ORGANIC LETTERS

1999
Vol. 1, No. 10
1571−1573

(*R*)- and (*S*)-3-Fluorothalidomides: Isosteric Analogues of Thalidomide

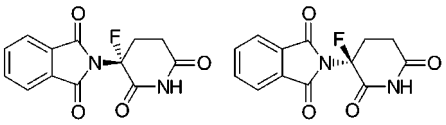
Yoshio Takeuchi,* Tomoki Shiragami, Kenichi Kimura, Emiko Suzuki, and Norio Shibata

Faculty of Pharmaceutical Sciences, Toyama Medical & Pharmaceutical University, Sugitani 2630, Toyama, 930-0194 Japan

takeuchi@ms.toyama-mpu.ac.jp

Received August 5, 1999

ABSTRACT



(3*R*)-fluorothlidomide (3*S*)-fluorothalidomide

3-Fluorothalidomide, a nonracemizable isosteric analogue of thalidomide, was successfully prepared by perchloryl fluoride fluorination of a 3-phthalimidopiperidin-2-one derivative followed by RuO_2 oxidation. In the preliminary biological evaluation of (*R*)- and (*S*)-enantiomers, it was shown that the (*S*)-isomer was found to be more active than both the (*R*)-isomer and the racemic thalidomide in lipopolysaccharide-induced TNF-α production enhancement produced from human peripheral blood lymphocytes cultivated in vitro.

Thalidomide (**1**) was first marketed in several countries as a clinically effective sedative hypnotic in 1956. Unexpected potent teratogenic side effects, leading to birth defects such as limb reduction, produced one of the most notorious medical disasters of modern medical history. Thalidomide was withdrawn from the market in 1962.[1] However, the strong teratogenicity notwithstanding, the unique and broad physiological effects of thalidomide[2] have prompted recent reevaluation of its medical applications.[3] Thus, thalidomide was recently approved in the USA for the treatment of a painful inflammation associated with leprosy.[4]

Thalidomide is marketed as a racemic mixture. Although it has been suggested that the sedative hypnotic effect is associated with the (*R*)-isomer of **1** and that the teratogenic effects are associated with the (*S*)-isomer,[5] recent reports show that the strongly acidic hydrogen atom at the asymmetric center of thalidomide rapidly epimerizes under physiological conditions,[6] rendering bioassay of enantiomers difficult. In strategies designed to develop drugs that might have the beneficial effects of thalidomide without the teratogenic side effects, synthesis of novel nonracemizable analogues of thalidomide has attracted much attention. In one approach, 3-methylthalidomide (**2**) was prepared.[7] Examination of the biological activity of optically active **2** by Knabe[8] and Hashimoto[9] revealed clear differences between the enantiomers. For example, while only (*S*)-**2** inhibits tumor necrosis factor-α (TNF-α) production in the 12-*O*-tetradecanoylphorbol 13 acetate/human leukemia HL-60 assay system, (*R*)-**2** is more active than (*S*)-**2** in the okadaic acid/HL-60 assay system.[9] Although the methyl group of **2**

(1) (a) McBride, W. G. *Lancet* **1961**, *2*, 1358. (b) Lenz, W. *Lancet* **1962**, *1*, 45.
(2) (a) Gunzler, V. *Drug Safety* **1992**, *7*, 116. (b) Sheskin, *J. Clin. Pharmacol. Ther*. **1965**, *6*, 303. (c) Makonkawkeyoon, S.; Limson-Pobre, R. N. R.; Moreira, A. L.; Schauf, V.; Kaplan, G. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5974.
(3) (a) Randall, T. *J. Am. Med. Assoc*. **1990**, *263*, 1467. (b) Skolnick, A. *J. Am. Med. Assoc*. **1990**, *263*, 1468. (c) Randall, T. *J. Am. Med. Assoc*. **1990**, *263*, 1474. (d) Muller, G. W. *Chemtech* **1997**, *27*, 21.
(4) *Chem. Ind.* **1998**, 591.
(5) (a) Wintersk, W.; Frankus, E. *Lancet* **1992**, *339*, 365. (b) Eriksson, T.; Bjorkman, S.; Roth, B.; Fyge, A.; Hoglund, P. *Chirality* **1996**, *7*, 44. (c) Shealy, Y. E.; Opliger, C. E.; Montgomery, J. A. *Chem. Ind.* **1965**, 1030.
(6) (a) Wnendt, S.; Finkam, M.; Winter, W.; Ossing, J.; Rabbe, G.; Zwingenberger, K. *Chirality* **1996**, *8*, 390. (b) Knoche, B.; Blaschke, G. *J. Chromatogr*. **1994**, *2*, 183. (c) Winter, W.; Frankus, E. *Lancet* **1992**, *339*, 365. (d) Nishimura, K.; Hashimoto, Y.; Iwasaki, S.; *Biochem. Biophys. Res. Commun*. **1994**, *199*, 455.
(7) Blaschke, G.; Graute, W. F. *Liebigs Ann. Chem*. **1987**, 647.
(8) Beuch, H. P.; Omlor, G.; Knabe, J. *Arzneim. Forsch*. **1990**, *40*, 32.
(9) (a) Nishimura, K.; Hashimoto, Y.; Iwasaki, S. *Chem. Pharm. Bull*. **1994**, *42*, 1157. (b) Miyachi, H.; Azuma, A.; Hiroki, E.; Iwasaki, S.; Kobayashi, Y.; Hashimoto, Y. *Biochem. Biophys. Res. Comm*. **1996**, *226*, 439.

