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Discovery and Initial Structure–Activity Relationships of Trisubstituted Ureas as Thrombin Receptor (PAR-1) Antagonists

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Abstract—Thrombin is the most potent agonist of platelet activation, and its effects are predominantly mediated by platelet thrombin receptors. Therefore, antagonists of the thrombin receptor have potential utility for the treatment of thrombotic disorders. Screening of combinatorial libraries revealed **2** to be a potent antagonist of the thrombin receptor. Modifications of this structure produced **11k**, which inhibits thrombin receptor stimulated secretion and aggregation of platelets.

The enzyme thrombin plays a central role in thrombosis and hemostasis, not only by enzymatic activation of coagulation factors, but also by direct stimulation of several cell types such as vascular endothelial cells, smooth muscle cells, and platelets.[1] Thrombin is the most potent agonist of platelet activation, and recently the mechanism of this activation has been revealed. In 1991 Coughlin et al. characterized the first thrombin receptor, which is expressed on platelets, endothelial cells, smooth muscle cells, and others.[2] This 7-transmembrane domain G-protein coupled receptor is activated by a novel mechanism whereby thrombin proteolytically cleaves part of the extracellular N-terminal domain of the receptor, revealing a new N-terminus ending in the residues SFLLR. This sequence then acts as a 'tethered ligand' and binds to a site on the receptor in an intramolecular fashion, causing receptor activation.[3] This protein was therefore named protease activated receptor-1 (PAR-1), and subsequent to this discovery, three other receptors that work by a similar mechanism, PAR-2, PAR-3, and PAR-4, have been identified.[4] Support for the 'tethered ligand' hypothesis comes from the observation that in cells expressing PAR-1, addition of the pentapeptide SFLLR-NH_2 (thrombin receptor activating peptide, 'TRAP') mimics the effect of thrombin on those cells, albeit at higher concentration.

Interest in developing antiplatelet agents for treatment of thrombotic disorders led us to consider the thrombin receptor as an attractive target.[5] Potential advantages over current therapies are that blockade of the receptor would prevent both platelet secretion of prothrombotic and inflammatory mediators as well as aggregation. Further, the enzymatic activity of thrombin would remain intact, allowing for its anticoagulant properties. Modulation of the thrombin receptor on other cell types (e.g., vascular endothelial and smooth muscle cells) may also play a beneficial role. A significant challenge in developing a thrombin receptor antagonist is the requirement to compete with an intramolecular ligand; however, the fact that the TRAP peptide is a weak agonist and the observation that the activated receptor has a limited lifetime[6] suggests that it is possible.

Two complementary approaches directed toward finding thrombin receptor antagonists are modification of the endogenous TRAP ligand and screening of a large,

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diverse sample collection.[7] Efforts to develop agonist and antagonist peptides and peptidomimetics based on the TRAP sequence have resulted in high-affinity ligands,[8] exemplified by **1a** (Fig. 1).[8e] Screening of combinatorial libraries[9] revealed urea **2** as a novel, non-peptide structure with sub-micromolar activity. Modification of **2** has resulted in the discovery of potent and selective thrombin receptor antagonists.

Chemistry

Preliminary investigations involved truncation of the structure as well as modifications around the central trisubstituted urea. Examination of the screening data from the other compounds in the combinatorial library that generated **2** revealed that the trisubstituted urea portion of the molecule contained most of the important binding elements. Thus, a series of simple amides that replace the terminal two ureas were prepared as outlined in Scheme 1.

The acetophenone derivative **3** was selectively reduced with (+)DIP-Cl[10] to afford the alcohol **4** which was converted to the amino alcohol **5** by heating in an excess of isopropylamine. Triphosgene mediated urea formation between **5** and three different anilines **6** ($n=0$, 1, and 2) gave **7** which, after removal of the *tert*-butyl carbamate protecting group, were coupled to a variety of carboxylic acids to give the amides listed in Table 1.

