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

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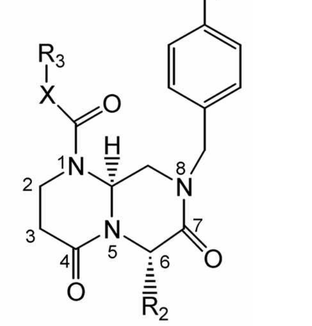
To develop a potent form of NF-kB inhibitors, β-turn peptidomimetics with a new scaffold (**1**),[1-6] as shown in Figure 1 were designed.

Previously,[7] we reported the synthesis and structure-activity relationships of new 1,6,8-trisubstituted tetrahydro-2*H*-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 aminoacetaldehyde 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



R_1 = OCH_3, -H, -F
R_2 = -Isopropyl, isobutyl
R_3 = Benzyl derivatives
X = O, S, N

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Figure 1

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-2*H*-pyrazino[1,2-*a*]pyrimidin-4,7-dione derivatives **7a-g** subjected to preliminary *in vitro* NF-kB inhibitory activity screening[8] 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***.

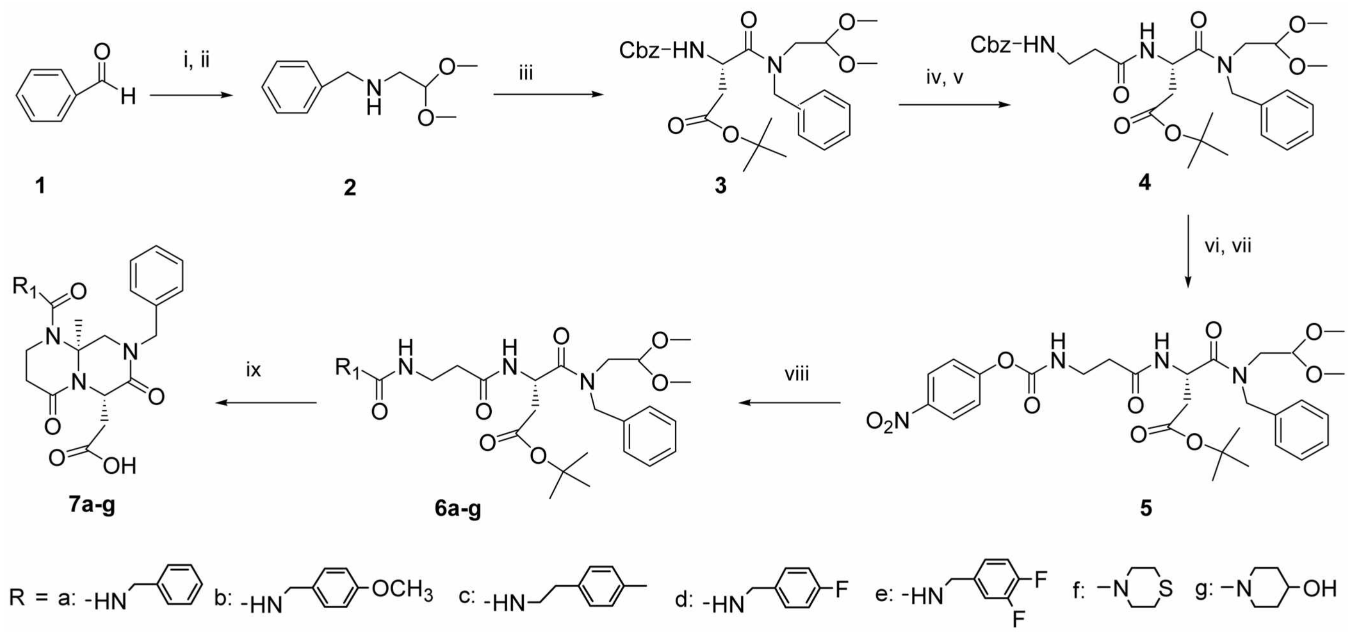
We found that introduction of carboxylic acid at the C-6 position of bicyclic β-turn mimetics did not affect biological activity compared with the alkyl group. It is of interest to investgate the fluoro substituent and this is in progress.

