Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3647–3651
Bioorganic & Medicinal Chemistry Letters

Himbacine derived thrombin receptor antagonists: Discovery of a new tricyclic core

Martin C. Clasby,[a,*] Samuel Chackalamannil,[a] Michael Czarniecki,[a] Darío Doller,[a] Keith Eagen,[a] William J. Greenlee,[a] Yan Lin,[a] Jayaram R. Tagat,[a] Hsingan Tsai,[a] Yan Xia,[a] Ho-Sam Ahn,[a] Jacqueline Agans-Fantuzzi,[a] George Boykow,[a] Madhu Chintala,[a] Yunsheng Hsieh[a] and Andrew T. McPhail[b]

[a]*Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*
[b]*Duke University, Durham, NC 27708, USA*

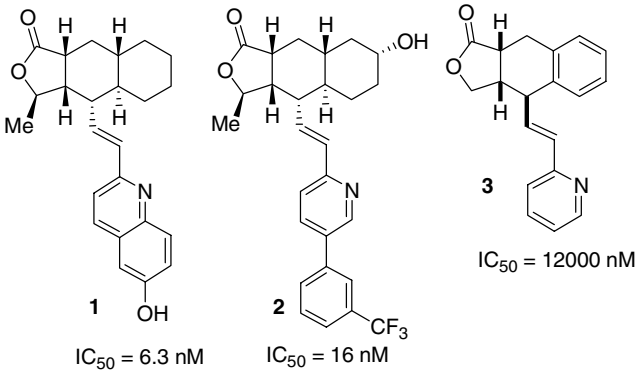
Received 8 March 2007; revised 6 April 2007; accepted 16 April 2007
Available online 25 April 2007

Abstract—The synthesis and biological activity of a novel series of thrombin receptor antagonists is described. This series of compounds showed excellent in vitro and in vivo potency. The most potent compound **40** had an IC_{50} of 7.6 nM and showed robust inhibition of platelet aggregation in a cynomolgus monkey model after oral administration.

Thrombin, the main effector protease of the coagulation cascade, converts soluble fibrinogen to fibrin and activates platelets which aggregate at the site of a vascular injury. Fibrin monomers polymerize to an insoluble fibrin meshwork that traps aggregated platelets and other plasma particle leading to the formation of a thrombus. The activation of platelets and other cell types by thrombin is mediated via proteolytic activation of specific cell surface receptors known as protease activated receptors (PARs).[1–6] Four PARs are known (PAR1-4). Among these, PAR-1, also known as thrombin receptor, is the most potent activator of human and primate platelets. Thrombin binds to PAR-1 through its exo-anion binding site. Cleavage of the extracellular domain at Arg^{41}-Ser^{42} reveals an amino terminus that then binds intramolecularly to the receptor.[7–9] This intramolecular activation mechanism (also known as the tethered ligand mechanism) makes it difficult to identify a small molecule antagonist for the activated thrombin receptor (PAR-1) due to entropic reasons.

It has been hypothesized that by targeting only the cellular action of thrombin, and not its role in the coagulation cascade, a thrombin receptor antagonist would be useful in the treatment of disorders such as arterial thrombosis, atherosclerosis and, restenosis. Such an agent could have a significant advantage in safety with regard to bleeding side effects over the current antithrombotic therapies.[10–13]

We have recently reported the synthesis and biological activity of a novel series of thrombin receptor (PAR-1) antagonists (e.g., **1** and **2**) based on the natural product himbacine[14–16] (Scheme 1). These compounds are potent antagonists of PAR-1,[17] and in the case of **2** show excellent activity when dosed orally in an ex-vivo model of