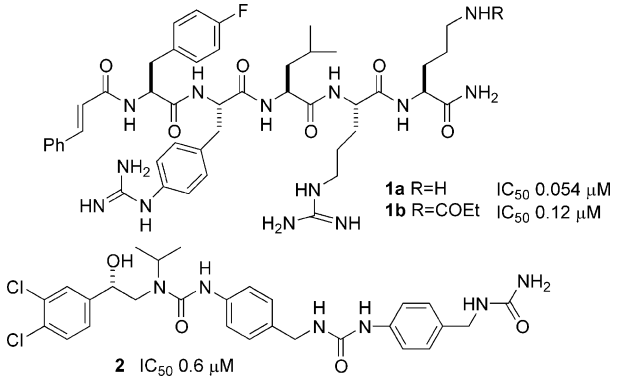
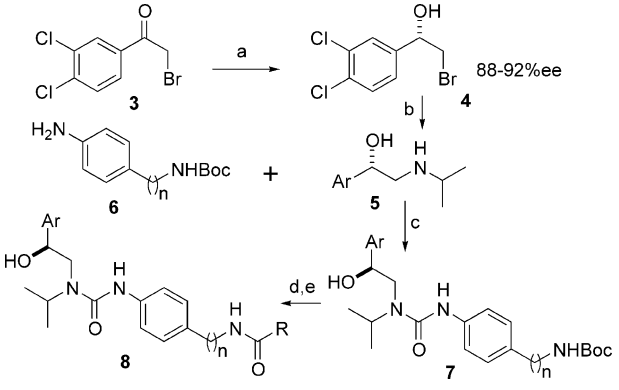


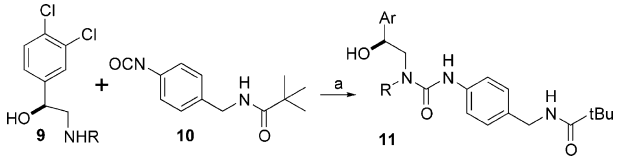
Figure 1. Lead structures of thrombin receptor antagonists.



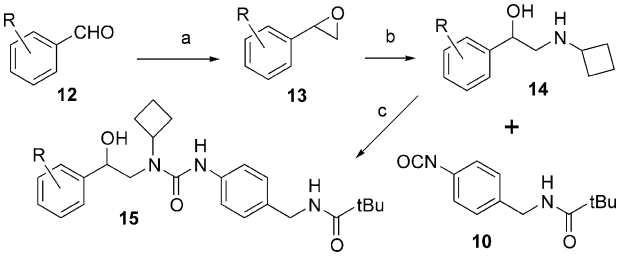
Scheme 1. Ar = 3,4-dichlorophenyl: (a) (+)DIP-chloride, THF, 0 °C; (b) i-Pr_2NH_2, neat, 60 °C; (c) triphosgene, Et_3N, CH_2Cl_2; (d) HCl, EtOAc; (e) RCO_2H, EDC, HOAt, DMF.

To examine replacements for the isopropyl group on the central urea, different primary amines were used to displace bromide **4** and the resulting secondary amines **9** were coupled to isocyanate **10**[11] to give ureas **11** (Scheme 2). To vary the dichlorophenyl portion of the lead, epoxides were prepared from a variety of aldehydes[12] and opened with excess cyclobutylamine (Scheme 3). Regioselectivity for amine attack at the least hindered carbon of the epoxide was greater than 5:1 under these conditions. The resulting β-amino alcohols were coupled to isocyanate **10** to give the desired urea analogues **15**. This method works in parallel synthesis format with many aldehydes and amines to provide an array of 1-aryl-*N*-alkyl-amino alcohols in good yield and purity.

