Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 101–103

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Biological Activities of Hapalosin Derivatives with Modification at the C12 Position

Nobuki Kashihara,[a] Sakino To-e,[b] Kensuke Nakamura,[c] Kazuo Umezawa,[b] Shosuke Yamamura[a] and Shigeru Nishiyama[a,*]

[a]*Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama 223-8522, Japan*
[b]*Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama 223-8522, Japan*
[c]*Institute of Medicinal Molecular Design, 5-24-4 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

Received 28 September 1999; accepted 6 November 1999

Abstract—Among the hapalosin derivatives synthesized, the compounds carrying methyl (**5a**), methylthioethyl (**5d**) and phenylmethyl (**5e**) groups at the C12 position possess only the *cis*-peptide structure, in contrast to the cases of **5b** and **5c**. In addition to their conformational stability, the biological activities of the compounds were determined in relation of the P-glycoprotein-mediated MDR-reversing activity and induction of apoptosis. © 2000 Elsevier Science Ltd. All rights reserved.

Hapalosin **1**, a cyclic depsipeptide isolated from the blue-green alga, *Hapalosiphon welwitschii* W. & G. S. West,[1] is of interest in view of its reversing activity against MDR (multidrug resistance) derived from P-glycoprotein which some cancers acquire during chemotherapeutic treatment (Fig. 1).[2–5] In addition to the comparable biological activity with that of verapamil,[6] this molecule possesses the characteristic feature of the peptide bond existing as a *trans*/*cis* mixture (1:3). Among a number of related investigations,[7–17] we reported a total synthesis of **1** and its *N*-demethyl analogue,[18,19] from which it was proposed the *cis*-peptide might be essential to express the MDR-reversing activity, since *N*-demethylhapalosin carrying only the *trans*-peptide exhibited no activities. During several structure–activity relationship studies, a proline-containing congener was reported to exhibit a more potent activity against MCF-7/ADR cells than **1**.[11,12] Against such background, we have attempted to produce hapalosin derivatives possessing a high ratio of *cis*-peptides.[20] Consequently, it was found that the alkyl substituents (the C12 position) of the valic acid part affects the peptide geometry. We disclose herein the synthesis and stereochemistry of the hapalosin derivatives, as well as their biological activities.

Synthesis of the Hapalosin Derivatives

As previously reported,[18,19] our synthesis of **1** was accomplished by the cyclization producing the peptide bond at the final stage, for its ready feasibility of amide bond formation compared to that of esters (Scheme 1). According to this synthetic strategy, the syntheses of the hapalosin derivatives (**5a**–**5e**) were commenced by catalytic hydrogenation of the benzyl ester **2**, obtained by the previously reported procedure.[18,19] The generated carboxylic acid was coupled with the appropriate allyl α-hydroxylate **3**[21–24] under the DCC conditions, leading to allyl ester **4** carrying the fully equipped carbon skeletons of the target molecule. After sequential deprotection of the TBS, allyl and Boc groups, cyclization under the DPPA conditions produced the desired cyclic products **5**.

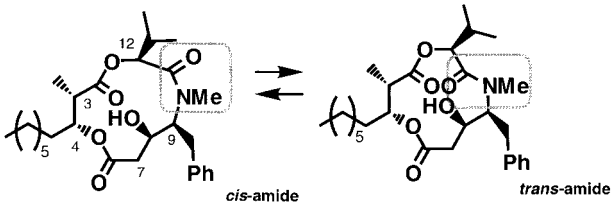


Figure 1. Hapalosin **1** (*cis*-amide:*trans*-amide = ca. 3:1).

Stereochemistry

In the previous investigation,[18,19] the peptide geometry was determined by spectroscopic means: the NOE correlation between H9 and H12 was observed in the *cis*-peptides, contrary to the *trans* cases. Based on this finding, it was determined that **5a**, **5d** and **5e** have the *cis*-peptides as single isomers, whereas **5b** and **5c** existed as 5:1 (*cis*:*trans*) and 1:1 (*cis*:*trans*) isomeric mixtures,

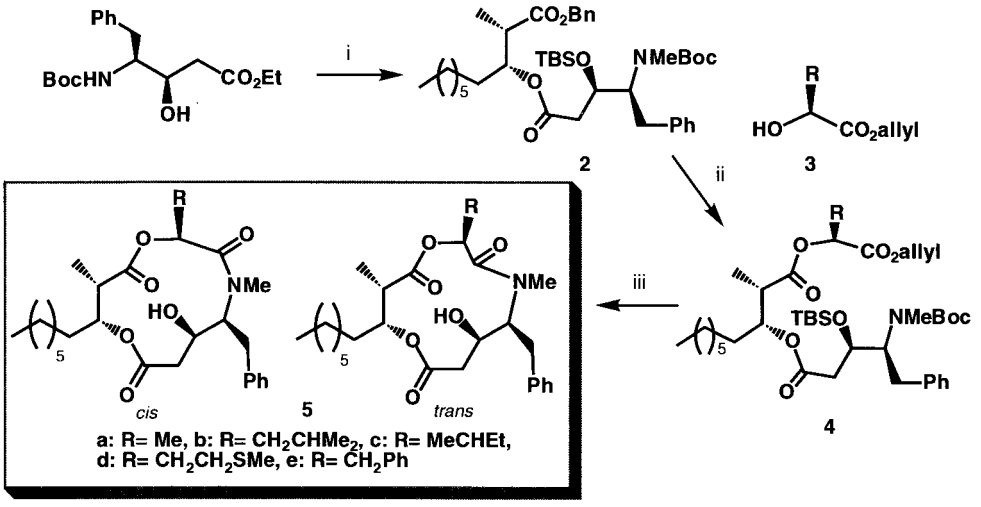
*Corresponding author.

