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Amino acid based enantiomerically pure 3-substituted benzofused heterocycles: A new class of antithrombotic agents

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ABSTRACT

A diverse group of novel medium ring heterocycles derived from naturally abundant proteinogenic amino acids were evaluated for their potency towards antithrombotic activity. The more potent benzofused oxazepine and oxazocine scaffolds were diversified by incorporating different amino acids at the position number 3. Further the effect of ring size has also been taken into account and it was observed that the eight-membered oxazocines ane more potent compared to the corresponding oxazepines.

Intravascular thrombosis leads to many acute cardiovascular diseases.[1] The resulting clinical ramifications of occlusive thrombus development, including myocardial infarction, deep vein thrombosis, pulmonary embolism, and stroke, annually affect millions of people worldwide and are the leading causes of mortality and morbidity in the industrialized world.[2] The origin of thrombosis is the failure of platelets to distinguish a damaged blood vessel wall in need of repair from a thrombogenic surface. In this case, self-aggregation of platelets occurs and that lead to vessel occlusion and the interruption of blood flow.[3] The current therapies include the use of anti-platelet agents; aspirin, ticlopidine, clopidogrel and anticoagulants such as heparin and warfarin. However, these drugs do not work in acute conditions and require extended periods of closely monitored therapy mainly because of the high risk of bleeding.[4–6]

The medium ring heterocycles constitute the core motif structures in a variety of natural products[7] and also well established pharmacophore in medicinal chemistry. Among the medium ring heterocycles, the benzofused seven and eight-membered heterocycles are having significant contribution. The benzodiazepines are a class of privileged structures having a wide range of biological activities.[8] Benzoxazepines show pharmacological activities such as antipsychotic, central nervous system along with antibreast cancer.[9] Benzothiazepines are active constituents of an important class of biologically active compounds such as bradykinin agonists.[10] Eight-membered benzannulated heterocycle benzodiazocine, such as Teleocidines activate protein kinase C (PKC) isozymes.[11] Benzoxazocine, such as Nefopam hydrochloride[12] is a non-narcotic analgesic drug with antidepressant properties.[13] Although various applications are reported, antithrombotic activities of this class of compounds are not explored much. In continuation of our research program towards synthesis and biological activity of amino acid based heterocycles[14] and natural products[15], some of the abundantly available benzofused heterocycles were evaluated for their efficacy as antithrombotic agents. The discovery program oriented towards the identification of antithrombotic agents was initiated by conducting in vivo screen of our chemical library of medium ring heterocycles.

Synthesis of seven and eight-member chiral heterocycles was undertaken. S-amino acid and substituted benzene derivatives were used as building blocks for the construction of benzannulated chiral heterocycles. The treatment of S-amino alcohol derivatives **1a–b** with *N*-tosyl derivative of methyl anthranilate and *N*-tosyl derivative of 2-nitro phenylacetic acid methyl ester under DEAD/PPh_3 condition furnished **2a–b, 3a–b**. The deprotection of Boc in **2a–b, 3a–b** by 6 N HCl in methanol furnished amine hydrochlorides **4a–b, 5a–b**. The primary amine functionality was converted to its tosyl derivatives **6a–b, 7a–b**. The reduction of **6a–b, 7a–b** by LAH at 0 °C afforded the alcohols **8a–b, 9a–b** which under Mitsunobu[16] cyclization conditions yielded the desired enantiomerically pure 3-substituted 1,4-benzodiazepines, benzo[*e*][1,4] diazocines **10a–b, 11a–b** (Scheme 1). To begin with, methyl

