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Discovery and SAR of small molecule PAR1 antagonists

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ABSTRACT

High-throughput screening resulted in the identification of a small molecule inhibitor of PAR1. Optimisation of the initial hit led to the discovery of compounds **34** and **49**, which displayed antithrombotic activity in an arteriovenous shunt model in the rat after iv administration.

Platelet activation in response to vascular injury occurs via a number of signalling pathways, for which a variety of agonists, such as thrombin, ADP, thromboxane A2, collagen, or epinephrine are known. Among these, thrombin is probably the most potent platelet activator, whose action is mediated via proteolytic cleavage of specific cell surface receptors known as protease activated receptors (PARs).[1] In humans, thrombin-induced platelet aggregation is mediated by one subtype of these receptors, termed PAR1.[2] Since inhibition of the PAR signalling pathway could reduce the number of activated platelets without inhibiting thrombin's roles in the coagulation cascade, it is thought that PAR1 antagonists may hold the key to antithrombotic therapies without the associated risk of bleeding side effects seen with current treatments. Indeed, SCH- 530348, a novel PAR1 antagonist, is currently in phase III clinical trials for the treatment of acute coronary syndrome.[3] Herein we wish to report our studies towards the development of selective PAR1 antagonists.

An in-house high-throughput screening program, based on inhibition of intracellular calcium release induced by a PAR1-selective agonist peptide (SFLLR) in CHO cells,[4] led to the discovery of compound **1** (Fig. 1), which was found to be a 96% antagonist at 10 μM. Analogues of **1** were synthesised via the route depicted in Scheme 1. All compounds were tested at 10 μM in the calcium release screening assay, and for compounds displaying greater than 75% inhibition, we also determined the pK_b for the inhibition of SFLLR-induced aggregation of human platelets.

Thus, nucleophilic substitution of commercially available alpha-bromo ketones was used to furnish thioethers in good yields. One-pot alpha halogenation of the crude products with NCS in CCl_4 followed by nucleophilic substitution of the intermediate chlorides

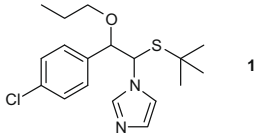
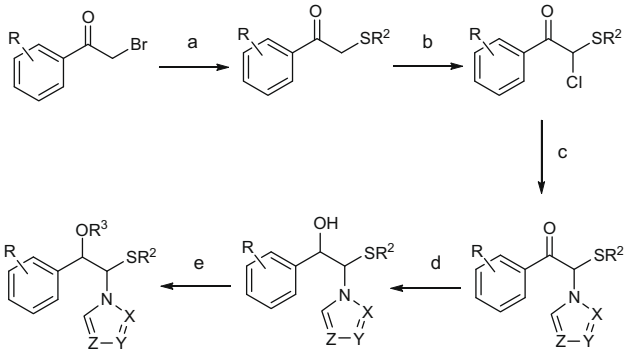


Figure 1. Initial PAR1 inhibitor hit.



Scheme 1. Reagents and conditions: (a) R^2SH, DIEA or NaH, THF, 0 °C, 90–100%; (b) NCS, CCl_4, rt, 30 min; (c) heterocycle, DCM, rt, 16 h, ca. 50% two steps; (d) $NaBH_4$, MeOH, rt, 70–80%; (e) R^3Br, NaH, DMF, 60 °C, 16 h, 50–80%.

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yielded alpha-disubstituted aryl ethanones in ca. 50% yield. Sodium borohydride reduction of the carbonyl group followed by alkylation of the resulting alcohols with alkyl bromides furnished the desired products as mixtures of enantiomers.

Initial optimization began with modification of the ether substituent (Table 1). In the screening assay, ethyl and methyl ethers (compounds **2** and **3**) were similar to hit structure **1**, whilst larger alkyl chains (compounds **5** and **6**) and the alcohol functionality (compound **4**) were much less active. The platelet model revealed that compounds **1** and **2** were equipotent whilst compound **3** was slightly more active.

