Synthesis and Evaluation of 3-Methyl-4-oxo-6-phenyl-4,5,6,7-tetrahydrobenzofuran-2-carboxylic Acid Ethyl Ester Derivatives as Potent Antitumor Agents

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For the construction of new combinatorial libraries, a lead compound was created by replacing the core structure of a hit compound discovered by screening for cytotoxic agents against a tumorigenic cell line. The newly designed compound maintained biological activity and allowed alternative library construction for antitumor drugs.

Key words antitumor; anticancer; cytotoxicity; 4,5,6,7-tetrahydrobenzofuran

In the process of new drug discovery, it is one of the most significant steps to generate lead compounds from hit compounds selected by random screening. Structurally-optimized derivatives are obtained from libraries constructed by referring to each lead compound, and the derivatives from each library are usually expected to possess various biological properties, which may sometimes show advantage as a desired drug.

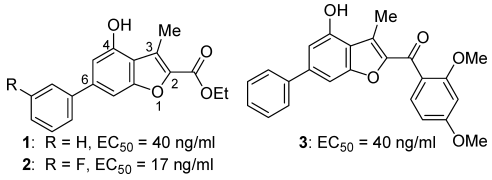

Fig. 1. Structures of a Hit Compound and a Metabolically Stable Derivative

From hit compound **1**, which was obtained by screening for anticancer agents displaying selective cytotoxicity against a tumorigenic cell line, optimized compounds **2** and **3** were designed (Fig. 1).[1—3] The phenyl substituent at the 6-position of the benzofuran core was tuned to introduce a fluorine substituent (**2**), and the biologically labile ethyl ester group was replaced with 2,4-dimethoxyphenyl ketone (**3**).

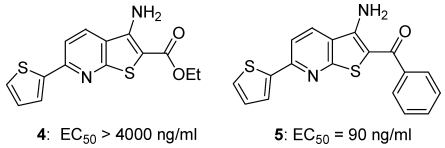

Fig. 2. Derivatives Consisting of an Alternative Core Structure

Thieno[2,3-b]pyridine derivative **5**, which has moderate similarity to 4-hydroxy-3-methylbenzofuran (**3**), was discovered and studied as a lead compound possessing a different core structure. The structure activity relationship (SAR), however, was significantly different from that of benzofuran derivatives; the corresponding ethyl ester **4** completely lost biological activity.[4] This result induced interest in the biological activities of the derivatives having a much more similar core structure to that of compound **1**.

Results and Discussion

As the most accessible compounds, 4,5,6,7-tetrahydrobenzofuran derivatives **7**, which was readily prepared from intermediates of benzofuran derivatives, were synthesized (Chart

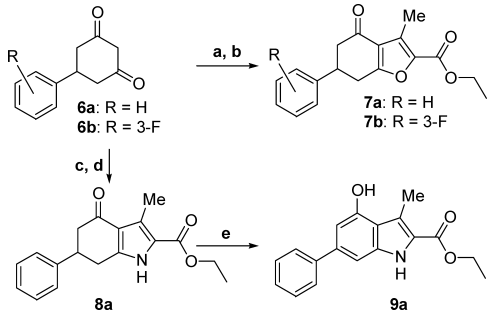

Chart 1. Synthesis of 4,5,6,7-Tetrahydrobenzofuran and 4,5,6,7-Tetrahydro-1*H*-indole Derivatives

Reagents: (a) ethyl 2-chloroacetoacetate, KOH in H_2O–MeOH; (b) AcOH in H_2O–MeOH; (c) ethyl acetylacetate, $NaNO_2$ in AcOH–H_2O; (d) zinc powder in AcOH; (e) see ref. 3.

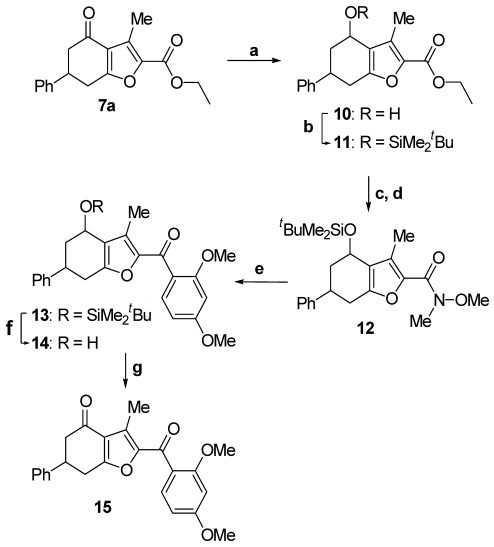

Chart 2. Conversion of Ethyl Ester to 2,4-Dimethoxyphenyl ketone

Reagents: (a) $NaBH_4$, $CeCl_3 \cdot 7H_2O$ in MeOH; (b) *t*-$BuMe_2SiCl$, imidazole in DMF; (c) NaOH in EtOH; (d) *N*,*O*-dimethylhydroxylamine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole hydrate (HOBt), Et_3N in DMF; (e) 2,4-dimethoxybromobenzene, *n*-BuLi in THF; (f) *n*-Bu_4NF, AcOH in THF; (g) Dess–Martin periodinane[6] in CH_2Cl_2.