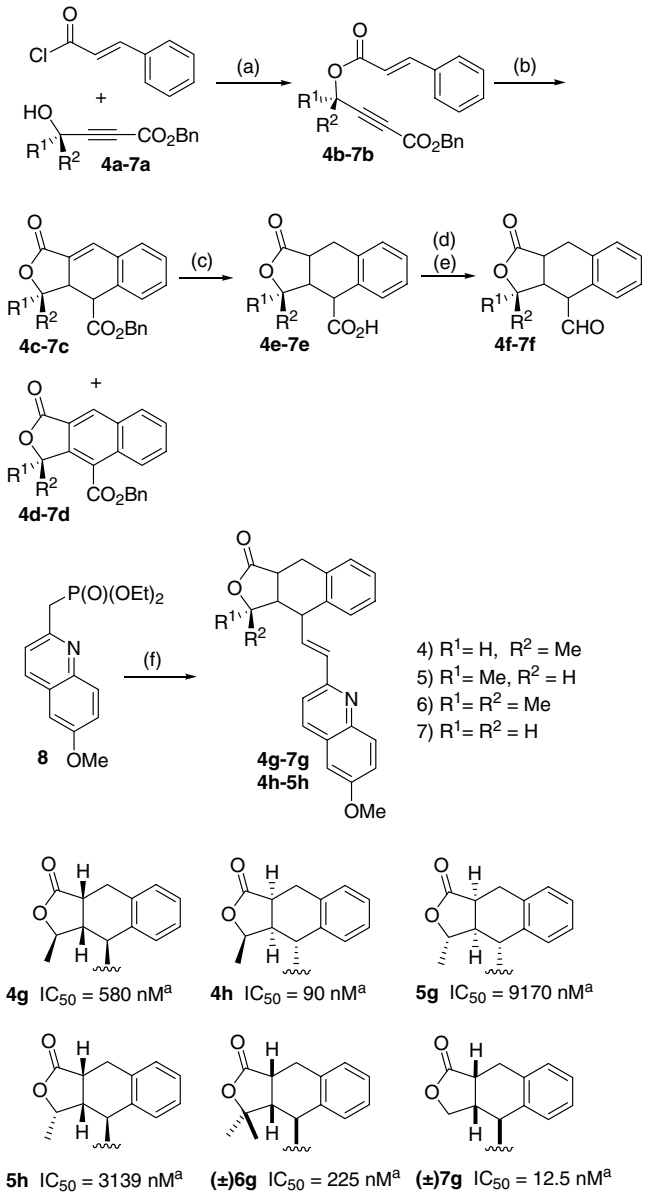
Scheme 1. Thrombin receptor antagonists.

Keywords: Thrombin; PAR1; Himbacine; Antagonist.
* Corresponding author. Tel.: +1 908 740 2244; fax: +1 908 740 7152; e-mail: martin.clasby@spcorp.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.061

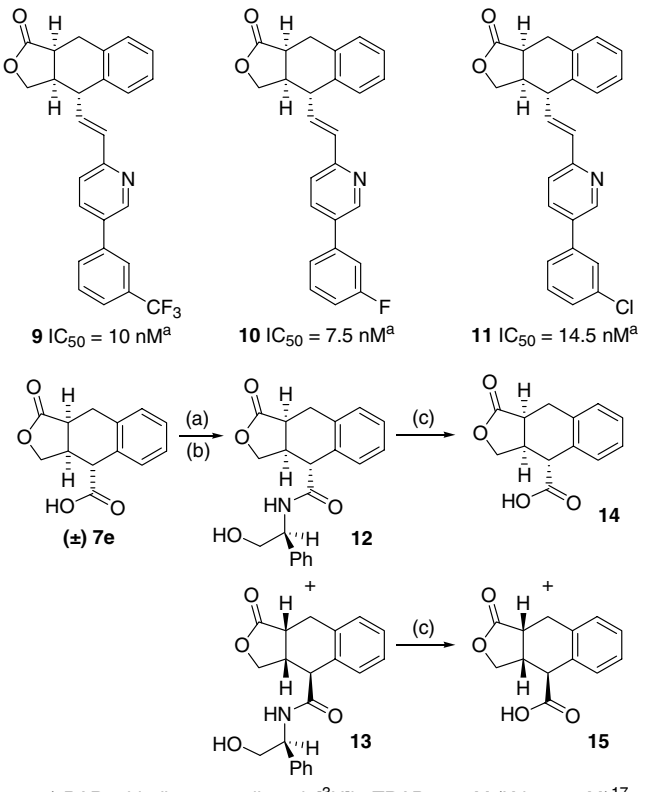
platelet aggregation using high affinity thrombin receptor-activating peptides (haTRAP) as the ligand.[18,19] A structurally related compound **3** was identified from our compound collection to be a mild antagonist of PAR-1 (IC_{50} = 12 μM). We were intrigued by the similarities to our current leads and embarked on an optimization study for this series of compounds. In this communication we describe the synthesis and biological activity of compounds such as **3**.