Summary

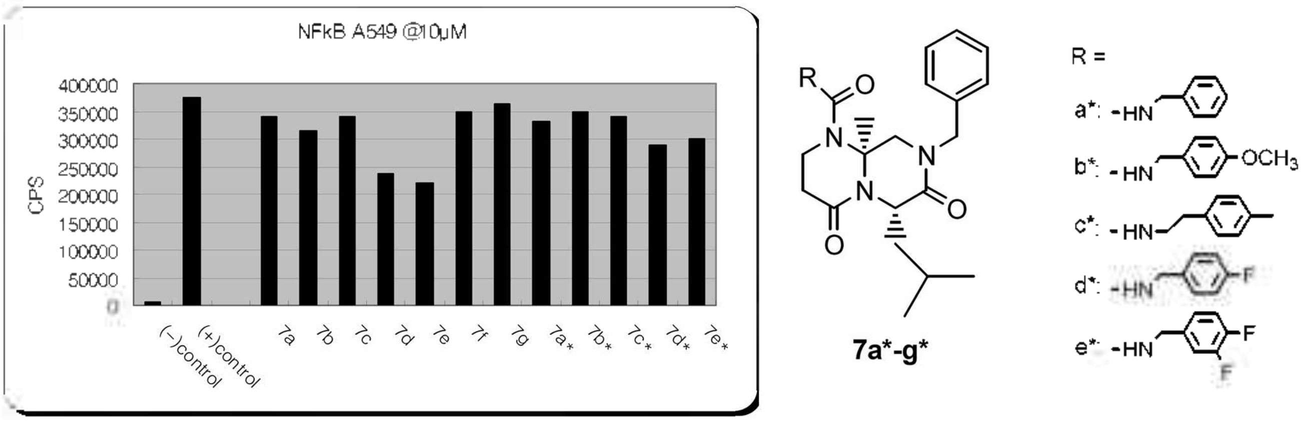
The solution-phase synthesis of a new series of 1,6,8-trisubstituted tetrahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-4,7-diones as bicyclic β-turn mimetics is described herewith. Their NF-kB inhibitory activities were tested and the effect of substituents of the bicyclic ring was investigated. Among these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

Experimental Part

Melting point (mp): Thomas Hoover apparatus, uncorrected. ^{1}H NMR spectra: Varian Gemini 300 spectrometer, tetra-



Scheme 1. i) Aminoacetaldehyde dimethyl acetal, toluene; ii) $NaBH_4$, 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, CH_2Cl_2:THF = 1 : 1; viii) Coresponding amines, CH_2Cl_2; ix) Formic acid



(-) control: None (+) control: phorbol myristate acetate NFkB A549@10μM

Figure 2. *In vitro* NFkB A549 inhibitory activity of **7a-g** and **7a*-g***.[8]

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 $NaBH_4$ (51.8 mmol, 2.0 g) at 0 °C and was stirred for 24 h at room temperature. The mixture was diluted with H_2O (40 mL), 1*N*-HCl and ethyl acetate (100 mL). The organic layer was dried over anhydrous Na_2SO_4, 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. ^{1}H-NMR ($CDCl_3$) 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 Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crude residue.

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. ^{1}H-NMR (CDCl$_3$) 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)propionylaminosuccinamic acid *t*-butyl 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-OH (20.0 mmol, 4.46 g), HOBT (20.0 mmol, 3.06 g) and DIC (20.0 mmol, 3.13 mL) in dry-DMF (20 mL) was added to the above solution 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 Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography with EtOAc/hexane (1 : 4) to give **4** (6.4 g, 83%) as a pale yellow oil. ^{1}H-NMR (CDCl$_3$) δ 1.40 (9H, d, J = 4.5 Hz), 1.64 (2H, m), 2.41 (2H, m), 3.36 (6H, m), 3.45 (2H, m), 3.57 (2H, m), 3.83 (1H, m), 4.50 (2H, m), 4.99 (1H, m), 5.08 (2H, s), 7.24 (10H, m).

