Solution-phase Synthesis and Preliminary Evaluation of 1,6,8-Trisubstituted Tetrahydro-2H-pyrazino[1,2-a]pyrimidin-4,7-dione Derivatives as a NF-kB Inhibitor

Jin-Woong Kim, Su-Chul Lee,† Hyeong Beom Park, Nam Hyun Jo, Kyung Ho Yoo, Jung-Hyuck Cho, and Chang-Hyun Oh*

Medicinal Chemistry Research Center, Korea Institute of Science and Technology, Seoul 130-650, Korea
*E-mail: choh@kist.re.kr
†Hawon Pharm. Co., Seoul 135-080, Korea
Received July 18, 2006

Key Words: Bicyclic β-turn mimetics, NF-kB inhibitor

To develop a potent form of NF-kB inhibitors, β-turn peptidomimetics with a new scaffold (1),1-6 as shown in Figure 1 were designed.

Previously,7 we reported the synthesis and structure-activity relationships of new 1,6,8-trisubstituted tetrahydro-2H-pyrazino[1,2-a]pyrimidin-4,7-dione derivatives to find the correlation between the polarity of the C-6 substituent and the inhibitory activity. However, we failed to introduce the carboxylic acid group at the C-6 position by solid phase method.

In this study, to investigate the effect of the carboxylic acid moiety at C-6 position of the bicyclic ring, bicyclic β-turn mimetics 7a-g were synthesized using solution phase, and their NF-kB inhibitory activities are discussed.

Chemistry

The β-turn mimetics were prepared from solution-phase synthesis, according to our previous solid-phase synthetic protocol.7 Benzaldehyde (1) was reacted with aminoacet-aldehyde dimethyl acetal, and subsequently treatment with sodium borohydride in MeOH gave the secondary amine 2, which was then coupled with the cbz-Asp(Obut)-OH with HOBT/DIC in DMF to give 3. Deprotection of the Cbz group 3 by catalytic hydrogenation in EtOH gave the amine compound, which was then coupled with Cbz-β-alanine to afford 4. After cleavage of the Cbz group of 4 by catalytic hydrogenation, the resulting compound was treated with the p-nitrophenyl chloroformate in the presence of DIEA to produce 5. The urea type compounds 6a-g were accomplished by treatment of compound 5 with the corresponding amines.

Cleavage of the acetal of 6a-g followed by stereoselective tandem acyliminium cyclization by treatment with formic acid at room temperature was carried out to give the 6,6-bicyclic β-turn mimetics 7a-g. All final products were purified by preparative TLC (silica gel) to afford the pure products.

Biological studies

All new 1,6,8-trisubstituted tetrahydro-2H-pyrazino[1,2-a]pyrimidin-4,7-dione derivatives 7a-g subjected to preliminary in vitro NF-kB inhibitory activity screening8 exhibited different biological properties, depending on the kind of substituents at N-1 position of the main bicyclic system. According to the results assembled in Figure 2, compounds 7d and 7e, which contain the fluorobenzyl groups at N-1 position, exhibited slightly better activity than their methoxybenzyl group 7b and benzyl group 7a. Tested at a concentration of 10 μM, both compounds showed a 40% inhibition against the target NFkB 549. The compounds 7a-g, having a carboxylic acid group at C-6 position, showed slight differences to their isobutyl group 7a*-g*.

We found that introduction of carboxylic acid at the C-6 position of bicyclic β-turn mimetics did not affect biological activity compared with the alkyl group. It is of interest to investigate the fluoro substituent and this is in progress.

Summary

The solution-phase synthesis of a new series of 1,6,8-trisubstituted tetrahydro-2H-pyrazino[1,2-a]pyrimidin-4,7-diones as bicyclic β-turn mimetics is described herewith. Their NF-kB inhibitory activities were tested and the effect of substituents of the bicyclic ring was investigated. Among these compounds, 7d and 7e showed the most potent activity.

Experimental Part

Melting point (mp): Thomas Hoover apparatus, uncorrected. 1H NMR spectra: Varian Gemini 300 spectrometer, tetra-
methylsilane (TMS), as an internal standard. The mass spectrometry system was based on a HP5989A MS Engine (Palo Alto, CA, USA). IR spectra: Perkin Elmer 16F-PC FT-IR.

N-(2,2-Dimethoxyethyl)benzylamine (2). To a stirred solution of aminoacetaldehyde dimethyl acetal (48.8 mmol, 5 mL) in dry toluene (60 mL) was added dropwise benzaldehyde (1, 48.8 mmol, 4.9 mL) and the reaction mixture was stirred for 3 h at 80 °C. Evaporation of the solvent in vacuo gave a crude residue, which was dissolved with MeOH (50 mL). To the resulting solution was added dropwise NaBH₄ (51.8 mmol, 2.0 g) at 0 °C and was stirred for 24 h at room temperature. The mixture was diluted with H₂O (40 mL), 1N-HCl and ethyl acetate (100 mL). The organic layer was dried over anhydrous Na₂SO₄, concentrated, and the resulting residue was purified by silica gel column chromatography with EtOAc/hexane (1 : 1.5) to give 2 (8.8 g, 92%) as a pale yellow oil. ¹H-NMR (CDCl₃) δ 2.76 (2H, d, J = 5.4 Hz), 3.37 (6H, s), 3.82 (2H, s), 4.50 (1H, t, J = 5.4 Hz), 7.37 (5H, m).