The general synthesis of analogs of **3** is described in Scheme 2. The tricyclic portion of the targets was synthesized using our reported literature procedure:[20,21] condensation of cinnamic acid chloride with propargylic alcohols **4a**–**7a** followed by thermolysis in *o*-xylene at 185–195 °C gave the tricyclic core **4c**–**7c** along with aromatic byproducts **4d**–**7d**. The core was then reduced to give the tricyclic acids **4e**–**7e**. The acids were then reduced to aldehydes **4f**–**7f** via reduction of the corresponding acid chlorides.[22] The aldehydes **4f**–**7f** were converted to the final targets **4g**–**7g** and **4h**–**5h** using a Horner–Emmons reaction. Compounds **4g**, **4h**, **5g**, and **5h** were synthesized from enantiopure **4a** and **5a**, compounds **6g** and **7g** were synthesized in racemic form. When tested in the PAR-1 binding screen compound **4g** was an immediate improvement over **3** with an IC_{50} = 580 nM, its enantiomer **5g** was much less potent, a similar trend was observed for **4h** (IC_{50} = 90 nM) and its enantiomer **5h**. Similarly to himbacine derived compounds **1** and **2**, it is the compounds derived from the (R)-alcohol that were more potent. Our attempts to epimerize the aldehyde **4f** to form compounds with the identical relative stereochemistry to **1** and **2** were met with failure. We were, however, pleased to see that the racemic compounds **6g** and **7g** were very potent with IC_{50} = 225 and 12.5 nM, respectively. Targets similarly derived from aromatic byproducts **4d**–**7d** were inactive. Compound **7g** was selected for further SAR study.



Scheme 2. Synthesis of analogs of compound **3**. Reagents and conditions: (a) TEA, THF, 0 °C; (b) 185–195 °C, *o*-xylene; (c) PtO_2, EtOAC, H_2; (d) $(COCl)_2$, DMF, CH_2Cl_2; (e) Bu_3SnH, $Pd(Ph_3P)_4$; PhMe, 0 °C; (f) BuLi, THF, then **4f**–**7f**.

Our previous studies with compounds related to **1** and **2** containing the himbacine tricycle showed that 5-phenylpyridines gave the greatest potency. This was also found to be the case for this new tricyclic aryl himbacine series. The best three examples, **9**, **10**, and **11**, are shown in Scheme 3. In general, the most potent compounds pos-

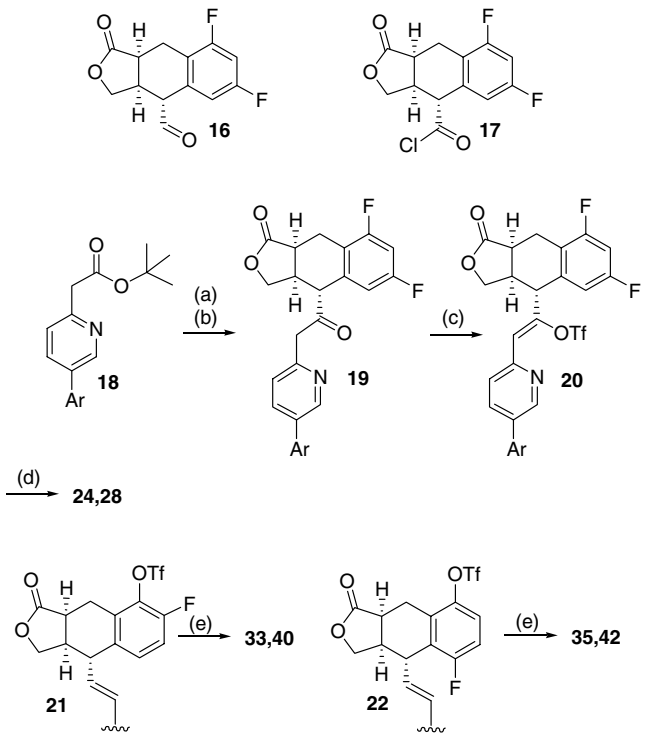


Scheme 3. Reagents and condition: (a) $(COCl)_2$, DMF, CH_2Cl_2; (b) (*S*)-2-phenylglycinol, TEA, THF (75%, 2 steps); (c) 4 M HCl in dioxane, reflux (99%).

sess a chloro, fluoro, or trifluoromethyl group at the *meta*-position of the phenyl ring of the 5-phenylpyridine. It should also be noted that these compounds are enantiopure. The resolution was achieved via chromatographic separation of the diastereomeric amides derived from the acid **7e** and (*S*)-2-phenylglycinol (Scheme 3). We were able to determine the absolute configuration of **13** via X-ray crystallographic analysis[23] and thus the absolute configuration of **12**. Acidic hydrolysis generated the enantiopure acids **14** and **15**. Compounds derived from **15** were found to be 10- to 15-fold less potent than compounds derived from **14**, the more potent enantiomer typically showed a 2-fold improvement over racemic compound. This resolution was found to be general for every tricycle of this kind studied, and allowed us to generate compounds with enantiomeric excess greater than 99% (determined by HPLC; CHIRALPAK® AD®, CHIRALCEL®OD®).