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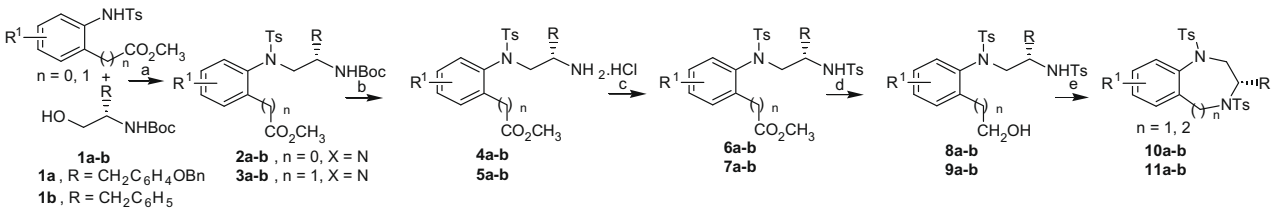
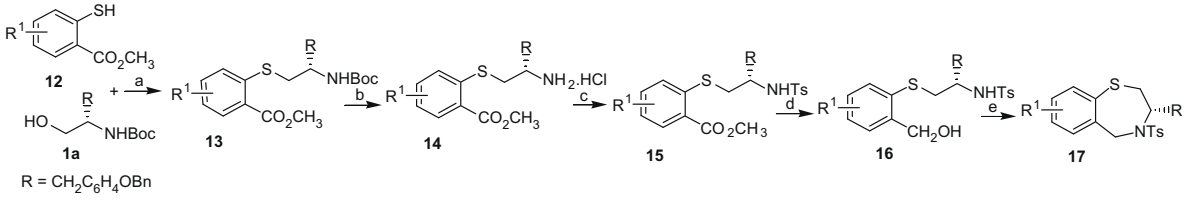


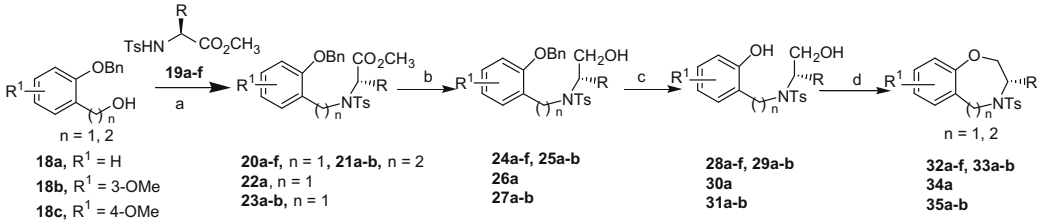
Scheme 1. Reagents and conditions: (a) DEAD, PPh_3, 0 °C (2 h) to rt (10 h); (b) 6 N HCl, MeOH, rt, 45 min; (c) TsCl, Et_3N, DCM, 1 h; (d) LAH, dry THF, 1 h; (e) PPh_3, DEAD, dry THF.

thiosalicylate **12** and Boc protected tyrosinol **1a** under Mitsunobu condition furnished the ester derivative **13**. The deprotection of Boc group with 6 N HCl afforded the free amine hydrochloride **14**, which was again converted to its tosyl derivative **15**. The reduction of ester group in **15** by LAH gave alcohol **16**. The intramolecular Mitsunobu cyclization furnished enantiomerically pure benzothiazepine derivative **17** in good yield (Scheme 2). To synthesize 3-substituted benzoxazepines and benzo[*f*][1,4]oxazepine derivatives, **18a, 18b, 18c** and *N*-tosyl amino acid methyl esters **19a–f** were used. The Mitsunobu reaction of *S*-amino acid derivatives **19a–f** with **18a, 18b, 18c** provided the esters **20a–f, 21a–b, 22a, 23a–b**. The Lithium aluminium hydride (LAH) reduction of these esters afforded the corresponding alcohols **24a–f, 25a–b, 26a, 27a–b** which on subsequent debenzylation by H_2/Pd (10% on carbon) gave **28a–f, 29a–b, 30a, 31a–b** containing free alcoholic and phenolic hydroxyl groups. Exposure of **28a–f, 29a–b, 30a, 31a–b** to Mitsunobu reaction conditions, that is, diethylazodicarboxylate (DEAD), triphenylphosphine (TPP) at 0 °C resulted in the formation of desired enantiomerically pure 3-substituted benzo[*f*][1,4]oxazepine, benzoxazepine derivatives **32a–f, 33a–b, 34a, 35a–b** (Scheme 3). Therefore, the selection of scaffold was initially guided by the application of the Topliss tree approach (Table 1) with the goal of gaining as much information from the least numbers of selected analogues.