Screening of thioether analogues (Table 2) showed that modification of the *tert*-butyl group led to a loss of potency. Surprisingly, isopropyl thioether **9** was found to be much less active than the corresponding *tert*-butyl thioether (compound **2**).

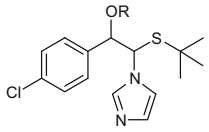
We then proceeded to make changes to the aryl group (Table 3). Amongst the compounds tested, only the 4-carboxymethyl- and 2-nitro-substituted phenyl were not potent enough in the screening assay not to be evaluated in the platelet model (compounds **17** and **19**, respectively).

The latter test revealed that with respect to an unsubstituted phenyl group (compound **13**), halogenated phenyls were more potent (compounds **2**, **11** and **12**), although the activity with respect to hit structure **1** was the same. A 5-substituted benzothiophene (compound **14**) was more potent than compound **1**, and a 4-trifluoromethylphenyl group (compound **15**) even more so. A 6-substituted 1,4-benzodioxane (compound **16**) was more active than the unsubstituted phenyl ring, but less potent than the initial hit structure.

We next turned our attention to modification of the imidazole ring. Thus, nucleophilic substitution of the alpha-chloro ketone intermediate in Scheme 1 with 1*H*-1,2,4-triazole led to a mixture of 1- and 4-substituted triazole isomers, which were easily separated by flash chromatography. Reaction with 4-chloro-1*H*-imidazole[5] gave a mixture of well-separated 4- and 5-substituted imidazoles, but 4-methyl-1*H*-imidazole and 4-(trifluoromethyl)-1*H*-imidazole[6] led to predominately the 4-isomer. The alpha-chloro intermediate was also reacted with 4,5-dimethyl-1*H*-imidazole[7] in order to avoid the problem of regioisomers. The positions of the substituent on the imidazole ring of the final products were determined by 2D NMR.

Replacement or substitution of the imidazole had a large impact upon antagonist activity (Table 4). In the screening test, pyrazole **20** was completely inactive. Potency was retained with triazole **21**, whereas isomer **22** was a weak inhibitor. Potency was also retained with a chlorine atom or a methyl group in the 4-position of the imidazole ring (compounds **23** and **25**) but not with chlorine in the 5-position (compound **24**). Compound **26**, a trifluoromethyl analogue of compound **25**, was very weak inhibitor. Finally, a loss of activity was found when the imidazole ring was substituted with a methyl group in the 2-position (compound **27**), or with two methyl groups (compound **28**). Out of the compounds tested in the platelet model, only the 4-methylimidazole (compound **25**) was found to be more potent than compound **1**.

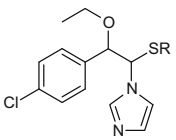
Table 1
Antagonist activity of compounds **1**–**6**



Compd	OR	% antag.@ 10 μM[a]	Hum. Plat. (pK_b)[b]
1	OPr	96	5.01
2	OEt	99	5.05
3	OMe	95	5.24
4	OH	11	—
5	OBu	33	—
6	OBn	23	—

[a] Inhibition of calcium release induced by 1 μM of SFLLR.
[b] Inhibition of SFLLR-induced human platelet aggregation.

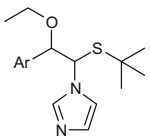
Table 2
Antagonist activity of compounds **2** and **7**–**10**



Compd	SR	% antag.@ 10 μM[a]	Hum. Plat. (pK_b)[b]
2	S*t*Butyl	99	5.05
7	S*n*Butyl	76	4.58
8	SPentyl	19	—
9	S*i*Propyl	40	—
10	SCyclohexyl	3	—

[a] Inhibition of calcium release induced by 1 μM of SFLLR.
[b] Inhibition of SFLLR-induced human platelet aggregation.