N-Benzyl-N-(2,2-dimethoxyethyl)-3-benzyloxycarbonyl-aminosuccinamic acid t-butyl ester (3). A solution of Cbz-Asp(OBut)-OH (5.6 mmol, 1.80 g), HOBT (5.6 mmol, 0.86 g), DIC (5.6 mmol, 0.9 mL) in dry-DMF (20 mL) was added to the solution of 2 (5.1 mmol, 1.0 g) in dry-DMF (20 mL) at room temperature and was stirred for 12 h at same temperature. The reaction mixture was poured into cold water and extracted with ethyl acetate. The organic layer was successively washed with water and dried over anhydrous Na₂SO₄. Evaporation of the solvent in vacuo gave a crude residue, resulting in a white solid.

Scheme 1. i) Aminoacetaldehyde dimethyl acetal, toluene; ii) NaBH₄, MeOH; iii) Cbz-ASP (OBut)-OH, 1,3-diisopropylcarbodiimide, DMF; iv) 10% Pd/C, THF:EtOH = 1 : 1; v) Cbz-b-Ala-OH, HOBT, DMF; 10% Pd/C, THF:EtOH = 1 : 1; vii) p-Nitrophenyl chloroformate, N,N-diisopropylethyl amine, CH₂Cl₂; THF = 1 : 1; viii) Corresponding amines, CH₂Cl₂; ix) Formic acid


Figure 2. In vitro NFkB A549 inhibitory activity of 7a-g and 7a*-g*.
which was purified by silica gel column chromatography with EtOAc/hexane (1:4) to give 3 (2.1 g, 70%) as a pale yellow oil. 3H-NMR (CDCl3) δ 0.85 (3H, dd, J = 6.6 and 13.8 Hz), 0.99 (3H, dd, J = 6.6 and 16.5 Hz), 1.32 (1H, m), 1.68 (2H, m), 3.37 (6H, m), 3.56 (2H, m), 4.57 (1H, t, J = 5.2 Hz), 4.76 (2H, s), 4.94 (1H, m), 5.10 (2H, d, J = 7.5 Hz), 7.27 (10H, m).

N-Benzyl-N-(2,2-dimethoxyethyl)-3-(3-benzyloxycarbonylamino)propionylaminosuccinic acid t-butyI ester (4). Compound 3 (13.4 mmol, 6.7 g) and 1.5 g of Pd/C (10%) were dissolved in THF and was hydrogenated at 50 psi for 2 h. The solution was filtered through celite and was evaporated to give a residue, which was used without further purification. A solution of Cbz-β-Ala-OMe (10.0 mmol, 3.13 g) and DIC (20.0 mmol, 3.13 mL) in dry CH2Cl2 (60 mL) was added slowly to the mixture and was stirred for 12 h at room temperature. The reaction mixture was neutralized with Et3N (20.6 mmol, 2.3 mL) and was stirred for 12 h at room temperature. The reaction mixture was concentrated in vacuo to give a residue, which was used without further purification.

N-Benzyl-N-(2,2-dimethoxyethyl)-3-(3-benzylureido)propionylaminosuccinic acid t-butyI ester (5). Compound 4 (11.2 mmol, 6.4 g) and 1.5 g of Pd/C (10%) were dissolved in THF and was hydrogenated at 50 psi for 2 h. The solution was filtered through celite and was evaporated to give a residue, which was used without further purification. A solution of Cbz-β-Ala-OMe (10.0 mmol, 3.13 g) and DIC (20.0 mmol, 3.13 mL) in dry CH2Cl2 (60 mL) was added slowly to the mixture and was stirred for 12 h at room temperature. The reaction mixture was neutralized with Et3N (20.6 mmol, 2.3 mL) and was stirred for 12 h at room temperature. The reaction mixture was filtered through celite and was evaporated to give a residue, which was used without further purification.

The synthesis of compounds 6b-g from 5 was carried out by the same procedure as described for the preparation of 6a.
486.1715, Found (M+) 486.1717.

7f: Yield 40%. 1H-NMR (CDCl3) δ 1.78 (2H, m), 2.37 (2H, m), 2.70 (4H, m), 3.29 (2H, m), 3.53 (4H, m), 4.38 (3H, m), 5.33 (2H, m), 6.03 (1H, q, J = 2.3 Hz), 7.29 (5H, m). -HRMS (FAB) Calcd. for C21H26N4O5S 446.1624, Found (M+) 446.1630.

7g: Yield 38%. 1H-NMR (CDCl3) δ 1.80 (2H, m), 1.64 (4H, m), 2.48 (2H, m), 3.02 (2H, m), 3.34 (4H, m), 3.37 (4H, m), 4.35 (2H, m), 5.32 (1H, dd, J = 3.0 and 9.0 Hz), 6.01 (1H, q, J = 6.0 Hz), 7.27 (5H, m). -HRMS (FAB) Calcd. for C22H28N4O6 444.2009, Found (M+) 444.2003.

Acknowledgements. We would like to thank Dr. Kahn and Dr. Masa for their helpful discussions and providing biological data the duration of this work. Finally, we wish to thank Hawon Pharmaceuticals co. which was supported with fund.

References