Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3271–3273

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Muscarinic M_2 Subtype Antagonistic Activity of Enantiomeric Pairs of 3-Demethylhimbacine (3-Norhimbacine) and Its C_4-Epimer

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Received 1 July 2002; accepted 20 August 2002

Abstract—In the course of our studies of the structure–activity relationships of himbacine **1**, a potent antagonist of the M_2 subtype of muscarinic receptor, the four title compounds, **2**, *ent*-**2**, **3**, and *ent*-**3**, were synthesized with a highly stereoselective intermolecular Diels–Alder reaction of tetrahydroisobenzofuran **4** with achiral furan-2(5*H*)-one **5** as a key step, followed by simultaneous optical resolution and epimer separation of the racemic intermediates. Among these compounds, 3-demethylhimbacine (3-norhimbacine) **2**, bearing an absolute configuration corresponding to that of **1**, was found to show more potent muscarinic M_2 subtype receptor binding activity than natural **1**.
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Himbacine (**1**) is a piperidine alkaloid isolated from the bark of *Galbulimima Baccata* of the magnolia family[1] and shows potent antagonistic activity against muscarinic M_2 subtype receptors, with 10–20-fold selectivity toward the M_1 subtype (Fig. 1).[2] Accordingly, it appears evident that **1** is the reasonable and attractive lead compound of a drug for the treatment of Alzheimer's disease.[3]

Recently, we reported the novel total synthesis of **1** by a method featuring a highly stereoselective intermolecular Diels–Alder reaction of the furan derivative with chiral furan-2(5*H*)-one as a key step.[4] The efficiency and directness of our explored synthetic route were demonstrated by the successful synthesis of various structural types of himbacine congeners and by the evaluation of their structure–activity relationships.[5] In the course of our study of **1** from the viewpoint of medicinal chemistry, we next focused on the effects of the lactone ring moiety, especially that of the C-3 methyl group, of **1** on the M_2 receptor subtype antagonistic activity. Thus, we designed enantiomeric pairs of 3-demethylhimbacine (3-norhimbacine) (**2** and *ent*-**2**) and its C_4-epimer (**3** and *ent*-**3**) for the purpose of further evaluating the structure–activity relationships, and in order to explore the advantages of our explored synthetic scheme. We wish to report here the successful total synthesis of **2**, *ent*-**2**, **3**, and *ent*-**3** and their muscarinic M_2 subtype receptor binding activity. Among these compounds, the synthetic himbacine **2**, which bears an absolute configuration corresponding to that of **1**, was found to show more potent receptor binding activity than natural **1**.

According to our reported procedure,[4] racemic *exo*-methylene compound **7** was prepared from the adduct **6**, which was prepared by a highly stereoselective intermolecular Diels–Alder reaction of tetrahydroisobenzofuran **4** and commercially available

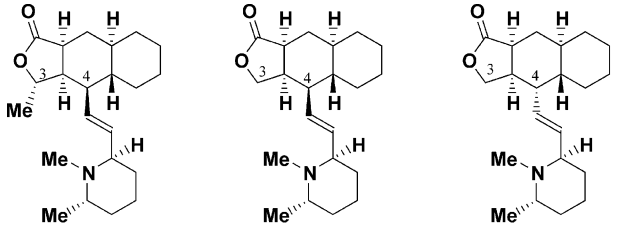

Figure 1.