10.1021/ol9902172 CCC: $18.00
Published on Web 10/10/1999

effectively blocks racemization as a matter of course, the methyl group also introduces substantial steric alterations into the molecule, alterations that could be expected to significantly modulate biological activity. Indeed, the sedative effect of **2** is weaker than that of **1**, and the TNF-α production-inhibiting activity of **2** is much stronger than that of **1**. As a result of our research designed to produce an analogue of thalidomide that is resistant to reacemization and is also a close structural mimic, we describe in this paper the design and synthesis of 3-fluorothalidomide (**3**), an isosteric analogue of thalidomide (Figure 1).[10]

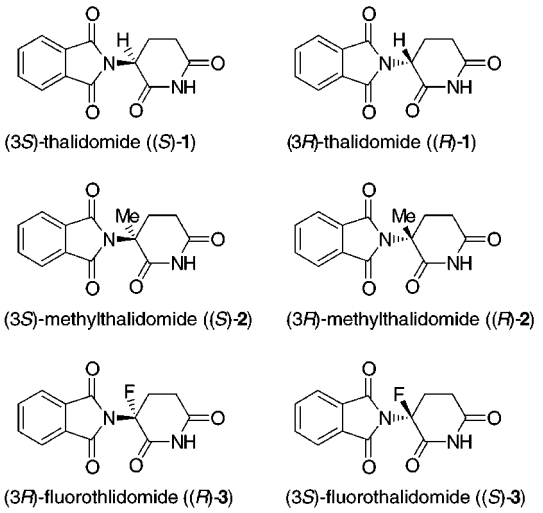
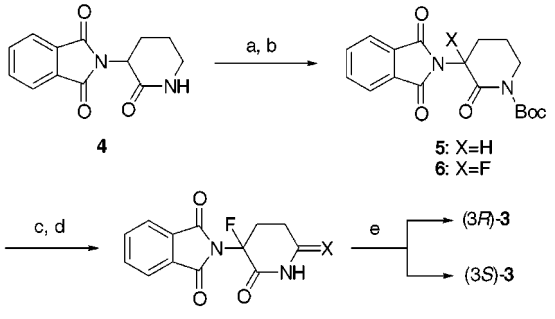


Figure 1.

The use of fluorine substitution in our approach has many precedents. Because the flourine atom is the smallest atom other than hydrogen, flourine is the best possible atom to substite for hydrogen in designing isosteric analogues.[11] High electronegativity and a strong C−F bond are added factors that have made the introduction of fluorine into biologically active compounds a very effective approach for developing new drugs.[12] In our example, the resistance of the enantiomers of 3-fluorothalidomide (**3**) to racemization and the close structural similarity to thalidomide itself render **3** an excellent candidate in the search for safe thalidomide-based drugs in which the sedative effects of **1** can be clearly separated from teratogenicity.

Attempted direct fluorination of thalidomide (**1**) or *N*-phthalylglutamic anhydride under various conditions did not give the corresponding fluorinated product. However, the preparation of **3** was finally carried out efficiently as follows: *N*-*tert*-butoxycarobonyl-3-phthalimidopiperidin-2-one (**5**), prepared from the readily available 3-phthalimidyl-piperidin-2-one (**4**)[13] by treatment with Boc_2O in acetonitrile, was deprotonated with 1.2 equiv of LiHMDS in THF at −40 °C (Scheme 1). Introduction of an excess of diluted perchloryl fluoride[14] gave fluorinated compound **6** in 71% yield. The Boc group of **6** was then removed by TFA treatment at room temperature to furnish **7**. Finally, oxidation[15] of **7** was

