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# Potent and Selective Bicyclic Lactam Inhibitors of Thrombin. Part 4: Transition State Inhibitors

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**Abstract**—Bicyclic piperazinone based thrombin inhibitors of general structure **2** were prepared and evaluated in vitro and in vivo. These inhibitors, having in common an electrophilic basic *trans*-cyclohexylamine $P_1$ residue, displayed high thrombin affinity, high selectivity against trypsin and good in vivo efficacy in the rat arterial thrombosis model. 

Bicyclic piperazinone thrombin inhibitors of general structure **1** have recently been shown by us to be highly potent and active in vivo.[1] Although these compounds have potency in the low nanomolar range, none of them had acceptable bioavailability or a high level of selectivity against other serine proteases, such as trypsin. In order to increase bioavailability as well as enzyme selectivity, we needed to replace the arginine moiety by a much more lipophilic and less basic group, such as a cyclohexylamine unit, to provide thrombin inhibitors of general structure **2**. This *trans*-cyclohexylamine moiety linked to a (D)-Phe-Pro dipeptide has been reported by others to afford selective thrombin inhibitors (Fig. 1).[2]

In this paper, we describe the preparation of cyclohexylamine based thrombin inhibitors of general structure **2** bearing a variety of different activated carbonyls at the $P_1'$ site, as well as their in vitro (potency, selectivity) and in vivo efficacy in the rat arterial thrombosis model.

## Chemistry

Since the known methods[2,3] for the preparation of the *trans*-cyclohexylamine moiety suffered from some limitations, we opted for a novel approach that is depicted in Scheme 1. Weinreb amide formation of the carboxylic acid[4] **3** followed by oxidation using TPAP afforded the ketone **4** in 57% overall yield. Reductive amination of the cyclohexanone **4** using sodium cyanoborohydride with an excess of ammonium acetate followed by protection with Mtr-Cl afforded, after purification to remove the minor *cis* isomer, the *trans*-cyclohexylamine **5** in overall yield of 38%. Addition of 2-lithiothiazole, 2-lithiobenzothiazole, or 2-lithio-3-vinyl-4-methyl thiazole to the Weinreb amide followed by acidic deprotection provided the cyclohexylamine **6a**, **6b** and **6c** in high yield. Coupling of these amines with the bicyclic template **7**[1] and deprotection yielded the thrombin inhibitors **2a** and **2b** in 20–40% yield. Coupling of the amine **6c** followed by oxidation afforded analogues **2c** bearing a carboxylic side chain on the thiazole ring.