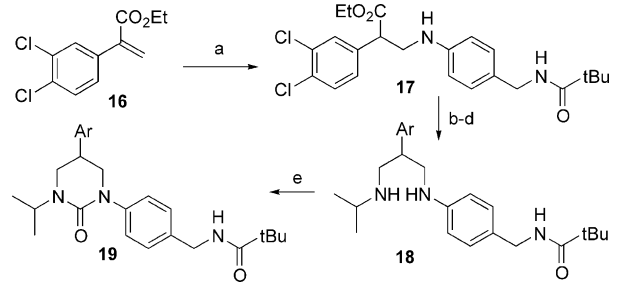
Several conformationally constrained derivatives were also prepared to try and deduce the bioactive conformation of the somewhat flexible lead. Cyclic urea **19** was synthesized as shown in Scheme 4. Michael addition of 4-(methylamino-*t*-butylcarbonyl)-aniline to acrylate **16** in the presence of $Yb(OTf)_3$ afforded the β-amino ester **17**. Ester reduction, mesylation and displacement with isopropylamine provided diamine **18** which was converted to the cyclic urea **19** upon treatment with phosgene. Five-membered cyclic urea **31** (Fig. 3) was prepared by the opening of 3,4-dichlorophenyloxirane with the sodium salt of *N*-phenyl ethylenediamine urea.



Scheme 2. Ar = 3,4-dichlorophenyl: (a) CH_2Cl_2, 23 °C.



Scheme 3. (a) Trimethylsulfonium iodide, KOH, H_2O, AcN, 60 °C; (b) cyclobutylamine, neat, 60 °C; (c) CH_2Cl_2.

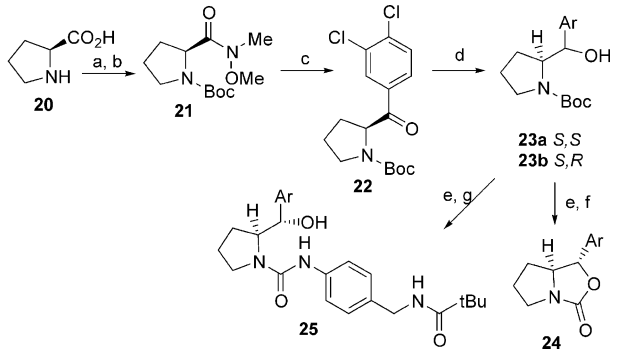


Scheme 4. Ar = 3,4-dichlorophenyl: (a) 4-(methylamino-*t*-butylcarbonyl)-aniline, $Yb(OTf)_3$, THF, 60 °C; (b) $LiBH_4$, THF; (c) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (d) i-$PrNH_2$, 70 °C; (e) phosgene, $NaHCO_3$, CH_2Cl_2.

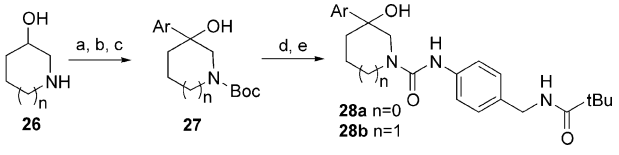
Proline, thioproline and pipecolic acid derivatives were prepared according to the synthetic sequence described in Scheme 5. Conversion of L-proline to its Boc derivative and coupling with *N*,*O*-dimethyl hydroxylamine provided Weinreb amide **21**. Lithiation of 3,4-dichloroiodobenzene under halogen–metal exchange conditions and addition to **21** lead to aryl ketone **22**. Reduction with $NaBH_4$ resulted in a 4:1 mixture[13] of diastereomeric benzylic alcohols **23a** and **23b** which could be separated by flash chromatography. Boc removal and coupling with 4-(methylamino-*t*-butylcarbonyl)-aniline in the presence of triphosgene afforded urea **25**. Stereochemical assignment of the carbinol center was achieved by conversion of **23a** to the corresponding fused bicyclic oxazolidinone **24** and its analysis by 1-D and 2-D NMR techniques. 5-Methyl and 5-ethyl proline were prepared according to the method of Kikugawa[14] as a *cis*/*trans* mixture, which could be separated by flash chromatography after installation of the 3,4-dichlorophenyl moiety.