Structure–activity relationships: The biological result of the medium ring heterocycles derived from naturally occurring Samino acids are shown in the Table 1. Our SAR studies begin with the comparison of the biological activity of various kinds of 3-substituted medium ring heterocycles towards antithrombotic activity.[19] Among the benzo- fused seven-member heterocycles, the compounds containing benzoxazepine ring showed the promising activity compared to the benzodiazepines and benzothiazepine. The benzothiazepines showed poor activity among all three types (benzofused seven-membered) of ring system. The synthesized benzodiazepines based on O-protected tyrosine **10a** and phenylalanine **10b** showed comparable antithrombotic activity of 20% protection with increase in bleeding time of 4% and 13%, respectively. The benzothiazepine scaffold derived from O-protected tyrosine gave only 10% protection. The benzoxazepine scaffold derived from alanine showed the antithrombotic activity comparable to benzodiazepines. Surprisingly, the compound **32b** was fully inactive while the corresponding benzodiazepine **10a** showed activity of 20%. On moving from methyl group at position-3 **32a** to 2-methyl propyl group **32c** further reduction in biological activity was observed. Furthermore, changing groups from methyl **32a** to isopropyl **32e** and benzyl **32d** gave the similar protection of 20%. The 3-substituted benzoxazepine derived from tryptophan which is having 3-methine indole as a side chain, showed the most promising antithrombotic activity of 40% protection which was comparable with aspirin. After getting lead scaffold among benzofused seven-membered heterocycles, we were interested to observe the antithrombotic activity of benzofused eight-membered heterocycles. In this context we tested few 3-substituted benzodiazocines and benzoxazocines. The benzodiazocine derived from O-protected tyrosine **11a** showed somewhat improved activity of 30% compare to corresponding diazepine **10a** which showed the protection of 20%. While there was no change in biological activity of benzodiazocine derived from phenylalanine **11b** with the corresponding benzodiazepine **10b**. In comparison of benzoxazepines the benzoxazocines were found to be more potent as indicated by the results shown in table; the benzoxazocine derived from alanine **33a** showed 40% protection compare to corresponding benzoxaze-

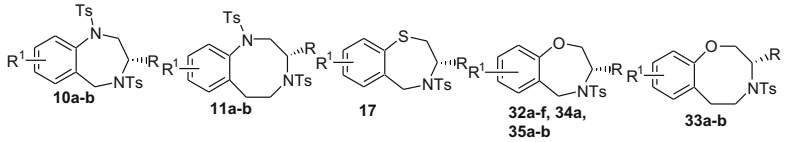


Scheme 2. Reagents and conditions: (a) DEAD, PPh_3, 0 °C (2 h) to rt (10 h); (b) 6 N HCl, MeOH, rt, 45 min; (c) TsCl, Et_3N, DCM, 1 h; (d) LAH, dry THF, 1 h; (e) PPh_3, DEAD, dry THF.



Scheme 3. Reagents and conditions: (a) DEAD, PPh_3, 0 °C (2 h) to rt (10 h); (b) LAH, dry THF, 0 °C; (c) H_2, 10% Pd/C, MeOH, rt, 2 h, 50 Psi; (d) DEAD, PPh_3, dry THF.

Table 1
In vivo assay of benzofused heterocycles derived from amino acids for antithrombotic activity



Compds	R^1	R[a]	Antithrombotic activity[17] (% protection)	Bleeding time[18] (% increase)
10a	H	$CH_2C_6H_4OBn$	20.00	4
10b	H	$CH_2C_6H_5$	20.00	13
11a	H	$CH_2C_6H_4OBn$	30.00	No change
11b	H	$CH_2C_6H_5$	20.00	22
17	H	$CH_2C_6H_4OBn$	10.00	28
32a	H	CH_3	20.00	25
32b	H	$CH_2C_6H_4OH$	0.00	No change
32c	H	$CH_2CH(CH_3)_2$	10.00	60
32d	H	$CH_2C_6H_5$	20.00	No change
32e	H	$CH_2(CH_3)_2$	20.00	22
32f	H	$CH_2C_8H_6N$	40.00	33
33a	H	CH_3	40.00	No change
33b	H	$CH_2C_6H_4OH$	40.00	No change
34a	3-OCH_3	$CH_2C_6H_4OH$	45.00	24
35a	4-OCH_3	$CH_2CH(CH_3)_2$	30.00	6
35b	4-OCH_3	$CH_2C_8H_6N$	20.00	12
36		Aspirin	37 ± 3	100 ± 20

[a] R means alkyl derivatives of different amino acids.

pine **32a** which showed only 20% of protection. The most interesting result observed in case of tyrosine based benzoxazocine which showed 40% protection compare to totally inactive corresponding benzoxazepine **32b**. Incorporation of 3-OCH_3 and 4-OCH_3 furnished better antithrombotic protection in **34a** and **35a** compared to **32b** and **32c**, respectively.

