Synthesis of Benzofuran Analogue of Go6976, an Isoform-Selective Protein Kinase C Inhibitor

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Based on the structure of Go6976, a known isoform-selective protein kinase C inhibitor, a benzofuran analogue (1) was designed. This analogue was synthesized by coupling of benzofuran 3-acetic acid and 8-oxo-tryptamine and subsequent intramolecular Dieckmann condensation, alkylation, oxidative photocyclization and cyanation reaction of mesylate.

Keywords Protein kinase C, Dieckmann condensation, oxidative photocyclization

PKCs are a growing family of isozymes involved in a wide variety of cellular processes.[1] Twelve distinct PKC isoforms have been cloned and classified into four subsets based on their different requirements for activation. The conventional PKC (cPKC) family members are the Ca^{2+}-, phospholipid- and diacylglycerol-dependent α, βI, βII, and γ isoforms. The new PKC (nPKC) members include δ, ε, η, and θ isoforms, which are activated by diacylglycerol (DAG) and phospholipid but are Ca^{2+}-independent and lack the Ca^{2+} binding region in their *N*-terminal domains. The atypical PKC (aPKC) members contain ζ, λ, and τ isoforms, which can be activated by *cis*-unsaturated fatty acids but not Ca^{2+}, phospholipid and DAG. The newly identified PKC isoform, PKC μ, is activated by DAG and phospholipid but not Ca^{2+}. Marked differences in tissue distribution and substrate specificities have suggested that these isozymes may play different roles in physiological and pathophysiological processes.[1,2] The isozyme-specific modulators are highly required in identifying these different roles, especially *in vivo*.[1,2] Recently, several isozyme-selective inhibitors for PKCs have been developed based on the structure modification of staurosporine, a potent but unselective inhibitor for PKC isozymes.[3-6] Among these selective inhibitor, Go6976 was found to inhibit PKC α and β1 at nanomolar concentrations but have no inhibition to PKC δ, ε, and ζ even at micromolar concentrations.[6] As a continuing program on the development of isoform-selective PKC modulators,[7] we became to interest in synthesis of benzofuran analogue **1**, in which the N-Me group of Go6976 was replaced with an oxygen. This subtle structure change would probably not only give structurally novel PKC inhibitor, but also alter the isoform-selectivity (Fig. 1). In this report we describe the synthesis of **1**.

As outlined in Scheme 1, we synthesized the target compound **1** based on Winterfeldt's strategy.[8] The coupling of benzofuran 3-acetic acid (**2**) with 8-oxo-tryptamine hydrochloride under the action of DCC provided amide **3**. Selective alkylation at 1-position of indole moiety of **3** with ICH_2CH_2OTHP mediated by potassium *tert*-butoxide afforded **4**, which was subjected to intramolecular Dieckmann condensation to gave lactam **5**. Direct oxidative photocyclization of **5** under typical condition ($h\nu/I_2$/air)[8,9] was found unable to give the

