Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 763–766

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Peptidomimetics of Efflux Pump Inhibitors Potentiate the Activity of Levofloxacin in *Pseudomonas aeruginosa*

Thomas E. Renau,* Roger Léger, Rose Yen, Miles W. She, Eric M. Flamme, Joan Sangalang, Carla L. Gannon, Suzanne Chamberland, Olga Lomovskaya and Ving J. Lee

Essential Therapeutics, Inc., 850 Maude Avenue, Mountain View, CA 94043, USA

Received 3 November 2001; accepted 14 December 2001

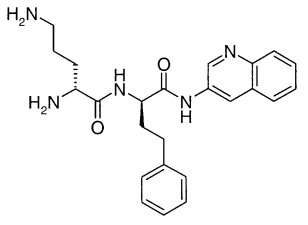
Abstract—Several classes of peptidomimetics of the efflux pump inhibitor D-ornithine-D-homophenylalanine-3-aminoquinoline (MC-02,595) have been prepared and evaluated for their ability to potentiate the activity of the fluoroquinolone levofloxacin in *Pseudomonas aeruginosa*. A number of the new analogues were as active or more active than the lead, demonstrating that a peptide backbone is not essential for activity.

Efflux mechanisms are increasingly recognized as causing resistance to many classes of antibacterial agents. While certain pumps selectively extrude specific agents, others, referred to as multidrug resistance (MDR) pumps, expel a variety of compounds, regardless of their structural class or mechanism of action.[1] Organisms such as *Pseudomonas aeruginosa* are increasingly refractory to available therapies in the clinic primarily because of their ability to develop high-level MDR due to an efflux mechanism.[2] Therefore, inhibition of efflux pumps may be an attractive avenue for improving the clinical activity of antibacterial agents that are substrates of such pumps.[1–4]

We recently reported the identification of a class of agents that potentiate the activity of the fluoroquinolone levofloxacin (LVFX).[5] These compounds function as efflux pump inhibitors (EPIs) in *P. aeruginosa*.[6] Although the original series of EPIs was unstable in several biological matrices such as mouse, rat, and human serum, we identified a class of stable, structurally related inhibitors, such as D-ornithine-D-homophenylalanine-3-aminoquinoline (MC-02,595, **1**; Chart 1), that maintained all of the favorable biological features of the original series.[7]

Based upon these results, **1** became the new lead in our program to explore the SAR of the EPIs. Since this series is based on a peptide backbone, we were interested in evaluating analogues in which this feature was modified. To this end we synthesized a variety of peptidomimetics of **1** and evaluated their ability to potentiate LVFX against *P. aeruginosa*. The present report highlights the preparation and activity of a representative sub-set of the scaffolds prepared for this study.

Schemes 1–5 illustrate the syntheses of the compounds prepared for this study. The analogues were assayed in the presence and absence of LVFX against PAM 1032, a laboratory strain of *P. aeruginosa* that overexpresses the MexAB-OprM pump.[8] As a means of tracking potentiation trends, the activity of the inhibitors was quantified by the term MPC_8. This value reports the minimum concentration (μg/mL) of inhibitor required to decrease (potentiate) the MIC of LVFX 8-fold. The MIC (μg/mL) of each compound was also determined to ensure that the potentiation effect observed was not due to intrinsic antibacterial activity of the putative