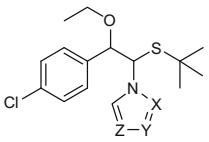
Table 3
Antagonist activity of compounds **2** and **11**–**19**



Compd	Ar	% antag.@ 10 μM[a]	Hum. Plat.[b] (pK_b)
2	4-Cl-Ph	99	5.05
11	4-Br-Ph	100	5.06
12	2,4-Cl-Ph	100	5.01
13	Ph	92	4.46
14	Benzothiophene	98	5.20
15	4-CF_3-Ph	100	5.38
16	Benzodioxane	88	4.79
17	4-CO_2Me-Ph	37	—
18	3-OMe-Ph	76	0
19	2-NO_2-Ph	33	—

[a] Inhibition of calcium release induced by 1 μM of SFLLR.
[b] Inhibition of SFLLR-induced human platelet aggregation.

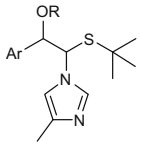
Table 4
Antagonist activity of compounds **2** and **20**–**28**



Compd	X	Y	Z	% antag.@ 10 μM[a]	Hum. Plat.[b] (pK_b)
2	CH	CH	N	99	5.05
20	N	CH	CH	0	—
21	N	CH	N	99	4.64
22	CH	N	N	34	—
23	CH	CCl	N	96	4.60
24	CCl	CH	N	59	—
25	CH	C–CH_3	N	92	5.36
26	CH	C–CF_3	N	20	—
27	C–CH_3	N	CH	63	—
28	C–CH_3	C–CH_3	N	64	—

[a] Inhibition of calcium release induced by 1 μM of SFLLR.
[b] Inhibition of SFLLR-induced human platelet aggregation.

Table 5
Antagonist activity of compounds **29**–**38**



Compd	Ar	OR	% antag.@ 10 μM[a]	Hum. Plat.[b] (pK_b)
29	2-Cl–Ph	OEt	100	5.21
30	2-Cl–Ph	OPr	97	5.14
31	3-Cl–Ph	OEt	100	4.96
32	4-Cl–Ph	OMe	96	4.42
33	4-Cl–Ph	OEt	92	5.36
34	4-Cl–Ph	OPr	99	5.71
35	4-Br–Ph	OEt	100	5.73
36	4-Br–Ph	OPr	76	5.99
37	2,4-Cl–Ph	OEt	93	5.84
38	2,4-Cl–Ph	OPr	88	5.95

[a] Inhibition of calcium release induced by 1 μM of SFLLR.
[b] Inhibition of SFLLR-induced human platelet aggregation.

Following these results, a library of compounds was synthesised using the optimal substituents. The most interesting results are shown in Table 5. Activity was found to be consistently higher with the 4-methylimidazole compounds than the non-substituted analogues (results not shown).

An additional pharmacological assay was then performed in order to better evaluate the antithrombotic activity of lead compounds. Thus, an in vivo model consisting of an arteriovenous extra-corporal shunt in anaesthetised rats was performed. The compounds were compared with a potent PAR1 antagonist, F16618, recently published by our group[8] (Table 6).

Compounds **34**, **36** and **38** are all potent antagonists of PAR1 in the human platelet aggregation model, although only compound **34** was found to be active in vivo. F16618 was more potent than compound **34** in the rat shunt model, despite its weaker activity in the human platelet model.

Further tests revealed that the metabolic stability of compounds **34**, **36** and **38** was reasonable against human liver microsomes but poor against rat liver microsomes (Table 7). This might partly explain the non-consistent results between the two models, although other pharmacokinetic parameters are likely to be involved.