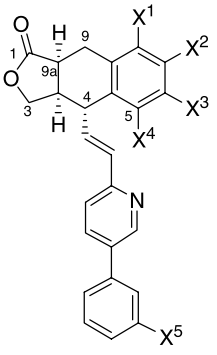
For compounds such as **1** and **2** we have used an ex-vivo model of platelet aggregation to determine efficacy in an animal model. The drug is usually administered orally to cynomolgus monkeys in 20% HPβCD at a dose of 3 mpk. Blood samples are then collected at various time intervals (1, 2, 3, 4, 5, 6, and 24 h) from both the treatment and control animals, and the aggregation response to 1 μM of haTRAP is measured in a whole blood aggregometer. This gives an aggregation versus time graph. Compounds **9** and **10** were inactive in this assay due to poor measured PK (AUC <300 ng h/ml). Only compound **11** (AUC = 6300 ng h/ml) was shown to have activity but with a limited duration (5 h). This was a very promising result but we required a compound that had at least 24 h duration at 3 mpk. In order to accomplish this goal we examined the effect of substitution on both the tricyclic and 5-phenylpyridine parts of the molecule.

One interesting aspect of this new series of compounds is the relative ease by which the SAR of aromatic ring of the tricycle can be studied; there are hundreds of commercially available cinnamic acids. We have studied the effect of substitution of various groups on the aromatic ring of the tricycle. Initial SAR showed that while simple alkyl, alkoxy, carboxy, and hydroxyl led to compounds with reduced activity ($IC_{50} > 100$ nM), simple halogen substitution gave very potent compounds. The synthesis used was identical to that described in Scheme 2 with notable exceptions shown in Scheme 4. The aldehyde **16** required for the synthesis of the 6,8-difluoro compounds (**24** and **28**) was unstable. To circumvent the use of this aldehyde we condensed the *tert*-butyl ester **18** with the acid chloride **17** followed by acid mediated decarboxylation to give **19**. Palladium mediated reduction of the derived enol-triflate **20** gave the desired compounds **24** and **28**. The 7-fluoro compounds (**33** and **40**) and the 5-fluoro compounds (**35** and **42**) were



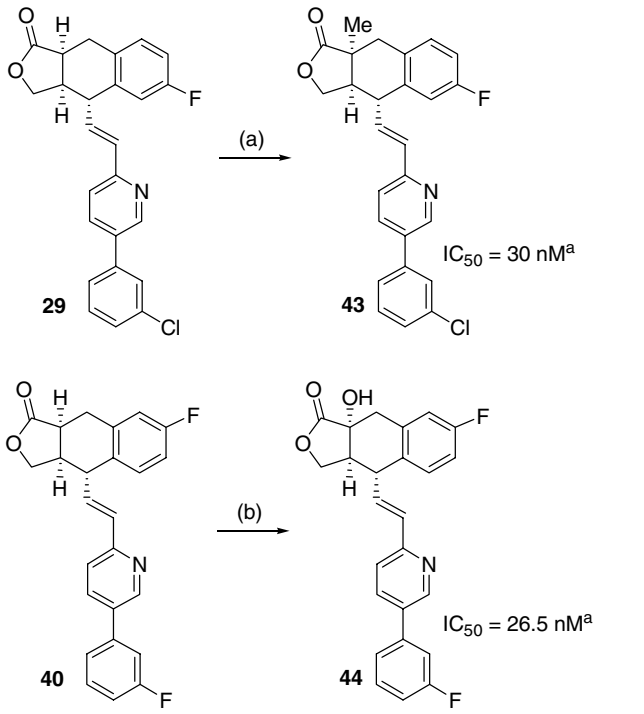
Scheme 4. Reagents and conditions: (a) BuLi, THF, then **17** (65%); (b) TFA, CH_2Cl_2, 0 °C (76%); (c) Tf_2O, TEA, CH_2Cl_2 (60%); (d) $Pd(OAc)_2$, HCO_2H, Ph_3P, DMF, 60 °C (59–76%); (e) $Pd(dppf)Cl_2$, HCO_2H, Ph_3P, DMF, 80 °C (20–74%).