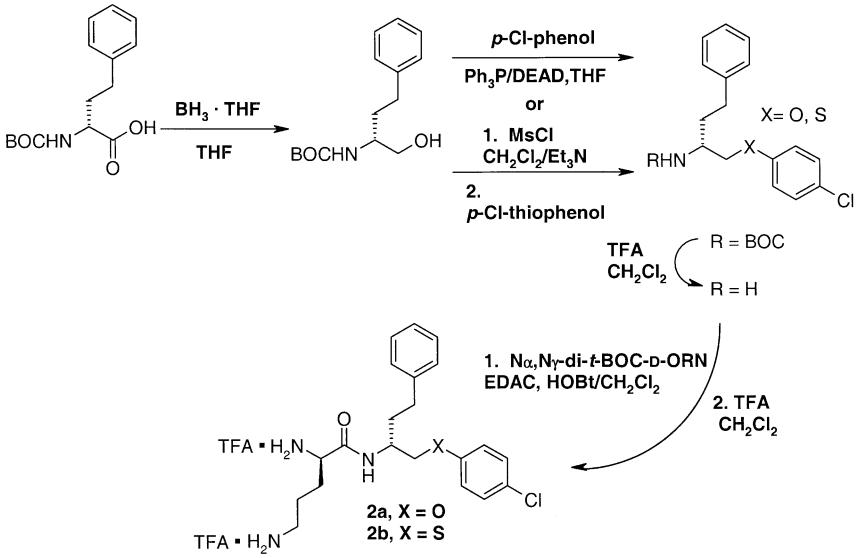


D-Orn-D-hPhe-3-NHQ (1, MC-02,595)

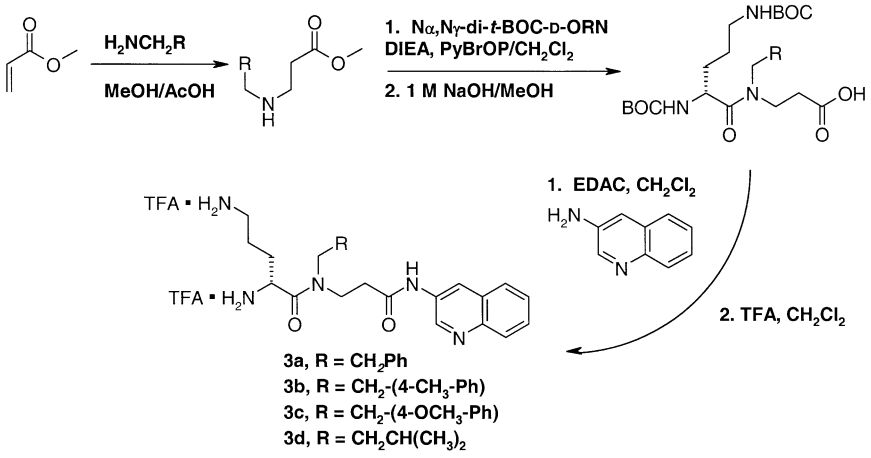
Chart 1.

*Corresponding author. Fax: +1-650-428-3550; e-mail: trenau@etrx.com

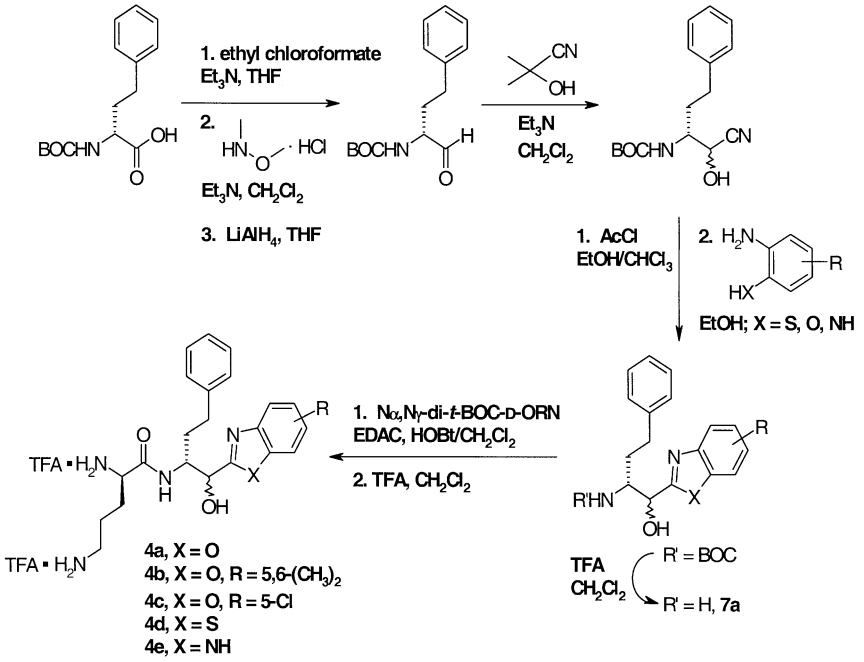
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00006-9



Scheme 1. Synthesis of the ether and thioethers **2a**–**2b**.