Pyrrolidine and piperidine derivatives **28a** and **28b** were prepared according to Scheme 6. The Boc derivative of 3-hydroxypiperidine was converted to the corresponding ketone. Lithiation of 3,4-dichloro-iodobenzene under halogen–metal exchange conditions and addition to the previous ketone provided racemic alcohol **27**. Boc removal and coupling with 4-(methylamino-*t*-butylcarbonyl)-aniline in the presence of triphosgene lead to urea **28**.

Morpholino derivative **30** was prepared according to Scheme 7.[15] Alkylation of bromide **3** with *N*-benzyl-ethanolamine resulted in the formation of hemiketal **29a**. Reduction with $NaBH_4$ to the corresponding diol and cyclization with HBr provided the morpholino derivative **29b**. Removal of the benzyl group and coupling with isocyanate **10** led to urea **30**.



Scheme 5. Ar = 3,4-dichlorophenyl: (a) Boc_2O, NaOH, dioxane, water; (b) MeNHOMe, pyBOP, *i*-Pr_2NEt; (c) 3,4-dichloro-iodobenzene, *n*BuLi, Et_2O, −78 °C; (d) $NaBH_4$, MeOH, 4:1 ratio **23a**/**23b**; (e) HCl(g), EtOAc; (f) 4-(methylamino-*t*-butylcarbonyl)-aniline, triphosgene, Et_3N, CH_2Cl_2; (g) carbonyldiimidazole, CH_2Cl_2.

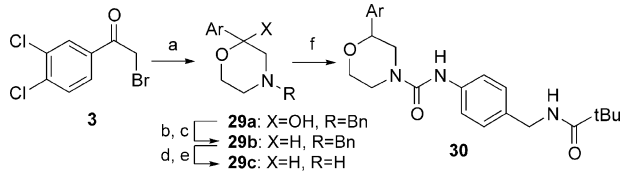


Scheme 6. Ar = 3,4-dichlorophenyl: (a) Boc_2O, CH_2Cl_2; (b) SO_3-pyridine, Et_3N, CH_2Cl_2; (c) 3,4-dichloro-iodobenzene, *n*-BuLi, Et_2O, −78 °C; (d) HCl(g), EtOAc; (e) 4-(methylamino-*t*-butylcarbonyl)-aniline, triphosgene, Et_3N, CH_2Cl_2.

Results and Discussion

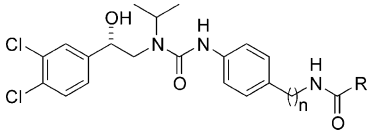
The compounds prepared in this study were evaluated for their ability to inhibit the TRAP-stimulated ($EC_{50} = 1\ \mu M$[8a]) secretion of ^3H radiolabeled serotonin from human washed platelets and are expressed as IC_{50} values.[16] As shown in Table 1, replacement of the two terminal ureas of **2** with a variety of amides results in compounds with similar potency to the lead with lower molecular weight and greater solubility. The optimal chain length appears to be $n = 1$; however, several of the heterocyclic derivatives could tolerate different chain lengths indicating some receptor flexibility. Efforts to further simplify the structure by removal of this amide or otherwise substitute the adjacent phenyl ring were accompanied by large losses of activity (data not shown); therefore, the 4-benzylic *tert*-butyl amide (e.g., **8e**) was used for subsequent studies.

Surprisingly, any modification to the 3,4-dichlorophenyl ring resulted in large losses in potency as shown in Table 2. Even the sterically similar 3,4-dimethyl substitution (**15g**) as well as other dichlorinated patterns were significantly less active than the parent compound. The 3,4-dibromophenyl (**15f**) and 4-cyanophenyl (**15i**) were the only other groups to show any promise in this area.



Scheme 7. (a) *N*-Benzyl-ethanolamine, CH_3CN; (b) $NaBH_4$, MeOH; (c) HBr, 80 °C; (d) α-chloroethyl chloroformate, CH_2Cl_2, 0–40 °C; (e) MeOH, 65 °C; (f) 4-(methylamino-*t*-butylcarbonyl)-aniline isocyanate **10**, CH_2Cl_2.