Table 1. Binding data of analogs of compound **7g**



Compound	X^1	X^2	X^3	X^4	X^5	IC_{50}/nM[a]
(±)**23**	F	H	H	H	CF_3	65
24	F	H	F	H	CF_3	21.5
25	H	H	F	H	CF_3	31.5
(±)**26**	H	H	Cl	H	CF_3	137.5
(±)**27**	Cl	H	H	H	CF_3	225
(±)**28**	F	H	F	H	Cl	46.5
29	H	H	F	H	Cl	10
(±)**30**	H	H	Cl	H	Cl	120
31	F	H	H	H	Cl	29
(±)**32**	Cl	H	H	H	Cl	328
33	H	F	H	H	Cl	13
34	F	F	H	H	Cl	15.5
(±)**35**	H	H	H	F	Cl	330
36	H	H	F	H	F	10
37	F	H	H	H	F	20.5
(±)**38**	Cl	H	H	H	F	63.5
39	H	H	Cl	H	F	14.5
40	H	F	H	H	F	7.6
41	F	F	H	H	F	7
(±)**42**	H	H	H	F	F	85.5

[a]PAR-1 binding assay ligand: [^{3}H]haTRAP, 10 nM (K_d = 15 nM).[17]



Scheme 5. Reagents and conditions: (a) LDA, THF, MeI, −78 °C (47%); (b) LHMDS, THF, −78 °C, (1*S*)-(10-camphorsulfonyl)oxaziridine (20%).

Table 2. In-vivo and ex-vivo data of selected compounds

Compound	Rat $AUC_{(0-6\ h)}$ h ng/ml (10 mpk HPβCD)	Ex-vivo platelet aggregation assay dose/duration (mpk/h)
24	nd	3 mpk/6 h
29	6720	3 mpk/24 h
33	3700	3 mpk/24 h (60% inh at 24 h)
34	264	3 mpk/inactive
36	5050	3 mpk/24 h
37	4590	3 mpk/minimal activity
40	3960 (M + 16 = 162)	2 mpk/24 h 1 mpk/6 h
41	nd	3 mpk/6 h

synthesized via a similar reduction of aryl triflates **21** and **22**. Representative data for these compounds are given in Table 1. Compounds of high interest have been highlighted; we found that fluoro substitution at either the X^2 or X^3 positions led to the most potent compounds (e.g., **29**, **36**, and **40**). Compounds substituted with fluorine at both X^1 and X^2 (**34** and **41**) gave compounds with similar potency to the mono-substituted compounds. Compounds in which X^5 was fluoro (**36**–**42**) were generally more potent than those substituted with chloro or trifluoromethyl. In addition we investigated the effect of substitution at the 9a position of the tricycle. Two representative examples are given in Scheme 5; treatment of **29** with LDA followed by methyl iodide gave **43**; similarly treatment of **40** with LHMDS followed by (1*S*)-(10-camphorsulfonyl)oxaziridine gave **44**. Both of these compounds were about 3-fold less active than their unsubstituted precursors; this was found to be typical for this type of substitution in this series of compounds. Selected compounds were tested in our ex-vivo platelet aggregation assay in cynomolgus monkeys; the data are shown in Table 2. A majority of the compounds tested showed activity at a dose of 3 mpk. Compound **40**, however, showed robust activity when dosed at 2 mpk for 24 h and intermittent activity at 1 mpk; this is the most potent compound discovered to date in this series. Compound **40** had excellent blood levels in both rats (Table 2) and cynomolgus monkeys ($AUC_{0-24\ h}$ = 6200 ng h/ml when dosed at 1 mpk). In addition compound **40** was shown to have a slow disassociation rate[14] from the receptor with a measured half-life of 139 min. We believe that because of the intramolecular nature of the PAR-1 mechanism this property will be important for any antagonist to be effective in a clinical setting.[12–14]

In summary, we have discovered a novel series of thrombin receptor antagonists based on the arylhimbacine series. Compound **40** was shown to be the most potent with activity in a monkey model of platelet aggregation at a dose of 2 mpk. This compound had good blood levels after oral dosing in both rats and monkeys. Compound **40** also has a slow disassociation rate from the receptor, thus giving it an excellent chance to compete with the intramolecular activation of PAR-1 initiated by thrombin. Results of additional studies will be reported in due course.

Acknowledgments

The authors thank Drs. Catherine Strader, John Piwinski, Michael Graziano, Ashit Ganguly, and Ronald White for helpful discussions, Drs. Birendra Pramanik and Pradip Das for mass spectral data and Drs. T.-M. Chan and Mohindar Puar for NMR data.