In summary, a diverse group of novel medium ring heterocycles derived from naturally abundant proteinogenic amino acids were evaluated for their potency towards antithrombotic activity. The more potent oxazepine and oxazocine scaffolds were diversified through incorporation of different amino acids at the position-3. Further, the effect of ring size on the biological activity was also explored and we found that the eight-membered benzoxazocines are more potent compared to the corresponding seven-membered benzoxazepines.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.126.

References and notes

1. Gent, M.; Blakely, J. A.; Easton, J. D. *Lancet* **1989**, *1*, 1215.
2. Rapaport, S. I.; Rao, L. V. *Arterioscler. Thromb.* **1992**, *12*, 1111.
3. Coller, B. S. In *The Heart and Cardiovascular System*; Fozzard, H. A., Haber, E., Jennings, R. B., Katz, A. M., Morgan, H. E., Eds., 2nd ed.; Raven Press: New York, 1991.
4. Monteal, M.; Lafoz, E.; Olive, A.; del Rio, L.; Vedia, C. *Thromb. Haemostasis* **1994**, *71*, 7.
5. Landefeld, C. S.; Rosenblatt, M. W.; Goldman, L. *Am. J. Med.* **1989**, *87*, 153.
6. Tabibian, N. *Am. J. Gastroenterol.* **1989**, *84*, 10.
7. Reviews: (a) Nubbemeyer, U. *Top. Curr. Chem.* **2001**, *216*, 125; (b) Maier, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 2073; (c) Evans, P. A.; Holmes, B. *Tetrahedron* **1991**, *47*, 9131; (d) Lindström, U. M.; Somfai, P. *Chem. Eur. J.* **2001**, *7*, 94; (e) Bieräugel, H.; Jansen, T. P.; Schoemaker, H. E.; Hiemstra, H.; van Maarseveen, J. H. *Org. Lett.* **2002**, *4*, 2673; (f) Derrer, S.; Davies, J. E.; Holmes, A. B. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2957; (g) Nicolaou, K. C.; Vourloumis, D.; Winssinger, N.; Baran, P. *Angew. Chem., Int. Ed.* **2000**, *39*, 44.
8. (a) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirschfield, J. *J. Med. Chem.* **1988**, *31*, 2235; (b) Sternbach, L. H. *J. Med. Chem.* **1979**, *22*, 1; (c) Bock, M. G.; Dipardo, R. M.; Evans, B. E.; Rittle, K. E.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Preidinger, R. M. *J. Med. Chem.* **1989**, *32*, 13; (d) Romer, D.; Buscher, H. H.; Hill, R. C.; Maurer, R.; Petcher, T. J.; Zeugner, H.; Benson, W.; Finnner, E.; Milkowski, W.; Thies, P. W. *Nature* **1982**, *298*, 759; (e) Komecki, E.; Ehrlich, Y. H.; Lenox, R. H. *Science* **1984**, *226*, 1454; (f) Hsu, M.-C.; Schutt, A. D.; Hooly, M.; Slice, L. W.; SherTnan, M. I.; Richman, D. D.; Potash, M. J.; Volsky, D. J. *Science* **1991**, *254*, 1799; (g) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; Clercq, E. D.; Janssen, P. A. J. *Nature* **1990**, *343*, 470.
9. (a) Liao, Y.; Venhuis, B. J.; Rodenhuis, N.; Timmerman, W.; Wikstrom, H.; Meire, E.; Bartoszyk, G. D.; Bottcher, H.; Seyfried, C. A.; Sundell, S. *J. Med. Chem.* **1999**, *42*, 2235; b Toshiyuki, H.; Tokuhiro, I., Hisao, Y. G. O. 2,014,223, 1969; *Chem. Abstr.* **1970**, *73*, 12069.; c Standridge, R. T. U.S. 4,125,538, 1978; *Chem. Abstr.* **1979**, *90*, 72246r.; (d) James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C., Jr. *Science* **1993**, *260*, 1937; (e) Díaz-Gavilán, M.; Rodríguez-Serrano, F.; Gómez-Vidal, J. A.; Marchal, J. A.; Aránega, A.; Gallo, M. Á.; Espinosa, A.; Campos, J. M. *Tetrahedron* **2004**, *60*, 11547.
10. (a) Milligan, G. *Trends Pharmacol. Sci.* **1993**, *14*, 413; (b) Amblard, M.; Daffix, I.; Bedos, P.; Berge, G.; Pruneau, D.; Paquet, J.-L.; Luccarini, J.-M.; Belichard, P.; Dodey, P.; Martinez, J. *J. Med. Chem.* **1999**, *42*, 4185.
11. (a) Ron, D.; Kazanietz, M. G. *FEBS J.* **1999**, *16*, 1358; (b) Kazanietz, M. G.; Caloca, M. J.; Fujii, T.; Eroles, P.; Garcia-Bermejo, M. L.; Reilly, M.; Wang, H. *Biochem. Pharmacol.* **2000**, *60*, 1417.
12. *The Merck Index*; Budavari, S., Ed., 12th ed.; Merck, Rahway: NJ, 1996; p 1105. and references cited therein.
13. Klohs, M. W.; Draper, M. S.; Petracek, F. J.; Ginzel, K. H.; Ré, O. N. *Arzneim.-Forsch. (Drug Res.)* **1972**, *22*, 132.
14. (a) Mishra, J. K.; Garg, P.; Dohare, P.; Kumar, A.; Siddiqi, M. I.; Ray, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1326; (b) Mishra, J. K.; Panda, G. *J. Comb. Chem.* **2007**, 321; (c) Shagufta; Panda, G. *Org. Biomal. Chem.* **2007**, *5*, 360; (d) Mishra, J. K.; Panda, G. *Synthesis* **2005**, 1881; (e) Mishra, J. K.; Rao, J. S.; Sastry, G. N.; Panda, G. *Tetrahedron Lett.* **2006**, *47*, 3357.
15. Srivastava, A. K.; Panda, G. *Chem. A Eur. J.* **2008**, *14*, 4675.
16. Mitsunobu, O. *Synthesis* **1981**, 1.
17. *Mouse thrombosis model*: Pulmonary thromboembolism was induced by a method as described by DiMinno and Silver (1983). The compounds to be tested (30 μM/kg), standard drugs or the vehicle were administered by oral