Table 6
Antagonist activity of F16618 and compounds **34, 36** and **38**

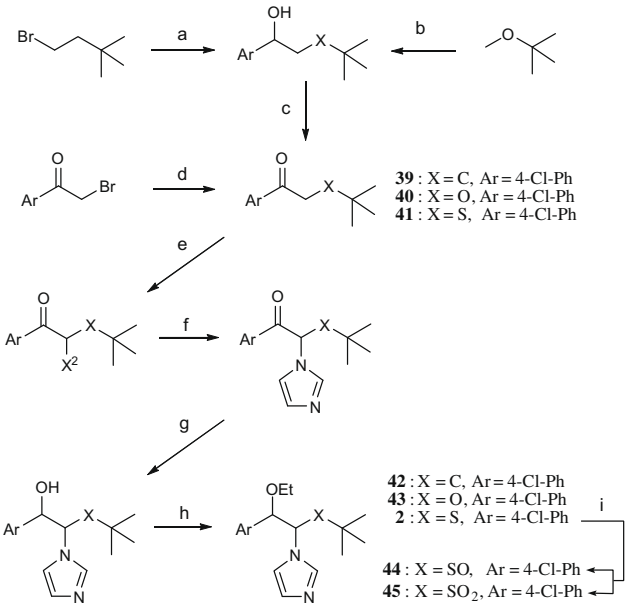
Compd	% antag.@ 10 μM[a]	pK_b[a]	Hum. Plat. (pK_b)[b]	AV Shunt[c] (iv,%)
F16618	99	—	5.30	58
34	99	5.51	5.71	45
36	76	—	5.99	0
38	88	—	5.95	7

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

Table 7
Metabolic stability of F16618 and compounds **34, 36** and **38**

Compd	Microsomal stability[a] (human)	Microsomal stability[a] (rat)
F16618	48	27
34	28	0
36	23	0
38	28	2

[a] % of parent compound remaining after 60 min incubation with rat or human hepatic microsomes.

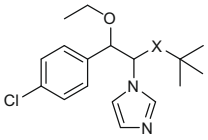


Scheme 2. Reagents and conditions: (a) (1) Mg, Et_2O, reflux, 2 h; (2) 4-chlorobenzaldehyde, rt, 16%; (b) (1) ^{s}BuLi, ^{t}BuOK, −78 °C; (2) 4-chlorobenzaldehyde, −78 °C to rt, 49%; (c) PCC, DCM, 100%; (d) X = S: ^{t}BuSH, NaH, THF, 0 °C to rt, 16 h, 100%; (e) X = C, X^2 = Br: Br_2, DCM; X = O, X^2 = Cl: SO_2Cl_2; X = S, X^2 = Cl: NCS, CCl_4; (f) imidazole, DCM (g) $NaBH_4$, MeOH, 63% three steps when X = C, 42% three steps when X = O, 49% three steps when X = S; (h) EtBr, NaH, DMF, 60 °C, 16 h, 60–70%; (i) mCPBA, DCM, 16% X = SO, 16% X = SO_2.

Further changes to the core structure were then performed in order to improve the metabolic stability against animal microsomes. Optimisation began with replacement of the thioether functionality of compound **2** with CH_2, O, SO and SO_2. The synthetic pathway to these compounds is shown in Scheme 2.

Thus, condensation of 4-chlorobenzaldehyde with a Grignard reagent derived from 1-bromo-3,3-dimethylbutane[9] gave an intermediate alcohol, which was oxidised to ketone **39** using PCC. Similarly, condensation of the same aldehyde with an organometallic resulting from the deprotonation of *tert*-butylmethyl ether[10] gave an intermediate alcohol which furnished ketone **40** after PCC oxidation. Ketone **41** was synthesised via nucleophilic substitution of an alphabromoketone with *tert*-butylthiol. The imidazole was introduced via halogenation of the alpha keto position, followed

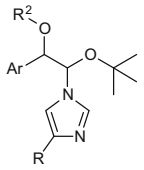
Table 8
Antagonist activity and metabolic stability of compounds **2** and **42**–**45**



Compd	X	% antag.@ 10 μM[a]	Hum. plat.[b] (pK_b)	Microsomal stability[c] (human)	Microsomal stability[c] (rat)
2	S	99	5.05	41	2
42	C	74	4.12	57	3
43	O	85	4.54	74	25
44	SO	15	—	59	39
45	SO_2	13	—	35	47

[a] Inhibition of calcium release induced by 1 μM of SFLLR.
[b] Inhibition of SFLLR-induced human platelet aggregation.
[c] % of parent compound remaining after 60 min incubation with rat or human hepatic microsomes.