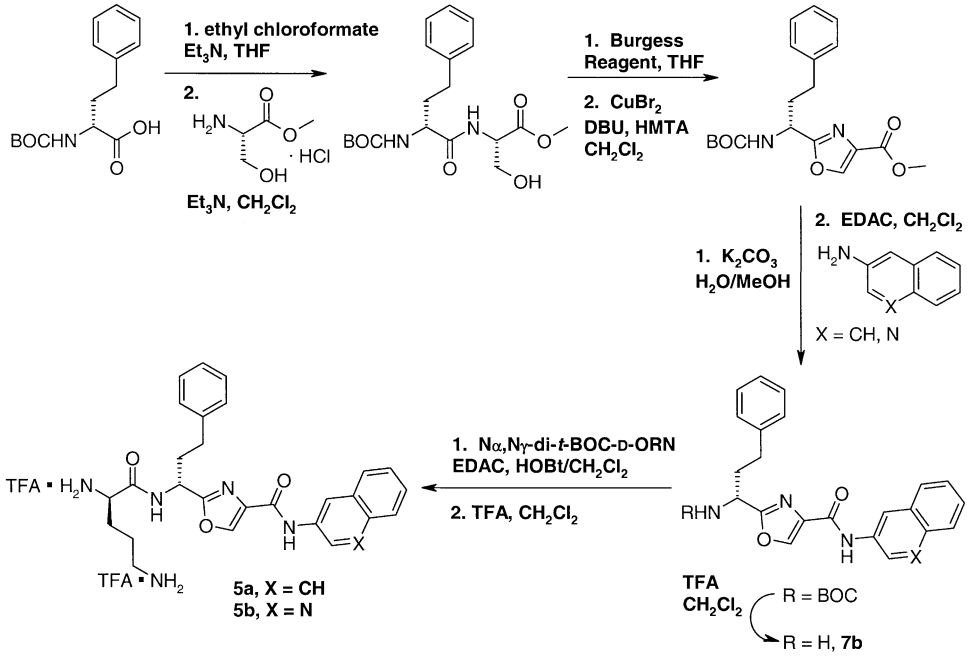
Scheme 2. Preparation of the tertiary amides **3a**–**3d**.



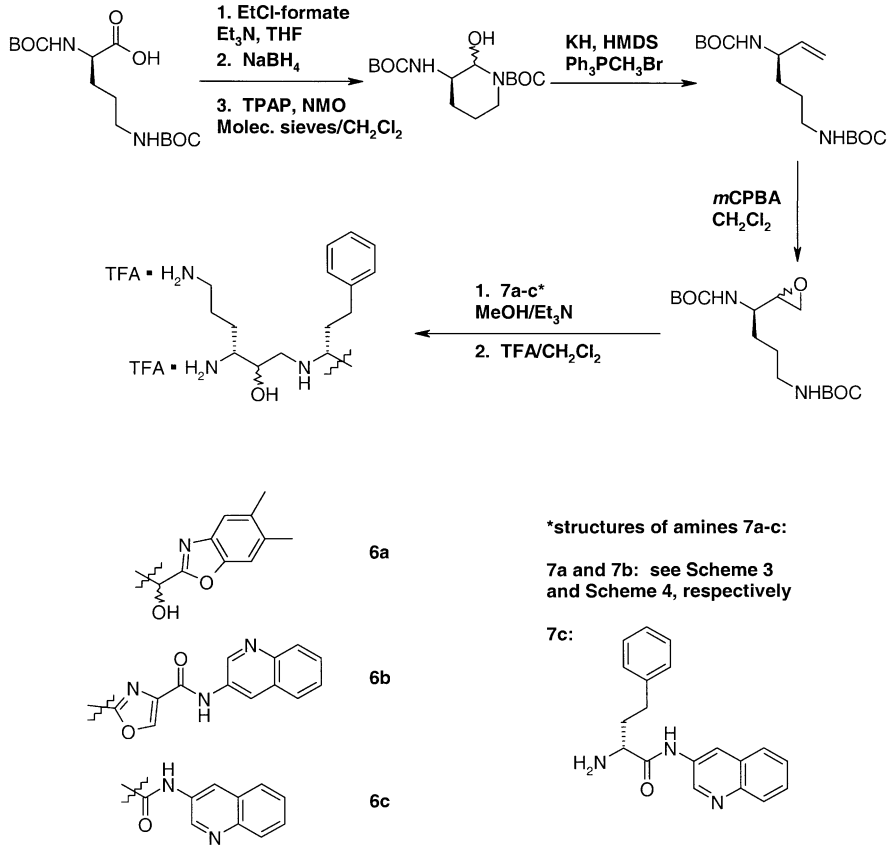
Scheme 3. Synthesis of analogues **4a**–**4e**.