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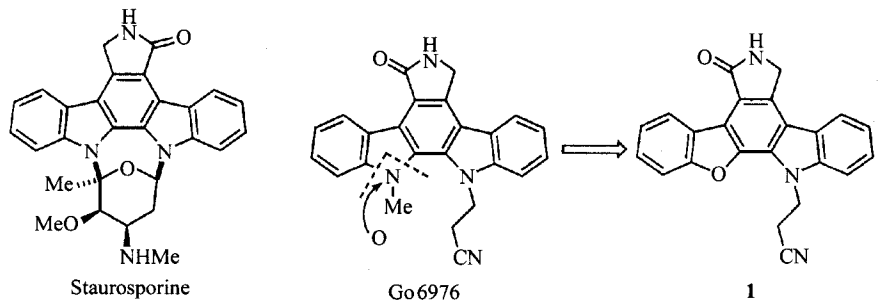
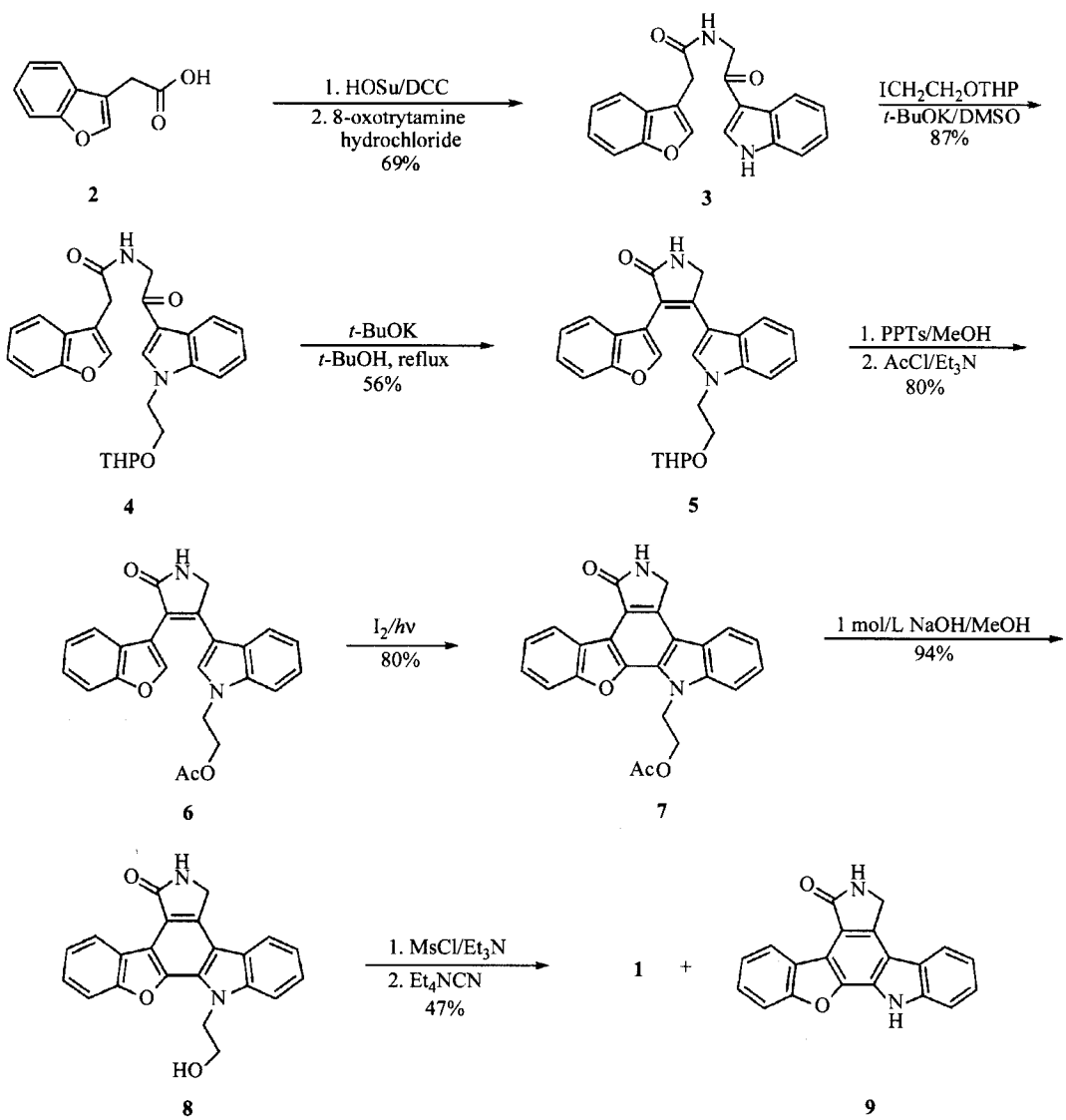


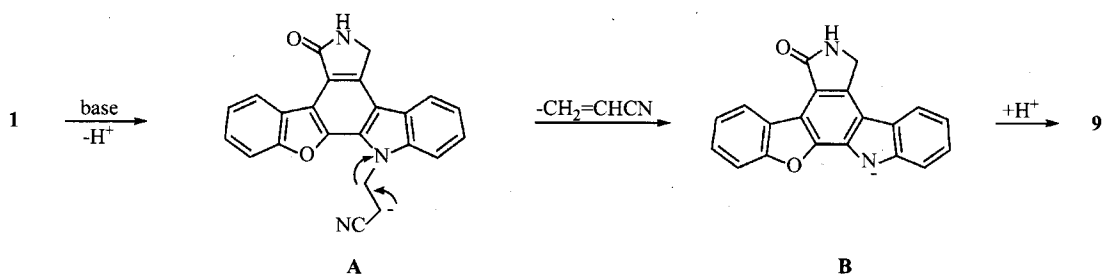
Fig. 1 Structure of staurosporine, Go6976 and design of benzoturan analogue.

Scheme 1



desired compound partially because the THP group was unstable under this reaction condition. We planned to solve this problem by switching the protecting group. Accordingly, treatment of **5** with PPTs/MeOH followed by reprotection with acyl group produced **6**. The oxidative photocyclization worked at this stage to provide **7** in 80% yield. Finally, hydrolysis of **7** gave alcohol **8**, which was transformed into mesylate and then reacted with tetraethylammonium cyanide to furnish **1** in 47% yield.[10] The major by-product was compound **9**, which might form through a mechanism indicated in Scheme 2. Deprotonation of **1** under the assistance of base gave intermediate **A**, which took elimination reaction to provide intermediate **B** and acrylonitrile. Protonation of **B** produced the compound **9**. This explanation could get support by the observation that when tetraethylammonium cyanide was replaced with more basic sodium cyanide only **9** was isolated as a major product.