Table 1. Terminal urea replacements



R	$n=0$ No.	$n=0$ IC_{50} (μM)[a]	$n=1$ No.	$n=1$ IC_{50} (μM)[a]	$n=2$ No.	$n=2$ IC_{50} (μM)[a]
tert-Butyl	**8a**	>10	**8e**	0.7	**8k**	>10
CMe_2NH_2	—	—	**8f**	1.0	—	—
2,4-Difluorophenyl	**8b**	>10	**8g**	0.9	**8l**	>10
3-Pyridyl	—	—	**8h**	0.7	**8m**	1.4
4-(SO_2NH_2)phenyl	**8c**	1	**8i**	0.85	**8n**	0.7
4-Benzimidazole	**8d**	1	**8j**	0.45	**8o**	0.36

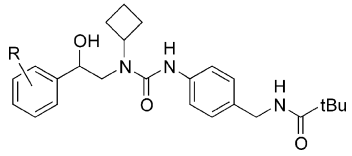
[a]Values represent inhibition of secretion of radiolabeled serotonin from washed human platelets stimulated by 3 μM of the TRAP peptide and represent the average of three or more determinations with the average standard error of the mean <10%.

The most fruitful area of study was substitution of the urea nitrogen with small alkyl groups. Table 3 demonstrates a progressive increase in activity as the size of the group increases, but with limits as cyclopentyl (**11g**) was much worse than cyclobutyl (**15a**) and *tert*-butyl (**11l**) was not as potent as *s*-butyl (**11j** and **11k**).

The conformation of a trisubstituted urea is determined in part by the nature of the substituents on the two nitrogen atoms. Regarding nitrogen N_1, both urea conformations A and B (Fig. 2) have to be considered. Larger, branched R groups usually favor conformation A and, as seen in Table 3, lead to potency enhancement. This effect may be the result of increased hydrophobic interaction with the receptor and/or enforcement of the bioactive conformation. Conformation A might also be stabilized by an intramolecular H-bond between the OH and N_2HAr, which is not possible within conformation B.[17]

We set out to examine various conformationally constrained derivatives of **2** in order to elucidate the bioactive conformation. Initial efforts to mimic conformation A or B lead to the preparation of six-membered cyclic urea **19** and five-membered cyclic urea **31**, respectively (Fig. 3). Both compounds were inactive, but in each case a potential H-bond acceptor or donor has been removed (the OH in the case of **19**, and the NH in the case of **31**).

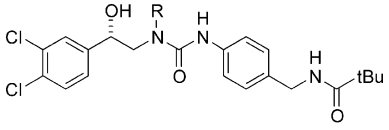
Table 2. Terminal aryl modification[a]



No.	R	IC_{50} (μM)[b]	No.	R	IC_{50} (μM)[b]
15a	3,4-Dichloro	0.23	**15f**	3,4-Dibromo	1
15b	2,4-Dichloro	10	**15g**	3,4-Dimethyl	>10
15c	2,3-Dichloro	>10	**15h**	3-Cyano	>10
15d	3,5-Dichloro	>10	**15i**	4-Cyano	6
15e	3,4-Difluoro	10			

[a]All compounds in this table are racemic except for **15a**, which has the (*S*) hydroxyl configuration.
[b]See Table 1, footnote a.

Table 3. Trisubstituted urea modification



No.	R	IC_{50} (μM)[a]	No.	R	IC_{50} (μM)[a]
11a	H	>40	**8e**	*iso*-Propyl	0.7
11b	Methyl	40	**11h**	*iso*-Butyl	>10
11c	Ethyl	3.8	**11i**	(±)2-Pentyl	1.0
11d	*n*-Propyl	1.1	**11j**	(*S*)*sec*-Butyl	0.4
11e	*n*-Butyl	>10	**11k**	(*R*)*sec*-Butyl	0.23
11f	Cyclopropyl	6.7	**11l**	*tert*-Butyl	1.7
15a	Cyclobutyl	0.23	**11m**	Phenyl	>10
11g	Cyclopentyl	>10			

[a]See Table 1, footnote a.