EPI. A more complete description of the methods used to monitor biological activity can be found in references 6 and 8.

The ether and thioether analogues **2a**–**2b** (Scheme 1) were prepared from the key intermediate *N*-Boc-D-homophenylalaninol, synthesized via borane reduction of the corresponding protected aromatic acid. Both compounds enhanced (potentiated) the MIC of LVFX with MPC_8s as low as 1.25 μg/mL (Table 1). Although the thioether **2b** displayed more intrinsic antibacterial activity than the ether **2a** (64 μg/mL vs 128 μg/mL, respectively), the overall selectivity (MIC/MPC_8) of **2b** was greater than **2a**.



Scheme 4. Synthesis of the oxazole derivatives **5a**–**5b**.



Scheme 5. Preparation of analogues **6a**–**6c**.

Table 1. Intrinsic antibacterial activity and potentiation activity of the peptidomimetic EPIs against PAM 1032[a]

Compd	MIC[b] (μg/mL)	MPC_8[c] (μg/mL)
1	>512	10
2a	128	5
2b	64	1.25
3a	>512	10
3b	256	5
3c	512	10
3d	>512	10
4a	>512	10
4b	128	2.5
4c	256	5
4d	512	20
4e	>512	20
5a	16	2.5
5b	128	5
6a	128	1.25
6b	64	5
6c	128	2.5

[a]Compounds were evaluated in PAM 1032, a strain of *Pseudomonas aeruginosa* derived from PA01 that over-expresses the MexAB-OprM efflux pump.[6,8]
[b]MIC: minimum concentration (μg/mL) of efflux pump inhibitor required to inhibit the growth of PAM 1032.
[c]MPC_8: minimum concentration (μg/mL) of efflux pump inhibitor required to reduce (potentiate) the MIC of levofloxacin 8-fold.

The tertiary amides **3a**–**3d** (Scheme 2), which lack one of the two stereocenters, were readily accessible from methyl acrylate and provided an opportunity to incorporate various structurally diverse building blocks that could not otherwise be prepared from commercially available amino acids. The aliphatic analogue **3d** was roughly as potent as the aromatic derivatives **3a**–**3c** (Table 1). In general, compounds from this class displayed a good separation between potentiation activity and antibacterial activity.

Various benzoxazole, benzothiazole, and benzimidazole derivatives were prepared via the modification of a literature procedure.[9] Scheme 3 outlines the general route for the preparation of a representative collection of these analogues. The key cyanohydrin intermediate was derived from the aldehyde of *N*-Boc-D-homophenylalanine, which was prepared by reduction of the corresponding Weinreb amide. Whereas the benzoxazole analogues **4a**–**4c** displayed good potency, the benzimidazole and benzothiazole analogues (**4d**–**4e**) were less active than the lead, **1** (Table 1).

Analogues **5a**–**5b** (Scheme 4) were prepared via the serine derivative, which, when treated with Burgess' reagent, dehydrated to furnish the oxazoline.[10] Oxidation of this intermediate with $CuBr_2$ afforded the oxazole,[11] which was further elaborated to furnish the desired compounds. The quinoline substituted analogue **5b** displayed similar activity but considerably less antibacterial activity than its partner, **5a** (Table 1).