PII: S0960-894X(99)00647-2

respectively. The spectroscopic observation was supported by Monte Carlo Conformation search (3000 steps/structure) with MM2* calculations employing GB/SA by taking the solvation effect of H_2O into account. Consequently, the energy differences (*trans*−*cis*: kcal/mol) were calculated to be +1.8 (**5a**), −0.1 (**5b**), −0.1 (**5c**), +1.6 (**5d**), and +3.3 (**5e**). Although the calculated relative energies do not exactly agree with the NMR ratio, it reproduces the qualitative preference for the *trans* isomers with **5b** and **5c** compared to **5a**, **5d** and **5e** (Fig. 2).[25]

Biological Activity

As can be seen in Table 1, the MDR-reversing activities of the synthesized hapalosin derivatives (**5a**–**5e**) were examined.[26] The C12-methyl derivative **5a**[27] showed high vincristine accumulation, which was superior to that of the reference, verapamil, along with hapalosin **1**. No remarkable participation of the peptide geometry was observed, since mixtures of the *cis*/*trans* isomers (**5b** and **5c**) exhibited rather better results than those of **5d**



Scheme 1. i. Refs 18 and 19. ii. (1) H_2, $Pd(OH)_2$; (2) **3**, DCC, DMAP, a: 70%, b: 91%, c: 87%, d: 82%, e: 91%. iii. (1) HF-pyr., a: 85%, b: 94%, c: 85%, d: 86%, e: 75%; (2) $(Ph_3P)_4Pd$, morpholine, then TFA, quantitative yields in all entries; (3) DPPA, EtN^iPr_2, a: 31%, b: 35%, c: 25%, d: 27%, e: 20%.

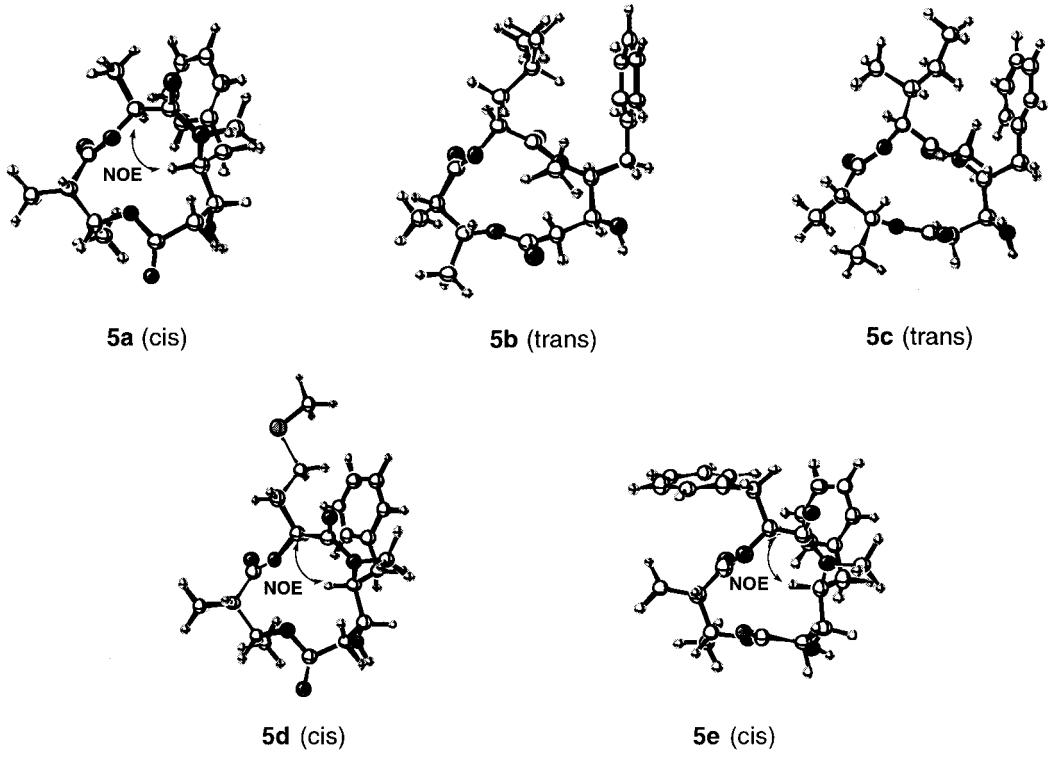


Figure 2. Lowest energy conformations of hapalosin derivatives. MM2* with GB/SA solvation model calculations.