Through examination of constrained analogues, which retained the OH and NH groupings, proline and thioproline derived ureas of type **25** were identified as potent antagonists of the thrombin receptor (Table 4).

Urea **25a** (derived from L-proline) blocked TRAP stimulated activation of the thrombin receptor with an IC_{50} of 1.5 μM (comparable to analogues **11c** and **11d**). The lack of activity observed with any other diastereomer of **25a** (**25b–d**) confirmed the binding specificity between antagonist **25** and the receptor. Piperidine derivative **25e** and thioproline derivative **25g** exhibited decreased activity, suggesting a five-membered or smaller ring as the proper conformational constraint. The lack of activity with sulfoxide derivative **25h** suggests a hydrophobic receptor pocket which accommodates the N-R substituent. It was shown in Table 3 that further branching of the *R* group (*S*-s-butyl) is beneficial to activity. In order to mimic this effect, we introduced alkyl substituents at the 5-position on the proline ring. As hoped, potency was enhanced: the (5*S*)-methyl derivative **25i** was found to be a 0.4 μM antagonist of PAR-1 while the (5*S*)-ethyl derivative **25k** was slightly less active. The most potent proline derivative **25i** was still less potent than the linear analogue **11k**, suggesting that a better substitution pattern or conformational constraint might be found.

Further studies uncovered piperidine derivative **28b** (Fig. 4), in which the placement of the binding elements is slightly altered, as a 0.5 μM antagonist of the thrombin receptor. Once again, ring size modification was detrimental to activity as the pyrrolidine analogue **28a** was much less potent (>40 μM). In an attempt to further refine the structure, the hydroxyl group was inserted in the six-membered ring to yield morpholino derivative **30**, which was also much less active than the hydroxy-piperidine derivative **28b**. This negative result further supports the identification of the hydroxyl group as an H-bond donor or acceptor with the receptor (vide supra). Racemic **28b** is significantly more potent than

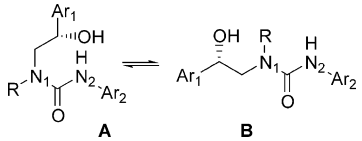


Figure 2. Possible ground state conformations of trisubstituted urea.

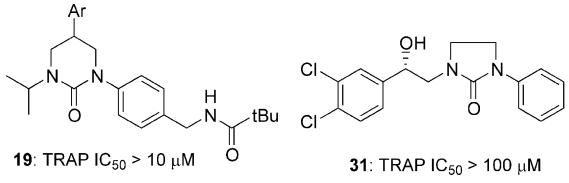


Figure 3. Cyclic urea derivatives.

its enantioenriched linear analogues **11c** and **11d**, so this result points the way to further pharmacophore elucidation and the design of more potent antagonists.

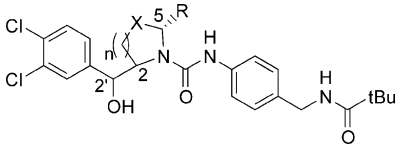
Several of the compounds were examined in a binding assay using radiolabeled **1b**[8e] as the ligand ($K_d = 13$ nM) with gel filtered platelets. As demonstrated in Table 5, the binding affinity paralleled the functional data, thereby supporting the notion that these compounds are indeed interacting with the platelet thrombin receptor.