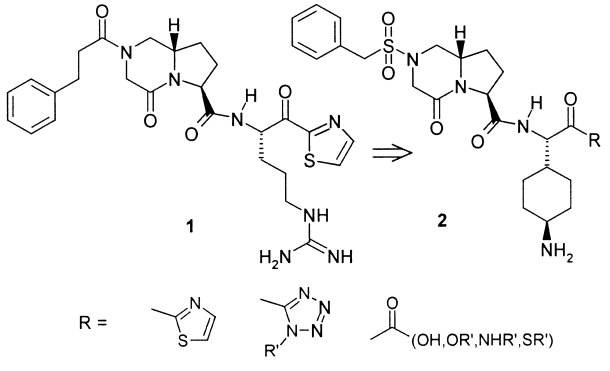


**Figure 1.**

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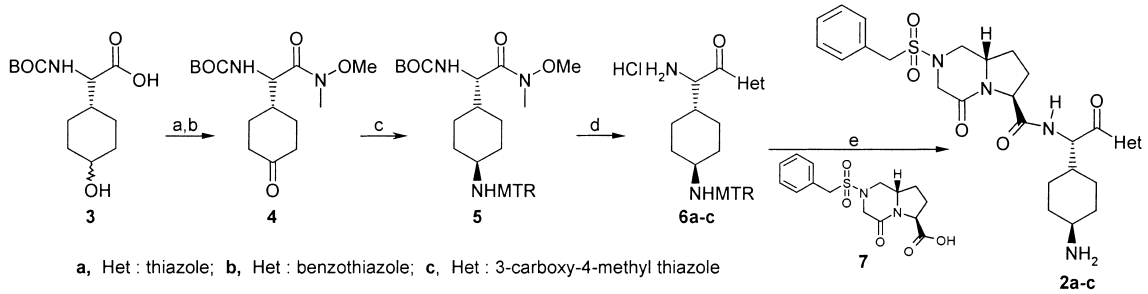
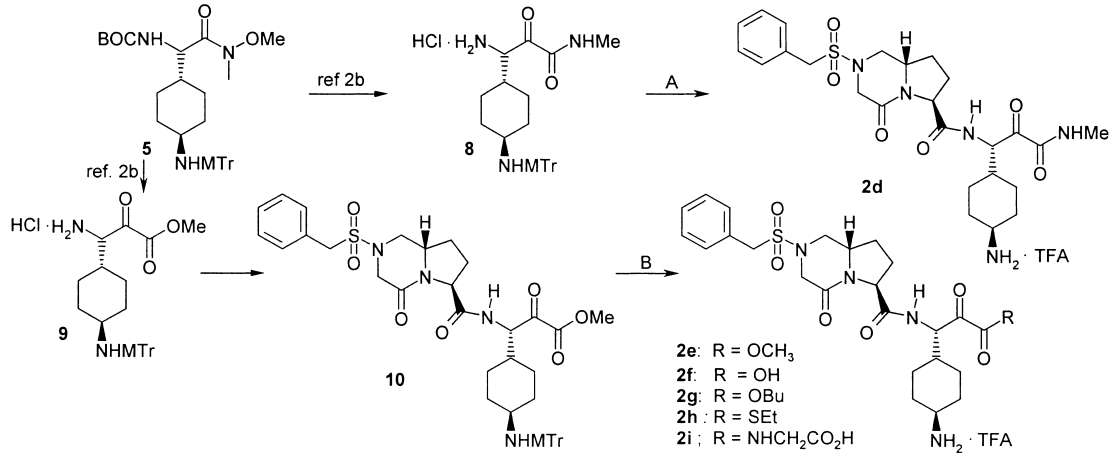
Scheme 2 summarizes the preparation of the 1,2-dicarbonyl analogues **2d–i**. Transformation of the Weinreb amide **5** to the ketoamide **8** was done using a known procedure.[2b] Using standard conditions, coupling with the bicyclic template **7** and removal of the Mtr protective group afforded the inhibitor **2d** in 44% yield. Preparations of the analogues **2e–i** were done slightly differently. The ketoester **9** was used as the reagent for coupling with the template that, upon further manipulations, afforded compounds **2e–i**.

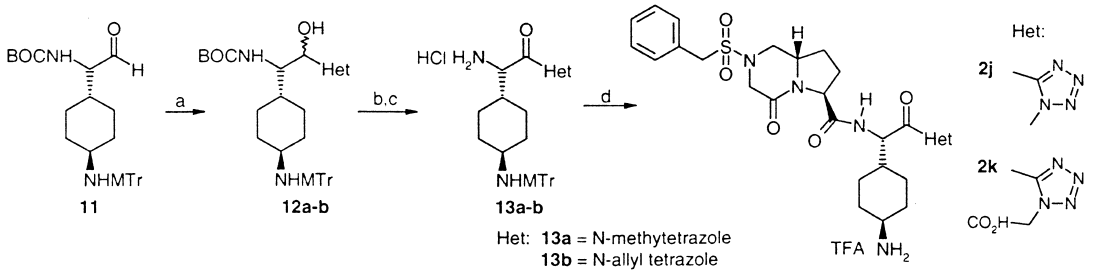
Preparations of analogues **2j–k** having a tetrazole unit are depicted in Scheme 3. Addition of lithioderivatives of *N*-methyl or *N*-allyl tetrazole to the aldehyde **11**




**Scheme 1.** (a) $CH_3NH(OCH_3)\cdot HCl$, DIEA, BOP, DMF (75%); (b) TPAP, NMO, MS 4 Å, DCM (73%); (c) (i) ammonium acetate (10 equiv), $NaCNBH_3$, isopropanol, MS 4 Å; (ii) MTr-Cl, DMAP, DIEA, DCM (38%, 2 steps); for **6a**: (d) (i) thiazole, *n*-BuLi, THF, −40 °C (75%); (ii) 4.0 M HCl, dioxane (100%); for **6b**: (d) (i) benzothiazole, *n*-BuLi, THF, −40 °C (%); (ii) 4.0 M HCl, dioxane (100%); for **6c**: (d) (i) 3-vinyl-4-methyl thiazole, *n*-BuLi, THF (95%); (ii) 4.0 M HCl, dioxane (100%); for **2a** and **2b**: (e) (i) HATU, NMM, DMF, bicyclic template **7** (78%); (ii) TFA, thioanisole, methanesulfonic acid (20-40%); for **2c**: (e) (i) HATU, NMM, DMF, bicyclic template **7** (72%); (ii) $O_3$, $CH_2Cl_2$, (100%); (iii) $AgNO_3$, NaOH, $THF/H_2O$ (85%); (iv) TFA, thioanisole, methanesulfonic acid (26%).