***N*-Benzyl-*N*-(2,2-dimethoxyethyl)-3-(*p*-nitrophenoxycarbonylamino)propionylaminosuccinamic acid *t*-butyl 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. To above solution of triethylamine (20.6 mmol, 3.6 mL) in dry CH_2Cl_2 (60 mL) was added slowly *p*-nitrophenyl chloroformate (20.6 mmol, 4.3 g) at 0 °C and was stirred for 1 h at same temperature. The mixture was diluted with H_2O (30 mL), CH_2Cl_2 (50 mL), and the organic layer was dried over anhydrous $MgSO_4$. The organic solvent was concentrated *in vacuo* to give a residue, which was used without further purification.

***N*-Benzyl-*N*-(2,2-dimethoxyethyl)-3-(3-benzylureido)-propionylaminosuccinamic acid *t*-butyl ester (6a).** To the solution of **5** (0.7 mmol, 0.4 g) in CH_2Cl_2 (20 mL) was added benzylamine (2.1 mmol, 0.23 mL) and was stirred for 2 h at room temperature. The reaction mixture was neutralized with 1*N*-HCl, diluted with water (20 mL) and CH_2Cl_2 (30 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the organic solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography with ethyl actate to give **6a** (0.16 g, 40%) as a pale yellow oil. ^{1}H-NMR (CDCl$_3$) δ 1.40 (9H, d, J = 4.5 Hz), 1.61 (2H, m), 2.36 (2H, m), 3.30 (6H, m), 3.54 (4H, m), 4.32 (2H, m), 4.50 (1H, m), 4.93 (2H, m), 5.41 (1H, q, J = 8.1 Hz), 7.24 (10H, m).

The synthesis of compounds **6b-g** from **5** was carried out by the same procedure as described for the preparation of **6a**.

6b: Yield 40%. ^{1}H-NMR (CDCl$_3$) δ 1.40 (9H, d, J = 4.5 Hz), 1.29 (2H, m), 2.38 (2H, m), 3.37 (6H, s), 3.51 (2H, m), 3.76 (2H, d, J = 1.5 Hz), 3.80 (3H, s), 4.26 (2H, t, J = 3.5 Hz), 4.72 (1H, m), 4.96 (2H, m), 5.19 (1H, q, J = 8.1 Hz), 6.86 (4H, m), 7.27 (5H, m).

6c: Yield 37%. ^{1}H-NMR (CDCl$_3$) δ 1.40 (9H, d, J = 4.5 Hz), 1.61 (2H, m), 2.36 (2H, m), 3.30 (6H, s), 3.51 (2H, m), 3.76 (2H, m), 4.52 (2H, m), 4.89 (1H, m), 4.98 (2H, m), 5.04 (1H, q, J = 8.1 Hz), 7.19 (3H, m), 7.24 (5H, m).

6d: Yield 35%. ^{1}H-NMR (CDCl$_3$) δ 1.40 (9H, d, J = 4.5 Hz), 1.61 (2H, m), 2.36 (2H, m), 3.31 (6H, s), 3.51 (2H, m), 3.76 (2H, m), 4.52 (2H, m), 4.89 (1H, m), 4.96 (2H, m), 5.49 (1H, q, J = 8.1 Hz), 7.02 (4H, m), 7.29 (5H, m).

6e: Yield 37%. ^{1}H-NMR (CDCl$_3$) δ 1.40 (9H, d, J = 4.5 Hz), 1.60 (1H, m), 2.33 (3H, s), 2.69 (2H, m), 2.95 (2H, m), 3.38 (6H, s), 3.45 (2H, m), 3.51 (2H, m), 4.52 (2H, m), 4.87 (1H, m), 4.95 (2H, m), 5.29 (1H, m), 7.12 (9H, m).

6f: Yield 32%. ^{1}H-NMR (CDCl$_3$) δ 1.40 (9H, d, J = 4.5 Hz), 1.60 (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 = 2.31 Hz), 7.29 (5H, m).