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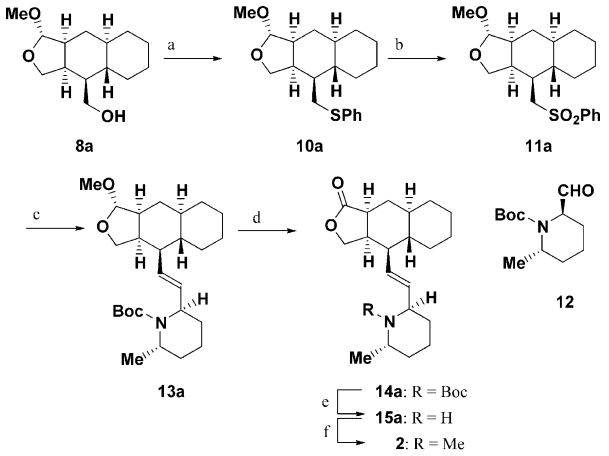
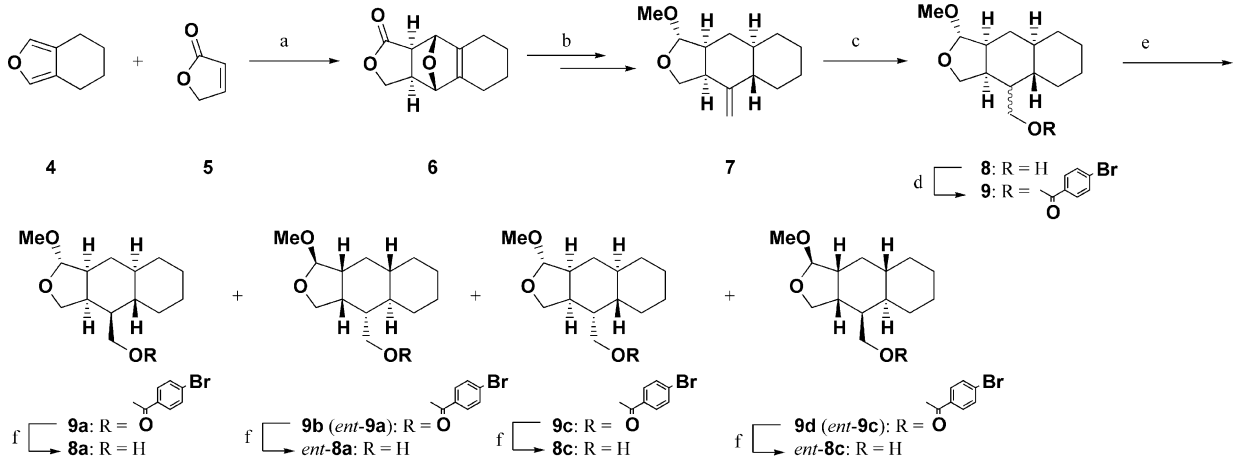
PII: S0960-894X(02)00695-9

achiral furan-2(5*H*)-one **5** (Scheme 1).[6] Hydroboration–oxidation of **7** afforded the carbinol **8** as an inseparable mixture of 4α, 4β-epimers in a ratio of 2:1, as demonstrated by ^{1}H NMR analysis. In our previous total synthesis of **1**, the stereoselectivity of the hydroboration–oxidation reaction was 1:8 (4α:4β).[4] This difference in stereoselectivity may have been due to the steric hindrance effects of the C-3 methyl group. Without separation, the racemic epimeric mixture was directly converted to the *O*-4-bromobenzoates **9** in a 99% yield. The compounds were subjected to simultaneous optical resolution and epimer separation by means of HPLC, using CHIRALCEL OD, giving four stereoisomers, **9a**–**9d**, each of which possessed high optical purity.[7] In order to determine the absolute configurations of these molecules, X-ray crystal structure analysis of **9a** and **9c** was performed.[8] Based on the results obtained by X-ray analysis, it was clearly established that **9a** bears an absolute configuration corresponding to that of **1**, and that **9c** is the 4α-epimer of **9a**. The absolute configurations of **9a**–**9d** were unambiguously determined, and are shown in Scheme 1. Accordingly, **9a** was transformed into the key intermediate sulfone **11a** by a three-step sequence involving alkaline hydrolysis, conversion to the phenyl sulfide **10a** using (cyanomethyl)-trimethylphosphonium iodide,[9] and *m*CPBA oxidation of **10a** (Scheme 2). A Julia–Lythgoe coupling reaction of **11a** with **12**[4] was quenched with excess benzoyl chloride, followed by treatment of the resulting diastereomeric mixture of *O*-benzoates with 5% Na–Hg in the presence of Na_2HPO_4, which gave rise to (*E*)-olefin **13a** in a 61% combined yield. Then, **13a** was sequentially subjected to oxidation of the hemiacetal moiety, deprotection of the *N*-Boc group, and reductive *N*-methylation, furnishing **2**[10,11] in a 40% combined yield. By the same sequences, **9b** (*ent*-**9a**), **9c**, and **9d** (*ent*-**9c**) were successfully converted to the corresponding target compounds *ent*-**2**, **3**, and *ent*-**3**, respectively.[10,11] To avoid confusion, the compounds carrying the natural configurations corresponding to that of **1** are only depicted in Scheme 2.



Scheme 2. (a) (Cyanomethyl)trimethylphosphonium iodide, thiophenol, *N*,*N*-diisopropylethylamine, MeCN, 80 °C, 2.5 h, 92%; (b) *m*CPBA, $NaHCO_3$, CH_2Cl_2, rt, 1.5 h, 82%; (c) (i) *n*BuLi, **12**, 1,2-dimethoxyethane, −78∼0 °C, 3 h; (ii) benzoyl chloride, −78 °C∼rt, 1 h; (iii) 3-(dimethylamino)propylamine, rt, 97% (a mixture of diastereomers); (iv) 5% Na–Hg, Na_2HPO_4, MeOH, rt, 1 h, 63%; (d) Jones reagent, acetone, rt, 1.5 h, 58%; (e) trifluoroacetic acid, CH_2Cl_2, rt, 0.5 h, 87%; (f) 37% HCHO aq $NaBH_3CN$, CH_3CN, rt, 1 h, 80%.