route 60 min prior to the thrombotic challenge. Ten mice were used for evaluating the effect of test compound, while a group of five mice was used to evaluate the effect of aspirin or vehicle. A mixture of collagen and adrenaline was injected into the tail vein to induce hind limb paralysis or death. Results have been reported as% protection, which represent protection against hind limb paralysis or death. The test compounds exhibiting protection equivalent to Aspirin (30 mg/kg) were considered as active molecules. (Dimnno, G.; Silver, M. J. *J. Pharmacol. Exp. Therap*. **1983**, *225*, 57–60).

18. *Bleeding time*: Bleeding time in mice was evaluated by the method of Dejana et al. (1979) to assess the increase in bleeding time in comparison to aspirin at its optimal antithrombotic dose. The tail 2 mm from tip of mice was incised and the blood oozed was soaked on a filter paper, which was monitored at an interval of 10–15 s till the bleeding stopped. The time elapsed from the tip incision to the stoppage of bleeding was recorded as the bleeding time. The tested compounds (30 μM/kg), aspirin (30 mg/kg) or vehicle was given orally 60 min prior to the tail incision in a group of 5 mice each. (Dejna, E.; Collioni, A.; Quntana, A. *Thromb. Res.* **1979**, *15*, 191).
19. (a) Batra, S.; Roy, A. K.; Patra, A.; Bhaduri, A. P.; Surin, W. R.; Raghavan, S. A. V.; Sharma, P.; Kapoor, K.; Dikshit, M. *Bioorg. Med. Chem.* **2004**, *12*, 2059; (b) Patra, A.; Batra, S.; Bhaduri, A. P.; Khanna, A.; Chander, R.; Dikshit, M. *Bioorg. Med. Chem.* **2003**, *11*, 2269; (c) Batra, S.; Srinivasan, T.; Rastogi, S. K.; Kundu, B.; Patra, A.; Bhaduri, A. P.; Dixit, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1905; (d) Saxena, A. K.; Pandey, S. K.; Seth, P.; Singh, M. P.; Dikshit, M.; Carpy, A. *Bioorg. Med. Chem.* **2001**, *9*, 2025.