Scheme 1



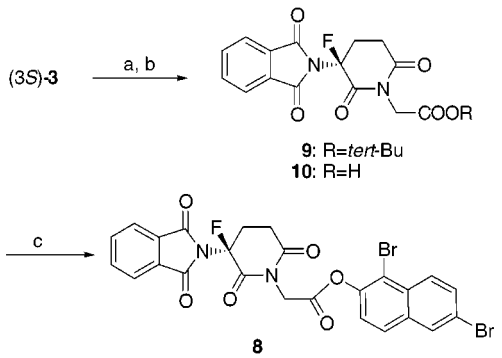
Reagents: a, Boc_2O, DMAP, acetonitrile, rt (92%); b, LiHMDS, $FClO_3$, THF, −40°C (71%) ; c, TFA / CH_2Cl_2 , rt (quant.); d, RuO_2, $NaIO_4$, AcOEt, CH_2Cl_2, H_2O (90%); e, HPLC separation by Daicel Chiralcel AD eluting with EtOH

performed using a catalytic amount of RuO_2 in the presence of excess sodium metaperiodate in a two-phase system[16] to give target compound **3** in 90% yield from **6**.

Optical resolution of racemic **3** by HPLC using Daicel Chiralcel AD (25 mm × 300 mm), eluting with ethanol, afforded as the first eluted isomer (t_R = 40 min), (*R*)-**3** with $[\alpha]^{27}_D$ = +257 ° (*c* 1.11, DMF), mp 237−241 °C, and as the second eluted isomer (t_R = 70 min) (*S*)-**3** with $[\alpha]^{27}_D$ = −263 ° (*c* 1.18, DMF), mp 237−240 °C. Each enantiomer was obtained with an optical purity of more than 99% ee.

The absolute stereochemistry of **3** was determined unambiguously by single-crystal X-ray crystallography[17] of the corresponding ester (*S*)-**8** (Scheme 2). This was prepared

Scheme 2



Reagents: a, *tert*-butyl bromoacetate, K_2CO_3, DMF, rt (quant.); b, TFA , CH_2Cl_2, rt (quant.); c, 1,6-dibromo-2-naphthol, DCC, DMAP, CH_2Cl_2, rt (quant.)

from the second eluted isomer of **3** by treatment with *tert*-butyl bromoacetate followed by removal of *tert*-butyl group and subsequent DCC-mediated coupling with 1,6-dibromo-2-naphthol.

In summary, we have synthesized the configurationally stable enantiomers of 3-fluorothalidomide (**3**), an isosteric

analogue of thalidomide. Although a variety of nonracemizable or difficultly racemizable analogues of thalidomide have been prepared,[7−9] our compound **3** is the closest structural mimic to thalidomide yet reported. In initial biological evaluation, (*S*)-**3** appears to be more active than both (*R*)-**3** and (±)-**1** in lipopolysaccharide (LPS) induced TNF-α production enhancement produced from human peripheral blood lymphocytes cultivated in vitro (Figure 2).[18−20] Teratogenicity of (*R*)-**3** and (*S*)-**3** has not been

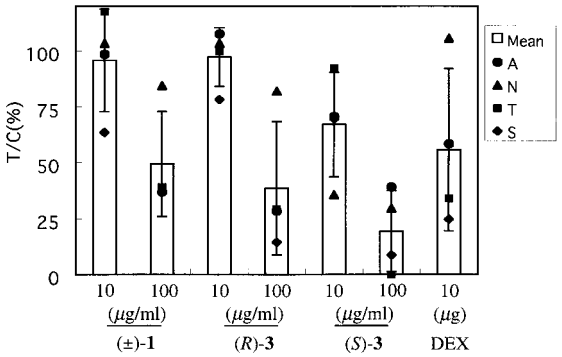


Figure 2. Suppressive effect of thalidomide analogues on LPS-induced TNF-α production human PBL in vitro. Levels of TNF-α in the condition medium prepared were measured by a commercially available ELISA kit (see Supporting Information). Results (individual data and mean and its standard derivation data) are expressed as relative to the control (T/C%). The amounts of TNF-α from control: A, 793.5 pg/mL; N, 751.1 pg/mL; T, 449.4 pg/mL; S, 867.4 pg/mL; DEX, dexamethasone.

measured yet because sufficient enantiomerically pure **3** has not yet been accumulated to permit animal experiments. However, we hope 3-fluorothalidomide (**3**) will be a key compound both to elucidate the mechanism for the teratogenicity of **1** and to determine if pure enantiomers can lead to safe thalidomide-based drugs. A large scale asymmetric synthesis of optically pure **3** for animal experiments is now in progress. Finally, we note that the strategy for fluorine substitution of hydrogen at an asymmetric center of biologically active compounds represents a novel and general approach for designing nonracemizable analogues of the parent molecules.

Acknowledgment. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan. N.S. wishes to thank the Kowa Life Science Foundation for support. We thank Mr. Toshihiko Akimoto (Daiichi Pharmaceutical Co. Ltd.) for assistance with assay of TNF-α produced from human peripheral blood lymphocytes.