Scheme 2



In conclusion, we synthesized a designed analogue of Go6976 using intramolecular Dieckmann condensation, oxidative photocyclization, and S_N2 reaction with tetraethylammonium cyanide as key steps. The biological evaluation of **1** is in progress and will report in due course.

Experimental

Coupling of benzofuran 3-acetic acid (**2**) *with 8-oxotryptamine hydrochloride*

To a mixture of benzofuran 3-acetic acid (**2**) (1.76 g, 10 mmol) and *N*-hydroxysuccinimide (2.30 g, 20 mmol) in 20 mL of methylene chloride was added dropwise a solution of DCC (4.12 g, 20 mmol) in 10 mL of methylene chloride at 0℃. After the addition the reaction mixture was stirred at room temperature for 1 h. The precipitate was filtered off and the filtrate was concentrated to dryness to give the corresponding activated ester. This ester was dissolved in 50 mL of THF and then 8-oxotryptamine hydrochloride (4.2 g, 20 mmol) was added. To this stirring mixture was added dropwise 10 mL of triethylamine. The resultant solution was stirred at room temperature until the activated ester disappeared monitored by TLC. The solvent was removed by rotavapor and the residue was partitioned between 100 mL of ethyl acetate and 20 mL of water. The aqueous layer was separated and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residual oil was chromatographed to afford 4.58 g of **3** (69%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.01 (s, 1H), 8.45 (d, J = 13.9 Hz, 1H), 8.17 (d, J = 6.2 Hz, 1H), 7.89 (s, 1H), 7.71 (d, J = 6.5 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.36—7.25 (m, 4H), 4.51 (s, 2H), 3.66 (s, 2H); MS m/z(%): 332 (M^+), 195, 144, 77; HRMS calcd for $C_{20}H_{16}N_2O_3$ 332.1161, found 332.1180 (M^+).

Alkylation of **3**

The amide **3** (3.0 g, 9.0 mmol) and potassium *tert*-butoxide (1.2 g, 10.8 mmol) were dissolved in 20 mL of dry DMSO. After the reaction solution was stirred for 1 h, a solution of ICH_2CH_2OTHP (3.5 g, 10.8 mmol) in 5 mL of DMSO was added. The mixture was stirred overnight at room temperature and then poured into a mixture of ethyl acetate and half-saturated aqueous NH_4Cl. After extraction, the organic layer was washed

with aqueous saturated $NaHCO_3$. The combined aqueous layers were extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated. Chromatography of the oily residue yielded 3.6 g of **4** (87%). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.18—8.14 (m, 1H), 7.85 (s, 1H), 7.60 (s, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.42 (d, J = 7.9 Hz, 1H), 7.33—7.17 (m, 5H), 6.83 (br s, 1H), 4.53 (d, J = 4.1 Hz, 2H), 4.40 (t, J = 3.2 Hz, 1H), 4.26 (t, J = 5.3 Hz, 2H), 4.01—3.94 (m, 1H), 3.66 (s, 2H), 3.63—3.61 (m, 1H), 3.42—3.40 (m, 1H), 3.31—3.27 (m, 1H), 1.65—1.32 (m, 6H); MS m/z (%): 460 (M^+), 376, 332, 272, 188, 144, 85, 77; HRMS calcd for $C_{27}H_{28}N_2O_5$ 460.1998, found 460.1998 (M^+).

Cyclization of **4**

A degassed solution of **4** (0.48 g, 1.06 mmol) in 30 mL of dry *t*-BuOH was heated to reflux. Potassium *tert*-butoxide (0.42 g, 3.61 mmol) in 10 mL of degassed *t*-BuOH was slowly added. After the solution was refluxed for 15 min, it was cooled to room temperature and poured into a well-stirred mixture of saturated NH_4Cl and ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed to afford 0.26 g (56%) of **5**. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 7.57—7.49 (m, 2H), 7.39—7.37 (m, 2H), 7.32—7.13 (m, 4H), 7.04—6.97 (m, 2H), 4.78 (s, 2H), 4.31 (t, J = 3.3 Hz, 1H), 4.15—4.11 (m, 2H), 3.86—3.84 (m, 1H), 3.5—3.41 (m, 2H), 3.36—3.34 (m, 1H), 1.61—1.28 (m, 6H); MS m/z (%): 442 (M^+), 358, 327, 270, 144, 77; HRMS calcd for $C_{27}H_{26}N_2O_4$ 442.1893, found 442.1865 (M^+).