Table 1. Comparison of vincristine accumulation in the multidrug resistant 2780AD cells, a subline of A 2780 ovarian carcinoma cells by hapalosin (**1**) and its derivatives (**5a**–**5e**)[a,b]

Additives						
5a	**5b**	**5c**	**5d**	**5e**	**1**	Verapamil
% of control						
441	288	308	269	254	267	350

[a]Concentration of the additives: 10 μg/mL.
[b]Control: absence of the additives.

and **5e** possessing only the *cis*-peptides. These observations suggested that exhibition of the significant activity requires additional factors, such as hydrophobic interaction of the C12-substituent with the receptor site. In contrast to the MDR-reversing activities, hapalosin **1** and its derivatives (**5a**–**5e**) showed no induction of apoptosis in human fibrosarcoma HT1080 cell line at the 10 μg/mL concentration.

Further investigation related to this structure–activity relationship is in progress.

Acknowledgements

The authors wish to thank the Screening Committee of New Anticancer Agents supported by Grant-in-Aid for Science Research on Priority Area 'Cancer' from the Ministry of Education, Science, Sports and Culture, Japan, for the evaluation of the MDR-reversing activities.

References and Notes

1. Stratmann, K.; Burgoyne, D. L.; Moore, R.; Patterson, M. L. *J. Org. Chem.* **1994**, *59*, 7219–7226.
2. Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1981**, *41*, 1967–1972.
3. Rogan, A. M.; Hamilton, T. C.; Young, R. C.; Klecker, R. W. Jr.; Ozols, R. F. *Science* **1984**, *224*, 994–996.
4. Broxterman, H. J.; Kuiper, C. M.; Schuurhuis, G. J.; Tsuruo, T.; Pinedo, H. M.; Lankelma, J. *Biochem. Pharm.* **1988**, *37*, 2389–2393.
5. Simon, S. M.; Schinller, M. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3497–3504.
6. The selective MDR-reversing activity was supported by the low cytotoxicity of this molecule ($IC_{50} = 5 \sim 15$ μg), see ref 1.
7. Dinh, T. Q.; Armstrong, R. W. *J. Org. Chem.* **1995**, *60*, 8118–8119.
8. Ghosh, A. K.; Liu, W.; Xu, L.; Chen, Z. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 74–76.
9. Dinh, T. Q.; Du, X.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 6606–6616.
10. Wagner, B.; Beugelmans, R.; Zhu, J. *Tetrahedron Lett.* **1996**, *37*, 6557–6560.
11. Dinh, T. Q.; Smith, C. D.; Armstrong, R. W. *J. Org. Chem.* **1997**, *62*, 790–791.
12. Dinh, T. Q.; Du, X.; Smith, C. D.; Armstrong, R. W. *J. Org. Chem.* **1997**, *62*, 6773–6783.
13. Dinh, T. Q.; Smith, C. D.; Du, X.; Armstrong, R. W. *J. Med. Chem.* **1998**, *41*, 981–987.
14. Wagner, B.; Gonzalez, G. I.; Dau, M. E. T. H.; Zhu, J. *Bioorg. Med. Chem.* **1999**, *7*, 737–747.
15. O'Connell, C.; Salvato, K. A.; Meng, Z.; Littlefield, B. A.; Schwartz, C. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1541–1546.
16. Pais, G. C. G.; Maier, M. E. *J. Org. Chem.* **1999**, *64*, 4551–4554.
17. Haddad, M.; Botuha, C.; Larcheveque, M. *SYNLETT* **1999**, 703–704.
18. Ohmori, K.; Okuno, T.; Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1996**, *37*, 3467–3470.
19. Okuno, T.; Ohmori, K.; Nishiyama, S.; Yamamura, S.; Nakamura, K.; Houk, K. N.; Okamoto, K. *Tetrahedron* **1996**, *52*, 14723–14734.
20. It was anticipated that alkyl groups such as ethyl or propyl, introduced at the N-position would conduct the *cis*-peptide geometry, according to computational calculations. However, coupling of the corresponding alkylated amino group, or insertion of alkyl groups to the peptide moiety were unsuccessful, owing to low reactivities.
21. The hydroxyl acids (>99% *ee*) were obtained by the stereoretentive conversion of the corresponding amino acids, via the diazonium intermediates.[22–24]
22. Scheibler, H.; Wheeler, A. S. *Chem. Ber.* **1911**, *44*, 2684–2690.
23. Abderhalden, E.; Weil, A. Z. *Physiol. Chem.* **1913**, *84*, 39–59.
24. Mori, K. *Tetrahedron* **1976**, *32*, 1101–1106.
25. The *cis*/*trans* ratio should be determined by the relative stability of the transition state of the cyclization rather than that of the cyclized products calculated in the present study. However, we assumed that the thermodynamic stability calculated correlates with the above-mentioned stability, based on the observation of a reversible change in the isomeric proportion (ca. 3:1) to 1.7:1 at 100 °C.[18,19]
26. Low cytotoxicities of **5a**–**5e** were determined.
27. During preparation of this paper, this sample was reported: see ref 15.