References and notes

1. Coughlin, S. R. In *Handbook of Cell Signaling*; Bradshaw, R. A., Dennis, E. A., Eds.; Elsevier: San Diego, USA, 2004; 1, pp 167–171.
2. Coughlin, S. R. *Cold Spring Harbor Symposia on Quantitative Biology* **2002**, *67*, 197.
3. Coughlin, S. R. *Thromb. Haemostasis* **2001**, *86*, 298.
4. Grand, R. J. A.; Turnell, A. S.; Grabham, P. W. *Biochem. J.* **1996**, *313*, 353.
5. Coughlin, S. R. *J. Thromb. Haemostasis* **2005**, *3*, 1800.
6. Coughlin, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11023.
7. Vu, T.-K. H.; Hung, D. T.; Wheaton, V. I.; Coughlin, S. R. *Cell.* **1991**, *64*, 1057.
8. Hung, D. T.; Vu, T.-H.; Nelken, N. A.; Coughlin, S. R. *J. Cell Biol.* **1992**, *116*, 827.
9. Vu, T.-K. H.; Wheaton, V. I.; Hung, D. T.; Charo, I.; Coughlin, S. R. *Nature* **1991**, *353*, 674.
10. Chackalamannil, S. *Curr. Opin. Drug Disc. Dev.* **2001**, *4*, 417.
11. Seiler, S. M.; Bernatowicz, M. S. *Curr. Med. Chem.; Cardiovsc. Hemat. Agents* **2003**, *1*, 13.

12. Chackalamannil, S.; Xia, Y. *Expert Opin. Ther. Patents* **2006**, *16*, 493.
13. Chackalamannil, S. *J. Med. Chem.* **2006**, *49*, 5389.
14. Chackalamannil, S.; Xia, Y.; Greenlee, W. J.; Clasby, M.; Doller, D.; Tsai, H.; Asberom, T.; Czarniecki, M.; Ahn, H.-S.; Boykow, G.; Foster, C.; Agans-Fantuzzi, J.; Bryant, M.; Lau, J.; Chintala, M. *J. Med. Chem.* **2005**, *48*, 5884.
15. Clasby, M. C.; Chackalamannil, S.; Czarniecki, M.; Doller, D.; Eagen, K.; Greenlee, W. J.; Lin, Y.; Tsai, H.; Xia, Y.; Ahn, H.-S.; Agans-Fantuzzi, J.; Boykow, G.; Chintala, M.; Foster, C.; Bryant, M.; Lau, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1544.
16. Clasby, M. C.; Chackalamannil, S.; Czarniecki, M. l.; Doller, D.; Eagen, K.; Greenlee, W.; Kao, G.; Lin, Y.; Tsai, H.; Xia, Y.; Ahn, H.-S.; Agans-Fantuzzi, J.; Boykow, George.; lton, K.; Bryant, M.; Hsieh, Y.; Lau, J.; Palamanda, J. *J. Med. Chem.* **2007**, *50*, 129.
17. Ahn, H.-S.; Foster, C.; Boykow, G.; Arik, L.; Smith-Torhan, A.; Hesk, D.; Chatterjee, M. *Mol. Pharm.* **1997**, *51*, 350, Assays were carried out in duplicate, compounds of high interest were assayed multiple times ($n > 5$, SEM ±20%).
18. Chackalamannil, S.; Asberom, T.; Xia, Y.; Doller, D.; Clasby, M. C.; Czarniecki, M. F. Preparation of himbacine analogs as thrombin receptor antagonists. U.S. Patent 6,063,847, May 16th, 2000.
19. Zhang, H.-C.; Derian, C. K.; Andrade-Gordon, P.; Hoekstra, W. J.; McComsey, D. F.; White, K. B.; Poulter, B. L.; Addo, M. F.; Cheung, W.-M.; Damiano, B. P.; Oksenberg, D.; Reynolds, E. E.; Pandey, A.; Scarborough, R. M.; Maryanoff, B. E. *J. Med. Chem.* **2001**, *44*, 1021.
20. Chackalamannil, S.; Doller, D.; Eagen, K. *Tetrahedron Lett.* **2002**, *43*, 5101.
21. Chackalamannil, S.; Doller, D.; Clasby, M.; Xia, Y.; Eagen, K.; Lin, Y.; Tsai, H.; McPhail, A. T. *Tetrahedron Lett.* **2000**, *41*, 4043.
22. Four, P.; Guibe, F. *J. Org. Chem.* **1981**, *46*, 4439.
23. CCDC 639630 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.