The four 3-demethylhimbacine (3-norhimbacine) derivatives, **2**, *ent*-**2**, **3**, and *ent*-**3**, were then subjected to receptor binding activity assay against M_1 and M_2 subtype muscarinic receptors (Table 1).[12] As was hoped, it was found that **2** bore an absolute configuration corresponding to that of **1**, and showed superior affinity to **1**. However, the other three stereoisomers, *ent*-**2**, **3**, and *ent*-**3**, exhibited only weak receptor binding activity compared with that of **1** and **2**. These results clearly demonstrated that the C-3 methyl group on the lactone ring moiety of **1** is not important for its strong muscarinic M_2 subtype antagonistic activity. In addition, the stereochemistry at the C-4 position was shown to play an important role in eliciting M_2 antagonistic activity, in a manner similar to that played by the stereochemistry of **1** and its 4-epimer.[5]



Scheme 1. (a) 5 M $LiClO_4$–Et_2O, 4,4′-thiobis(6-*tert*-butyl-*m*-cresol), rt, 48 h, 49%; (b) ref 4, 18% (eight steps); (c) (i) BH_3·THF, THF, 0 °C∼rt, 7 h; (ii) 10% H_2O_2, 10% NaOH, 0 °C, 0.5 h, 82%; (d) (i) 4-bromobenzoic acid, $SOCl_2$, 80 °C, 1 h; (ii) **8**, Et_3N, CH_2Cl_2, 0 °C∼rt, 2 h, 99%; (e) CHIRALCEL OD, C_6H_{14}/2-propanol = 98:2, **9a**: 19%, **9b**: 18%, **9c**: 30%, **9d**: 32%; (f) 10% NaOH, EtOH, rt, 0.5 h, **8a**: 100%, *ent*-**8a**: 100%, **8c**: 100%, *ent*-**8c**: 91%.

Table 1. In vitro binding activity of 3-norhimbacines

Entry	Compd	$-\log K_i$	
		M_1 (cortex)	M_2 (brainstem)
1	**1**	7.1	7.9
2	**2**	7.4	8.1
3	*ent*-**2**	6.0	6.1
4	**3**	6.2	6.4
5	*ent*-**3**	6.3	6.4

In conclusion, we have succeeded in the synthesis of four novel himbacine congeners, 3-demethylhimbacines (3-norhimbacines) (**2**, *ent*-**2**, **3**, and *ent*-**3**) lacking a methyl group at the C-3 position on the lactone ring of **1**. Among them, **2**, which bears an absolute configuration corresponding to that of **1**, exhibited more potent binding activity against the M_2 subtype receptor than did natural himbacine **1**. Further investigation of the pharmacological profile of **2** is therefore in progress.

Acknowledgements

We are grateful to Dr. H. Ohkubo and Dr. T. Ishizaki, Kyorin Pharmaceutical Co. Ltd., for their encouragement and many valuable suggestions. We would also like to thank Dr. Y. Fukuda, Kyorin Pharmaceutical Co. Ltd., for his suggestions and helpful input in discussions of this study. The in vitro binding activity assay was carried out by Dr. T. Kawai, Kyorin Pharmaceutical Co. Ltd., to whom our thanks are also due.

