylamino	F (*cis*)	85
28	2-Chlorophenyl		F (*cis*)	23
29	2-Cyanophenyl	4-Fluorobenzylamino	F (*cis*)	40
30	2-Cyanophenyl		F (*cis*)	68
31	2-Cyanophenyl		F (*trans*)	33

* Inhibition of calcium release induced by 1 μM of SFLLR-NH_2. Data are expressed as mean SEM of at least two independent determinations performed in triplicate.

easily prepared from piperidin-4-one (Scheme 5). Fluoride analogs **26–32** were prepared from the same starting material using the three-step sequence already published to introduce the fluorine atom.[9]

Determination of all compound potencies was based on the inhibition of fluorescent calcium release induced by the PAR1 selective agonist peptide (SFLLR-NH_2) in CHO cells (FLIPR).[10] Starting with the left-hand portion of **1**, fusing the phenyl and the alkene functions of the cinnamoyl motif (Table 1, Series I) resulted in inactive compounds, whereas more success was obtained with the cyclobutene-1,2-dione analogs (Table 1, Series II). Indeed, very potent antagonist of PAR1, such as compounds **7**, **11** and **12**, could be obtained after careful tuning of the substitution pattern on the cinnamoyl aromatic with the ligand on the piperazine ring. In the pyridines series (Table 2), more potent antagonists of PAR1 where obtained when the pyridine nitrogen was located in the *meta* position from the carbonyl function (Series IV). Again in this series, it was difficult to find general rules for structure–activity relationship. Therefore, it was crucial for every ligand R on the pyridine ring to explore many substitution patterns on the cinnamoyl aromatic, in order to properly determine their potential. In the piperidine series (Table 3), a small improvement of the antagonist potency was obtained through the introduction of a fluorine atom as shown with compounds **25** and **26**. Moreover, in all instances *cis* analogs were more potent than *trans* analogs as shown with compounds **30** and **31**.

The pharmacological properties of the best compounds in each series were further evaluated (Table 4). A concentration–response study using the same model as in the screening allowed the discrimination of partially versus fully competitive antagonists. In the case of full antagonist the pA_2 was determined. Anti-aggregant properties were assessed using an SFLLR-induced human platelet aggregation model,[2] and their antithrombotic potential was determined through an arteriovenous extra-corporal shunt model in anesthetized rats.[4] The best results were obtained with the last two series of analogs. Even though some cyclobutene-1,2-dione analogs behaved as strong PAR1 antagonists (compounds **11** and **12**), no anti-aggregant properties were detectable in our in vitro human platelet model. Conversely, pyridine analog **24** revealed a very comparable pharmacological profile with respect to compound **1**, and piperidine analog **30** displayed significant improved activity in the rat antithrombotic model.

In conclusion, two new series of PAR1 receptor antagonists have been identified. In each series, one analog showed good in vitro anti-aggregant properties combined with potent in vivo antithrombotic activity in a rat pharmacodynamic model. Both compounds were selected for more extensive biological evaluation.

Table 4
Antagonist activity results for analogs series V of compound **1**

Compds	% Antag. at 10μM[a]	pA_2[b]	Human platelets[c] (pKb)	AV Shunt[d] (%)
1	93	6.42	5.52	28
7	85	Partial	—	—
11	97	6.41	4	—
12	84	6.37	4	—
21	97	Partial	—	—
24	84	6.03	5.05	28
27	85	6.41	4	—
30	68	6.23	5.68	53

[a,b] Inhibition of calcium release induced by 1 μM of SFLLR-NH_2: % at 10 μM or pA_2.
[c] Inhibition of SFLLR-induced human platelet aggregation.
[d] % Increase of the occlusion time in an arteriovenous shunt in rat at 1.25 mg/kg iv.

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