The hydroxyethylamino derivatives **6a**–**6c** were prepared from a key epoxide derivative (Scheme 5), which was prepared by oxidation of the corresponding olefin.[12,13] The alkene, in turn, was prepared by Wittig olefination of the aldehyde of *N*,*N*′-di-Boc-D-ornithine, isolated as the di-protected hemiaminal. The epoxide, when treated with various amines (whose syntheses are described in Schemes 3 and 4) afforded the analogues **6a**–**6c.** These derivatives, which incorporate the structural features found in **1** and compounds **4**–**5**, had MPC_8s as low as 1.25 μg/mL (Table 1).

The results for compounds **2**–**6**, when viewed collectively, demonstrate that a peptide backbone is not essential for the potentiation activity of this class of compounds. They suggest that a di-cationic compound, with an appropriate lipophilicity range, coupled with an overall topology comparable to that of MC-02,595 appear to be features that lead to the inhibition of MDR pumps in *P. aeruginosa*. Efforts are underway to substantiate these predictions.

Acknowledgements

This work was principally funded by Daiichi Pharmaceuticals, Ltd. The authors thank Scott Hecker, William Watkins, Dean Boojamra and Bret Benton for their review of the manuscript.

References and Notes

1. Lomovaskaya, O.; Watkins, W. *J. Mol. Microbiol. Biotechnol.* **2001**, *3*, 225.
2. Poole, K. *J. Mol. Microbiol. Biotechnol.* **2001**, *3*, 255.
3. Ryan, B. N.; Dougherty, T. J.; Beaulieu, D.; Chuang, J.; Dougherty, B. A.; Barrett, J. F. *Exp. Opin. Invest. Drugs* **2001**, *10*, 1409.
4. Guz, N. R.; Stermitz, F. R.; Johnson, J. B.; Beeson, T. D.; Willen, S.; Hsiang, J.-F.; Lewis, K. *J. Med. Chem.* **2001**, *44*, 261.
5. Renau, T. E.; Léger, R.; Flamme, E. M.; Sangalang, J.; She, M. W.; Yen, R.; Gannon, C. L.; Griffith, D.; Chamberland, S.; Lomovskaya, O.; Hecker, S. J.; Lee, V. J.; Ohta, T.; Nakayama, K. *J. Med. Chem.* **1999**, *42*, 4928.
6. Lomovskaya, O.; Warren, M. S.; Lee, A.; Galazzo, J.; Fronko, R.; Lee, M.; Blais, J.; Cho, D.; Chamberland, S.; Renau, T.; Léger, R.; Hecker, S.; Watkins, W.; Hoshino, K.; Ishida, H.; Lee, V. *Antimicrob. Agents Chemother.* **2001**, *45*, 105.
7. Renau, T. E.; Léger, R.; Flamme, E. M.; She, M. W.; Gannon, C. L.; Mathias, K. M.; Lomovskaya, O.; Chamberland; Lee, V. J.; Ohta, T.; Nakayama, K.; Ishiada, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 663.
8. Lomovskaya, O.; Lee, A.; Hoshino, K.; Ishida, H.; Mistry, A.; Warren, M. S.; Boyer, E.; Chamberland, S.; Lee, V. J. *Antimicrob. Agents Chemother.* **1999**, *43*, 1340.
9. Edwards, P. D.; Meyer, E. F., Jr.; Vijayalakshmi, J.; Tuthill, P. A.; Andisk, D. A.; Gomes, B.; Strimpler, A. *J. Am. Chem. Soc.* **1992**, *114*, 1854.
10. Hu, J.; Miller, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 3462.
11. Pihko, P. M.; Koskinen, A. M. P.; Nissinen, M. J.; Rissanen, K. *J. Org. Chem.* **1999**, *64*, 652.
12. Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487.
13. Rich, D. H.; Sun, C.-Q.; Prasad, J. V. N. V.; Pathiasseril, A.; Toth, M. V.; Marshall, G. R.; Clare, M.; Mueller, R. A.; Houseman, K. *J. Med. Chem.* **1991**, *34*, 1222.