References and Notes

1. Brown, R. F. C.; Drummond, R.; Fogerty, A. C.; Hughes, G. K.; Pinhey, J. T.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1956**, *9*, 283.
2. (a) Kozikowski, A. P.; Fauq, A. H.; Miller, J. H.; McKinney, M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 797. (b) Malaska, M. J.; Fauq, A. H.; Kozikowski, A. P.; Aagaard, P. J.; McKinney, M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1247. (c) Malaska, M. J.; Fauq, A. H.; Kozikowski, A. P.; Aagaard, P. J.; McKinney, M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 61. (d) Doller, D.; Chackalamannil, S.; Czarniecki, M.; McQuade, R.; Ruperto, V. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 901.
3. (a) McKinney, M.; Miller, J. H.; Aagaard, P. J. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 74. (b) Doods, H. N.; Quirion, R.; Mihm, G.; Engel, W.; Rudolf, K.; Entzeroth, M.; Schiavi, G. B.; Ladinsky, H.; Bechtel, W. D.; Ensinger, H. A.; Mendla, K. D.; Eberlein, W. *Life Sci.* **1993**, *52*, 497. (c) McKinney, M.; Coyle, J. T. *Mayo Clinic Proc.* **1991**, *66*, 1225. (d) Iversen, L. L. *Trends Pharmacol. Sci. Suppl.* **1986**, 44.
4. Takadoi, M.; Katoh, T.; Ishiwata, A.; Terashima, S. *Tetrahedron Lett.* **1999**, *40*, 3399.
5. Takadoi, M.; Katoh, T.; Ishiwata, A.; Terashima, S. *Tetrahedron*. Submitted for publication.
6. The Diels–Alder cycloadduct **6** was also synthesized by reacting **4** with maleic anhydride followed by $NaBH_4$ reduction of one of the carbonyl groups. See: Tochtermann, W.; Bruhn, S.; Wolff, C. *Tetrahedron Lett.* **1994**, *35*, 1165.
7. HPLC conditions were as follows: CHIRALCEL OD column (DAICEL Chemical Industries, Ltd.) (1 × 25 cm), mobile phase [hexane/2-propanol = 10:1 (v/v)], flow rate (1.0 mL/min), and temperature (40 °C). The effluent was monitored at 254 nm. The retention times and ee values were as follows: **9c**, 21.2 min, 99% ee; **9b** (*ent*-**9a**), 22.7 min, 99% ee; **9a**, 23.5 min, 94% ee; **9d** (*ent*-**9c**), 33.6 min, 98% ee.
8. The crystallographic data (excluding structure factors) for structures **9a** and **9c** reported in this paper have been deposited to the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 183427 and 183426, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44–1223–336033; e-mail: http://www.deposit@ccdc.cam.ac.uk).
9. (a) Zaragona, F. *Tetrahedron* **2001**, *57*, 5451. (b) Zaragona, F.; Stephensen, H. *J. Org. Chem.* **2001**, *66*, 2518.
10. All compounds were characterized by ^1H and ^{13}C NMR, IR, and mass spectroscopic methods. Yields of each reaction are as follows: (a) thiophenylation; *ent*-**10a**: 88%, **10c**: 79%, *ent*-**10c**: 76%; (b) *m*CPBA oxidation, *ent*-**11a**: 99%, **11c**: 86%, *ent*-**11c**: 82%; (c) (i) Julia–Lythgoe coupling reaction (isolated as *O*-benzoates), (ii) 5% Na–Hg, *ent*-**13a**: 52% from *ent*-**11a**, **13c**: 77% from **11c**, *ent*-**13c**: 59% from *ent*-**11c**; (d) Jones oxidation, *ent*-**14a**: 62%, **14c**: 60%, *ent*-**14c**: 70%; (e) removal of the *N*-Boc group, *ent*-**15a**: 94%, **15c**: 98%, *ent*-**15c**: 99%; (f) reductive methylation, *ent*-**2**: 59%, **3**: 78%, *ent*-**3**: 63%. Details will be reported in a separate paper.
11. **2**: Colorless oil, $[\alpha]_D^{24}$ +69° (*c* 0.44, $CHCl_3$), HRMS (FAB) (*m*/*z*): calcd for $C_{21}H_{34}NO_2$ ($M^+ + H$): 332.2590. Found, 332.2609, *ent*-**2**: colorless oil, $[\alpha]_D^{24}$ −72° (*c* 0.30, $CHCl_3$), HRMS (FAB) (*m*/*z*): calcd for $C_{21}H_{34}NO_2$ ($M^+ + H$): 332.2590. Found, 332.2579, **3**: mp 86–88 °C, $[\alpha]_D^{24}$ −9.1° (*c* 0.42, $CHCl_3$), HRMS (FAB) (*m*/*z*): calcd for $C_{21}H_{34}NO_2$ ($M^+ + H$): 332.2590. Found, 332.2573, *ent*-**3**: mp 85–87 °C, $[\alpha]_D^{24}$ +9.7° (*c* 0.47, $CHCl_3$), HRMS (FAB) (*m*/*z*): calcd for $C_{21}H_{34}NO_2$ ($M^+ + H$): 332.2590. Found, 332.2569.
12. Binding assay: the receptor binding analysis of the M_1 and M_2 subtypes of muscarinic receptors was performed using homogenates of the cerebral cortex and the brainstem of the rat, respectively. The radioligands used were [^3H]-pirenzepine for the cerebral cortex and [^3H]-quinuclidinyl benzilate (QNB) for the brainstem, respectively. The homogenates were incubated in a 50 mM Tris–buffer (pH 7.4) at 25 °C for 90 min, and rapidly filtrated on Whatman GF-B filters. The radioactivities were counted using a liquid scintillation counter. Non-specific binding was defined in the presence of 2 μM atropine. Test compounds were dissolved in DMSO and diluted with buffer to the final concentrations. The competition binding experiments were performed in the presence of less than 0.1% DMSO, which did not affect the specific binding. The equilibrium dissociation constants (K_i) were calculated using the Cheng–Prusoff equation, $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d were the concentration and the dissociation constant of the radioligand, respectively. The K_d values were determined by a Scatchard analysis.