**Scheme 2.** (**A**) (i) HATU, NMM, DMF, bicyclic template **7** (69%); (ii) TFA, thioanisole, methanesulfonic acid (58%); (**B**) for **2e**: (i) TFA, MSA, thioanisole, HPLC purification (24%); for **2f**: (i) $LiOH\cdot H_2O$, $THF/H_2O$ (94%); (ii) TFA, MSA, thioanisole, HPLC purification (24%); for **2g**: (i) *n*-BuOH, EEDQ (44%); (ii) TFA, MSA, thioanisole, HPLC purification (69%); for **2h**: (i) EtSH, EDC, DMAP (23%); (ii) TFA, MSA, thioanisole, HPLC purification (59%) for **2i**: (i) $ClH_3NCH_2CO_2Bn$, HATU, 2,4,6-collidine (63%); (ii) $H_2$, Pd/C 5%, methanol (83%); (iii) TFA, MSA, thioanisole, HPLC purification (26%).




**Scheme 3.** (a) (i) *N*-Alkyltetrazole, *n*-BuLi, THF, −40 °C (63% for *N*-methyl for **12a**, 55% for *N*-allyltetrazole **12b**); (b) (i) Dess–Martin, $CH_2Cl_2$ (79% for **13a**, 55% for **13b**); (ii) 4.0 M HCl, dioxane (100%). For **2j**: (d) (i) HATU, NMM, DMF, bicyclic template **7** (74%); (ii) TFA, thioanisole, methanesulfonic acid (73%). For **2k**: (d) (i) HATU, NMM, DMF, bicyclic template **7** (71%); (ii) $O_3$, (96%); (iii) $AgNO_3$, TFA, thioanisole, methanesulfonic acid (60%).

provided the corresponding alcohol **12a–b** in good yield. Dess–Martin oxidation of the alcohol followed by acidic deprotection furnished ketones **13a–b**, which after coupling with bicyclic template **7**, oxidation of the vinyl group with ozone followed by silver oxide afforded after usual deprotection analogues **2j–k**.

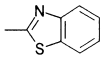
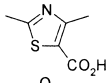
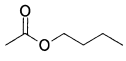
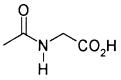
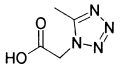
## Biology

Inhibition of the proteolytic activity of thrombin and trypsin ($K_i$), in vivo coagulation parameters such as the mean occlusion time (MOT), the activated partial thromboplastin time (aPTT), the thrombin time (TT) and bioavailability were measured according to published procedures.[5]

## Results and Discussion

All compounds prepared and listed in the Table 1 displayed high potency towards thrombin with $K_i$'s ranging from 0.09 (**2f**) to 5 nM (**2j**) except for compound **2k**, which has a $K_i$ of 2.6 μM. The use of benzylsulfonamide at the $P_3$ site was necessary to obtain a high level of potency. For example, compound **2a** has a $K_i$ of 3 nM, which represents a 10-fold increase in potency compared to the previously reported analogue having a phenylpropanoyl group at the $P_3$ site.[6] As can be seen, the presence of a cyclohexylamine moiety substantially increased the selectivity over trypsin. In fact, the analogue of compound **2a**, which had a regular arginine at the $P_1$ instead of a *trans*-cyclohexylamine, did not show any selectivity.[7] From Table 1, all compounds produced good selectivity ratios ranging from 150 for compound **2k** to a maximum value of 24,400 displayed by compound **2f**. In some cases, the presence of a carboxylic function seemed to increase the selectivity ratio, for example if one compares compound **2g** (Tryp/Thr: 5500) with compound **2f** (Tryp/Thr: 24,400) or compound **2d** (Tryp/Thr: 7340) with compound **2i** (Tryp/Thr: 12,960). But in other cases the presence of the same functionality decreased the selectivity ratio if one compares compounds **2a** and **2b** (Tryp/Thr: 2500

**Table 1.** In vitro activity against human α-thrombin and trypsin, selectivity, in vivo activity and bioavailability of inhibitors **2a–k**[a] in the rat arterial thrombosis model

| | | | | | Rat arterial thrombosis model[b] | | | |
|---|---|---|---|---|---|---|---|---|
| Entry | R | $K_i$ (nM) (Thrombin) | $K_i$ (nM) (Trypsin) | Tryp/Thr | MOT[e] (min) | APTT[f] (s) | TT[g] (s) | % F[h] |
| **2a** |  | 3.2(S)[c] | 8000 | 2500 | >60 | 66 ± 6 | 829 ± 208 | 1.8 |
| **2b** |  | 0.72(S) | 1400 | 1930 | >60 | 25 ± 5 | 339 ± 55 | 0.6 |
| **2c** |  | 80 | 65,000 | 812 | 16 ± 1 | 20 ± 1 | 99 ± 9 | 0.4 |
| **2d** |  | 0.33(S) | 2400 | 7340 | 20 ± 4 | 47 ± 6 | 999 | nd[i] |
| **2e** |  | 0.13(S) | nd | nd | >60 | 46 ± 4 | 999 | 1.1 |
| **2f** |  | 0.09(S) | 2200(S) | 24,400 | >60 | 54 ± 2 | 938 ± 75 | 0.4 |
| **2g** |  | (0.1)[d](S) | (550)[d] | 5500 | 47 ± 17 | 53 ± 10 | 883 ± 143 | 0.7 |
| **2h** |  | 0.26(S) | 150 | 580 | 49 ± 14 | 33 ± 1 | 999 | 0.3 |
| **2i** |  | 2.14(S) | 29,000 | 12,960 | nd | nd | nd | nd |
| **2j** |  | 5.0 | 35,000 | 7000 | 46 ± 12 | 46 ± 2 | 322 ± 38 | nd |
| **2k** |  | 2600 | >400,000 | 150 | nd | nd | nd | nd |