Table 9
Antagonist activity of compounds **43** and **46–50**



Compd	Ar	R	R^2	% antag.@ 10 μM[a]	Hum. plat. (pK_b)[b]
43	4-Cl–Ph	H	Et	85	4.54
46	3,4-Cl–Ph	H	Pr	93	5.04
47	4-F–Ph	H	Pr	18	—
48	4-CF_3–Ph	Me	Et	98	5.20
49	3,4-Cl–Ph	Me	Pr	81	5.73
50	2,4-Cl–Ph	Me	Et	98	5.32

[a] Inhibition of calcium release induced by 1 μM of SFLLR.
[b] Inhibition of SFLLR-induced human platelet aggregation.

by reaction of the crude intermediate chloride or bromide with imidazole. Sodium borohydride reduction of the carbonyl group followed by alkylation of the resulting alcohol with bromoethane yielded products **2**, **42** and **43** as mixtures of enantiomers. mCPBA oxidation of product **2** led to a mixture of sulfoxide **44** and sulfone **45**. The results of the modifications upon the activity and metabolic stability of the compounds are shown in Table 8.

Replacement of sulfur (compound **2**) by carbon (compound **42**) resulted in a loss of potency and no increase in stability against rat microsomes. The oxygen analogue (compound **43**) was also less active than compound **2**, although more potent than compound **42**. However, a large increase in metabolic stability was observed. Oxidation of the thioether to a sulfoxide or a sulfone (compounds **44** and **45**, respectively) resulted in almost inactive molecules, but which displayed increased stability against rat microsomes.

We then proceeded to synthesise a small library of oxygen analogues to see if the potency could be improved with retention of metabolic stability. The compounds were synthesised by the same route as depicted in Scheme 2. The results are shown in Table 9.

As with the sulfur compounds, a methyl group in the 4-position of the imidazole ring gave more potent antagonists with respect to the unsubstituted analogues, best shown by the platelet model for compounds **46** and **49**. Unfortunately, the metabolic stability observed with compound **43** was not retained (Table 10). Nevertheless, compound **49** was found to possess potent antithrombotic activity in vivo, and its overall profile was similar to that of

Table 10
Antagonist activity and metabolic stability of F16618 and compounds **43, 49** and **50**

Compd	% antag.@ 10 μM[a]	Hum. plat.[b] (pK_b)	AV shunt[c] (iv,%)	Microsomal stability[d] (human)	Microsomal stability[d] (rat)
F16618	99	5.30	58	27	48
43	85	4.54	0	74	25
49	81	5.73	43	23	3
50	98	5.32	0	57	3

[a] Inhibition of calcium release induced by 1 μM of SFLLR.
[b] Inhibition of SFLLR-induced human platelet aggregation.
[c] % increase in occlusion time of an arteriovenous shunt in the rat (1.25 mg/kg iv).
[d] % of parent compound remaining after 60 min incubation with rat or human hepatic microsomes.

compound **34**. Despite its metabolic stability, compound **43** was inactive in the shunt model. Although this compound was a much weaker antagonist in the platelet model, the lack of in vivo activity is likely due to other pharmacokinetic parameters.

In conclusion, we have identified a series of novel PAR1 antagonists through screening of our in-house library. SAR studies resulted in an increase in potency, and compounds **34** and **49** were found to display strong antithrombotic activity in vivo. Although the compounds lacked metabolic stability against rat microsomes, the compounds were found to be sufficiently stable against human liver microsomes. Work is underway in order to further optimise these compounds.

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