One of the most potent compounds from this series, **11k**, was examined in further detail. The compound was shown not to be an inhibitor of thrombin's catalytic activity ($K_i > 10\,\mu M$) and did not significantly prevent ADP or collagen induced platelet activation ($IC_{50} \gg 10\,\mu M$), implying that it is indeed mediating its effects through the platelet thrombin receptor. In the secretion assay, **11k** was again tested but with 1 nM thrombin as the agonist. Disappointingly, **11k** was only able to elicit a 38% decrease in secretion at 1 μM and no further reductions were possible at even higher concentrations of antagonist.[18] This behavior was also noted for peptide antagonists such as **1a** and the reason for diminished activity versus thrombin could be due to a number of factors. The compounds may exhibit different binding affinity for cleaved (thrombin stimulated) and uncleaved (TRAP stimulated) receptors. Alternatively, this class of compounds may have a fast off-rate, thereby giving the tethered ligand more opportunities to bind. There is also the possibility of involvement of the PAR-4 platelet thrombin receptor. Further studies are required to precisely establish the importance of these and other potential factors. As a final measure of in vitro activity, **11k** was evaluated for its ability to block platelet aggregation using 1 nM thrombin as agonist.[16] As shown in Figure 5, at the higher doses, **11k** delays aggregation for several minutes as measured by increases in light transmittance. Given the sub-optimal activity of the compound in the secretion assay with thrombin as the agonist, the delay of aggregation in this assay is encouraging.

Conclusion

Screening of combinatorial libraries has identified the novel, nonpeptide thrombin receptor antagonist **2**. Examination of structure–activity relationships revealed that the central disubstituted urea could be replaced with a variety of simple amides, whereas the trisubstituted urea was less tolerant to change. Several constrained variants were prepared with piperidine **28b** being the most promising, and refinement of the acyclic lead provided the more potent antagonist **11k**. In vitro binding and functional assays of this compound suggest that it is indeed acting via the thrombin receptor and has some activity when thrombin is used to generate the tethered ligand. This structural class represents a promising area for further study with the hope that further potency enhancements will improve activity versus thrombin-stimulated platelets.

Table 4. Proline derived ureas



No.	X	R	Stereo (2,2′,5)	*n*	IC_{50} (μM)[a]
25a	CH_2	H	*S,S*	1	1.5
25b	CH_2	H	*S,R*	1	>10
25c	CH_2	H	*R,S*	1	>10
25d	CH_2	H	*R,R*	1	>10
25e	CH_2	H	*S,S*	2	4.3
25f	CH_2	H	*S,R*	2	>10
25g	S	H	*S,S*	1	2.5
25h	SO	H	*S,S-rac*	1	>10
25i	CH_2	Me	*S,S,S*	1	0.4
25j	CH_2	Me	*S,S,R*	1	>10
25k	CH_2	Et	*S,S,S*	1	0.7

[a]See Table 1, footnote a.

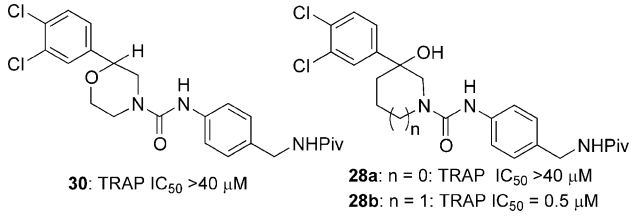


Figure 4. Piperidine and morpholine derivatives.

Table 5. Binding data for selected compounds

No.	TRAP IC_{50} (μM)	Bind IC_{50} (μM)[a]
8e	0.7	0.7
8i	0.85	1.2
11b	40	6.6
11f	6.7	2.1
11k	0.23	0.12
25a	1.5	1.1
25i	0.4	1.0

[a]Values represent the average of four or more determinations with the average standard error of the mean <10%.

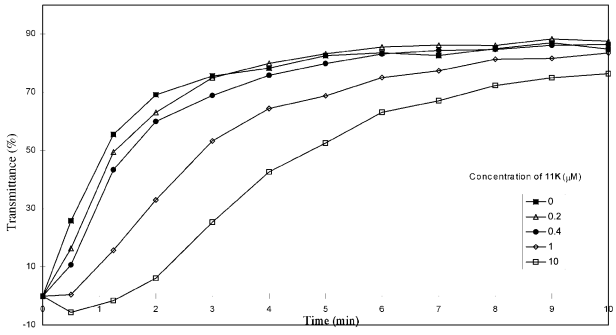


Figure 5. Inhibition of 1 nM thrombin induced platelet aggregation by **11k**.

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