[a]All new targets were characterized by $^1$H NMR, reverse HPLC and mass spectroscopy.
[b]Dose: intravenous bolus dose (0.75 mg/kg) followed by an infusion (50 μg/min).
[c](S) = slow binder.
[d]$IC_{50}$.
[e]Mean occlusion time (control: 17–19 min).
[f]Activated partial thromboplastin time (20–22 s).
[g]Thrombin time (control: 40–45 s).
[h]Bioavailability, based on oral administration (30 mg/kg).
[i]Not determined.

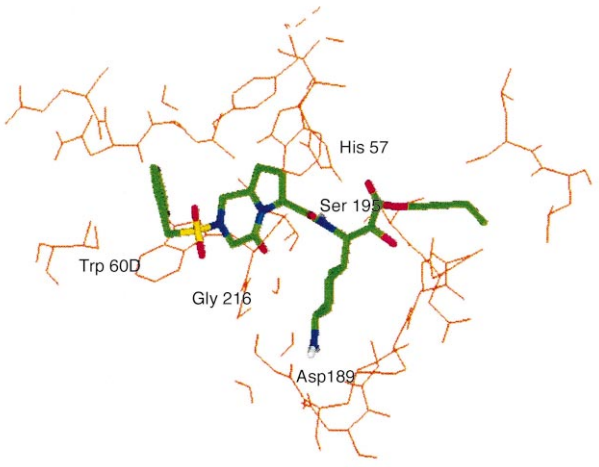



**Figure 2.**

and 1930, respectively) with compounds **2c** (Tryp/Thr: 812) or even more dramatically when one compares analogue **2j** with **2k** (Tryp/Thr: 7000 and 150, respectively). All these data taken together suggest that the cyclohexylamine moiety is obviously essential to obtain a good level of selectivity and moreover that this selectivity could be substantially varied by having a polar group on $P_1'$.

Most of the compounds with the exception of **2c** and **2d** displayed a substantial increase (2- to 3-fold) in antithrombotic activity in the rat arterial thrombosis model as measured by the MOT. Unfortunately, when these compounds were given orally to rats, low levels of bioavailability were observed for all the compounds listed in Table 1.

Figure 2 shows the X-ray structure[8] of inhibitor **2g** with thrombin. The mode of interaction is very similar to the structure already reported by us on a compound having a regular arginine at $P_1$.[1] Briefly, the benzylsulfonamide group penetrates deeply in the $S_3$ pocket, while the amino group of *trans*-cyclohexylamine makes a salt bridge interaction with the Asp 189 at the bottom of the $S_1$ pocket. Since thrombin has a more lipophilic $S_1$ pocket (Ala 190) than trypsin (Ser 190), this difference might explain why the more lipophilic $P_1$ side chains are preferred in thrombin compared to trypsin. This suggests why all the analogues prepared in this paper showed remarkably good selectivity. Finally, the activated carbonyl group makes a strong covalent bond with the Ser 195 forming a tetrahedral intermediate providing additional binding affinity.

## Conclusion

We have demonstrated that incorporation of a cyclohexylamine unit in the $P_1$ site bearing an electrophilic carbonyl affords very potent thrombin inhibitors in the low nanomolar range. These compounds, moreover, displayed good antithrombotic activity in the rat arterial thrombosis model system, doubling and even tripling the mean occlusion time in some cases.

## Acknowledgements

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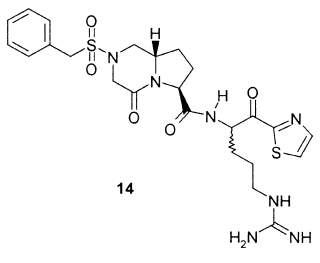
7. Compound **14**, $K_i$ (thrombin) 0.23 nM, $K_i$ (trypsin) 0.23 nM; Siddiqui, M. A., Unpublished results




8. The authors have deposited X-ray crystallographic data with the Brookhaven Protein Data Bank. Deposition code 1G37.