3-Benzofuran-3-yl-4-(1-((2-acetoxy) ethyl)-1H-indole-3-yl)-1,4-2H-pyrrolidine-2-one (**6**)

A solution of **5** (100 mg, 0.23 mmol) and PPTs (5 mg, 0.02 mmol) in 5 mL of methanol was stirred at room temperature for 5 h. The solvent was evaporated *in vacuo* and the resulting residue was partitioned between ethyl acetate (30 mL) and water (10 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The crude product was chromatographed to afford the corresponding alcohol, which was dissolved in 2 mL of dry methylene chloride. To this solution was added triethylamine (0.11 mL, 0.8 mmol) and acetyl chloride (40 mg, 0.5 mmol) by syringe. After it was stirred for 30 min, the reaction mixture was partitioned between methylene chloride and water. The organic layer was washed with water and dried over Na_2SO_4. Evaporation of solvent followed by chromatography of the residue to afford 73 mg (80%) of **6**. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 7.61—6.95 (m, 10H), 4.74 (s, 2H), 4.20—4.13 (m, 4H), 1.88 (s, 3H); MS m/z (%): 400 (M^+), 399, 382, 371, 270, 326, 43; HRMS calcd for $C_{24}H_{20}N_2O_4$ 400.1423, found 400.1418 (M^+).

Oxidative photocyclization of **6**

A solution of **6** (270 mg, 0.67 mmol) and 30 mg of I_2 in 20 mL of benzene was irradiated with a high-pressure mercury lamp. During the irradiation air was bubbled through for 15 h. The reaction mixture was concentrated and the residue was chromatographed to yield 240 mg (80%) of **7**. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.12 (br s, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.69—7.39 (m, 7H), 5.05 (t, J = 5.1 Hz, 2H), 4.98 (s, 2H), 4.66 (t, J = 5.2 Hz, 2H), 1.75 (s, 3H); MS m/z (%): 398 (M^+), 355, 339, 325, 43; HRMS calcd for $C_{24}H_{18}N_2O_4$ 398.1267, found 398.1265 (M^+).

Hydrolysis of **7**

To the solution of **7** (259 mg, 0.65 mmol) in 2 mL of methanol was added 0.7 mL of 1 mol/L sodium hydroxide at room temperature. The reaction mixture was stirred until the reaction was completed as monitored by TLC. Ethyl acetate work up followed by chromatography yielded 210 mg (94%) of **8**. ^1H NMR (300 MHz, DMSO-d_6) δ: 9.03 (d, J = 7.2 Hz, 1H), 8.09 (d, J = 7.5 Hz, 1H), 7.85—7.83 (m, 2H), 7.61—7.67 (m, 2H), 7.49 (t, J = 7.5 Hz, 2H), 7.37 (t, J = 7.5 Hz, 2H), 5.00 (s, 2H), 4.94 (t, J = 5.5 Hz, 2H), 3.95 (t, J = 5.5 Hz, 2H); MS m/z (%): 356 (M^+), 339, 339, 325, 43; HRMS calcd for $C_{22}H_{16}N_2O_3$ 356.1161, found 356.1166 (M^+).

Conversion of the alcohol **8** *to* **1**

To a stirred solution of alcohol **8** (40 mg, 0.14 mmol) in 5 mL of dry THF, mesyl chloride (25 mg, 0.22 mmol) and triethylamine (30 μL, 0.22 mmol) were added at −30℃. Then the reaction mixture was stirred at room temperature for 5 h. Water was added to quench the reaction. Ethyl acetate extract work up provided the corresponding mesylate, which was dissolved in 2 mL of methylene chloride. To this stirring solution Et_4NCN (10 mg, 0.06 mmol) was added under nitrogen atmosphere. The reaction mixture was stirred at 40℃ for 24 h before it was evaporated. The crude product was purified by chromatography to provide 24 mg of **1** (47%) and 18 mg of **9**. **1**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.13 (d, J = 7.3 Hz, 1H), 7.96 (d, J = 7.9 Hz, 1H), 7.71—7.26 (m, 7H), 6.29 (s, 1H), 5.17 (t, J = 6.6 Hz, 2H), 5.02 (s, 2H), 4.11 (t, J = 6.6 Hz, 2H); MS m/z(%): 365 (M^+), 325, 312, 299, 283, 43; HRMS found m/z (%): 365.1168 (M^+), $C_{23}H_{15}N_3O_2$ requires 365.1164. **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.08 (d, J = 7.4 Hz, 1H), 8.01 (s, 1H), 7.94 (d, J = 7.9 Hz, 1H), 7.70—7.26 (m, 7H), 6.29 (s, 1H), 5.01 (s, 2H); MS m/z(%): 312 (M^+), 283, 43; HRMS calcd for $C_{21}H_{12}N_2O_2$ 312.0894, found 312.0918 (M^+).

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