6g: Yield 50%. ^{1}H-NMR (CDCl$_3$) δ 1.40 (9H, d, J = 4.5 Hz), 1.60 (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).

{(6S)-8-Benzyl-1-[(benzylamino)carbonyl]tetrahydro-2H-pyrazino[1,2-a]pyrimidin-4,7-dione-6-ly}acetic acid (7a). A solution of **6a** (0.14 mmol, 82 mg) and formic acid (7 mL) in CH_2Cl_2 (300 mL) was stirred for 12 h at room temperature. Evaporation of the solution *in vacuo* gave a crude residue, which was purified by silica gel column chromatography with EtOAc/acetone (3 : 1) to give **7a** (19.0 mg, 30%) as a foamy solid. ^{1}H-NMR (CDCl$_3$) δ 1.78 (2H, m), 2.37 (2H, m), 3.29 (4H, m), 4.38 (3H, m), 5.33 (2H, m), 6.03 (1H, q, J = 2.3 Hz), 7.29 (10H, m). -HRMS (FAB) Calcd. for $C_{24}H_{26}N_4O_5$ 450.1903, Found (M$^+$) 450.1907.

The synthesis of compounds **7b-g** was carried out by the same procedure as described for the preparation of **7a**.

7b: Yield 35%. ^{1}H-NMR (CDCl$_3$) δ 1.82 (2H, m), 2.53 (2H, m), 3.31 (4H, m), 3.80 (3H, s), 4.35 (4H, m), 5.35 (1H, dd, J = 3.0 and 9.0 Hz), 5.99 (1H, q, J = 9.0 Hz), 6.86 (2H, d, J = 6.0 Hz), 7.30 (7H, m). -HRMS (FAB) Calcd. for $C_{25}H_{28}N_4O_6$ 480.2009, Found (M$^+$) 480.2005.

7c: Yield 38%. ^{1}H-NMR (CDCl$_3$) δ 1.82 (2H, m), 2.43 (2H, m), 3.33 (4H, m), 4.35 (4H, m), 5.32 (1H, dd, J = 3.0 and 9.0 Hz), 6.01 (1H, q, J = 6.0 Hz), 7.17 (8H, m). -HRMS (FAB) Calcd. for $C_{26}H_{30}N_4O_5$ 478.2216, Found (M$^+$) 478.2220.

7d: Yield 37%. ^{1}H-NMR (CDCl$_3$) δ 1.82 (2H, m), 2.48 (2H, m), 3.34 (4H, m), 4.35 (4H, m), 5.32 (1H, dd, J = 3.0 and 9.0 Hz), 6.01 (1H, q, J = 6.0 Hz), 7.00 (2H, t, J = 8.7 Hz), 7.27 (7H, m). -HRMS (FAB) Calcd. for $C_{24}H_{25}FN_4O_5$ 468.1809, Found (M$^+$) 468.1808.

7e: Yield 38%. ^{1}H-NMR (CDCl$_3$) δ 1.82 (2H, m), 2.34 (3H, s), 2.39 (2H, m), 2.80 (2H, t, J = 6.6 Hz), 3.30 (2H, m), 3.48 (4H, m), 4.74 (2H, m), 5.32 (1H, dd, J = 3.0 and 9.0 Hz), 5.99 (1H, q, J = 6.0 Hz), 7.09 (4H, dd, J = 7.8 and 21.9 Hz), 7.28 (5H, m). -HRMS (FAB) Calcd. for $C_{24}H_{24}F_2N_4O_5$

486.1715, Found (M^-) 486.1717.

7f: Yield 40%. ^{1}H-NMR ($CDCl_3$) 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 $C_{21}H_{26}N_4O_5S$ 446.1624, Found (M^+) 446.1630.

7g: Yield 38%. ^{1}H-NMR ($CDCl_3$) 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 $C_{22}H_{28}N_4O_6$ 444.2009, Found (M^-) 444.2003.

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