Supporting Information Available: Full experimental procedures for synthesis of all new compounds and X-ray crystallographic analysis of **8** and a protocol and results of inhibition studies of TNF-α production by (±)-**1**, (*R*)-**3**, and (*S*)-**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL9902172

(10) For example; (a) Muller, G. W.; Chen, R.; Huang, S.-Y.; Corral, L. G.; Wong, L. M.; Patterson, R. T.; Chen, Y.; Kaplan, G.; Stirling, D. I. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 1625. (b) Miyachi, H.; Azuma, A.; Ogasawara, A.; Uchimura, E.; Watanabe, N.; Kobayashi, Y.; Kato, F.; Kato, M.; Hashimoto, Y. *J. Med. Chem*. **1997**, *40*, 2858. (c) Muller, G. W.; Corral, L. G.; Shire, M. G.; Wang, H.; Moreira, A.; Kaplan, G.; Stirling, D. I. *J. Med. Chem*. **1996**, *39*, 3238. (d) Turk, B. E.; Jiang, H.; Liu, J. O. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 7552. (e) Niwayama, S.; Turk, B. E.; Liu, J. O. *J. Med. Chem*. **1996**, *39*, 3044. (f) Corral, L. G.; Muller, G. W.; Moreiram. A.; Chen, Y.; Wu, Mingdan; Stirling, D. I.; Kaplan, G. *Mol. Med.* **1996**, *2*, 506.

(11) Fusso & Yakugaku; Kobayashi, Y., Kumadaki, I., Taguchi, T., Eds.; Hirokawa Publishing Co.: Tokyo, 1992.

(12) (a) Biomedical Aspects of Fluorine Chemistry; Filler, R., Kobayashi, Y., Eds.; Kodansha/Elsevier Biomedical Press: Tokyo, Amsterdam-New York-Oxford, 1982. (b) *Biomedical Frontiers of Fluorine Chemistry* (ACS Symposium Series 639); Ojima, I., McCarthy, J. R., Welch, J. T., Eds.; American Chemical Society: Washington, DC, 1996.

(13) (a) Oklobdzija, M.; Comisso, G.; Decorte, E.; fajdiga, T.; Gratton, G.; Moimas, F.; Toso, R.; Sunjic, V. *J. Heterocycl. Chem*. **1983**, *20*, 1329. (b) Belyaev, A. A.; Krasko, E. V. *Synthesis* **1991**, 417.

(14) (a) Gensler, W. J.; Ahmed, Q. A.; Leeding, M. V. *J. Org. Chem*. **1968**, *33*, 4279. (b) Sharts, C. M.; Sheppard W. A. *Org. React*. **1974**, *21*, 125. (c) Barth-Wehrenap, G. *J. Inorg. Nucl. Chem*. **1956**, *2*, 266. (d) Leech, H. R. *Chem. Ind*. **1960**, 242. (e) Takeuchi, Y.; Nagata, K.; Koizumi, T. *J. Org. Chem.* **1989**, *54*, 3.

(15) Attempted oxidation of **7** by the use of *m*-CPBA met failure.

(16) (a) Sheehan, J. C.; Tulis, R. W. *J. Org. Chem*. **1974**, *39*, 2264. (b) Tanaka, K.; Yoshifuji, S.; Nitta, Y. *Chem. Pharm. Bull*. **1987**, *35*, 364.

(17) X-ray data for (*S*)-**8** is available in the Supporting Information: $C_{25}H_{15}Br_2FN_2O_6$; crystal size, 0.2 × 0.08 × 0.03 mm; $M_r = 618.21$; triclinic; space group *P*1, $a = 10.0383(15)$ Å, $b = 11.7550(12)$ Å; $c = 5.2155(19)$ Å; $\alpha = 96.067(16)°$; $\beta = 95.938(21)°$; $\gamma = 75.485(10)°$; $V = 590.55(21)$ Å^3; $Z = 1$; $R[I > 3.00\sigma(I)] = 0.031$; $R_w = 0.044$; temp = 23 °C.

(18) The assay was carried out in accordance with Kaplans' procedure (ref 19). See the Supporting Information.

(19) (a) Sampaio, E. P.; Sarno, E. N.; Galilly, R.; Cohn, Z. A.; Kaplan, G. *J. Exp. Med*. **1991**, *173*, 699. (b) Moreira, A. L.; Sampaio, E. P.; Zmuidzinas, A.; Frindt, P.; Smith, K. A.; Kaplan, G. *J. Exp. Med*. **1993**, *177*, 1675.

(20) Since the difference in the activities between individual enantiomers may not be significant, biological activities in other assay systems are being determined.