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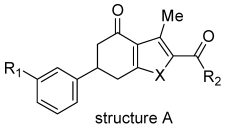
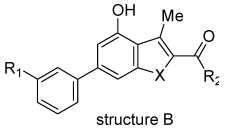
Table 1. Cytotoxicity of Synthesized Derivatives and Related Compounds



structure A



structure B

Entry	R_1	R_2	X	Cytotoxicity of structure A		Cytotoxicity of structure B	
				Compd.	EC_{50}, ng/ml[a)]	Compd.	EC_{50}, ng/ml[a)]
1	H	OEt	O	**7a**	22	**1**	40
2	F	OEt	O	**7b**	78	**2**	17
3	H	OEt	NH	**8a**	72	**9a**	470
4	H	2,4-dimethoxyphenyl	O	**15**	>4000	**3**	40

a) Selectivity against tumor cell was confirmed.

1). The corresponding dimethoxyphenyl ketone (**15**), which was expected to be metabolically stable,[1)] was converted from **7a** (Chart 2). For the purpose of comparing the SAR of indole derivative **9a**, 4,5,6,7-tetrahydro-1*H*-indole derivative **8a** was also synthesized as shown in Chart 1.

The cytotoxicity of the synthesized derivatives and related compounds are summarized in Table 1. The potency of compound **7a** was stronger than corresponding benzofuran compound **1**. A derivative with a fluorine substituent (**7b**) also possessed significant biological activity. These results indicate that 4,5,6,7-tetrahydrobenzofuran is an alternative core structure that shows selective cytotoxicity as benzofuran core. Nitrogen analogues (**8a**)[5)] maintained cytotoxicity, nevertheless dimethoxyphenyl ketone (**15**) completely lost biological activity.

Although ketone derivative **15** was designed to avoid metabolic deactivation by the enzymatic hydrolysis of the ethyl ester and this was successful in the benzofuran derivatives, this approach was unfortunately revealed to be inapplicable to 4,5,6,7-tetrahydrobenzofuran derivatives.

In conclusion, 4,5,6,7-tetrahydrobenzofuran was recognized to be a new core structure for compound libraries targeting antitumor drugs. The derivatives based on this different core structure are expected to have different biological properties, which expand the possibilities to be new antitumor drugs.

Experimental

Melting points (mp) were determined with a Yanaco melting point apparatus and not corrected. Infrared (IR) spectra were measured with a Nic 5SXC FT-IR spectrophotometer. NMR spectra were recorded on a JEOL JNM-GX 270 FT-NMR or a Varian Mercury 400 spectrophotometer. Chemical shifts are expressed in δ ppm from the internal standard tetramethylsilane (TMS). Mass spectra were obtained on a JEOL HX-100, SX-102A or JMS-AX-505H mass spectrometer instruments by applying an electric ionization (EI) method, a fast atom bombardment (FAB) ionization method, or an electrospray ionization (ESI) method. Column chromatography was carried out using SK-85 (230—400 mesh). Preparative thin-layer chromatography (PTLC) was performed using 60 F_{254} plates (Merck art. 5744). Yields were not optimized.

Evaluation of Biological Activity Human tumorigenic cell line VA13 (CCL-75.1) and its parental normal cell line WI-38 (CCL-75) were obtained from the American Tissue Culture Collection (ATCC) and were maintained and assayed in Minimum Essential Medium-Eagle (MEM-E) with Earl's salts (11095-080, Gibco BRL) with 100 U/ml of penicillin and 100 μg/ml of streptomycin supplemented with 10% fetal calf serum (Hyclone). Exponentially growing VA-13 cells and WI-38 cells were collected and distributed into 96-well plates (3598, Corning-Coaster) at a density of 1.5—3.0$\times10^3$ cells and 4.8—9.6$\times10^3$ cells, respectively, at a final volume of 200 μl/well. After cultivation for two or three days, test compounds dissolved in DMSO were diluted with fresh growth medium and then added to the plates. The final concentrations of DMSO were always kept at less than 0.5%. The plates were incubated for 24 h. Then the culture medium was removed and washed. Finally, 150 μl/well of fresh medium was added to the cells. After cultivation for three days, 50 μl/well of MEM-E containing 1 mg/ml of 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanide (XTT: X-4251, Sigma) and 25 mM of phenazine methosulfate (P-9625, Sigma) was added and the plates were incubated for 2.5 h. OD450 was measured by a SPECTRA MAX 250 (Molecular Devices) and cell viability was calculated using the following formula:

$$\text{cell viability (\%)} = (WC - BG) \times 100 / (RF - BG)$$

WC: OD450 for well containing cells treated with compounds
RF: OD450 for well containing cells and no compounds
BG: OD450 for well containing neither cells nor compounds

A dose–response curve was drawn and the 50% effective concentration (EC_{50}) was determined as an indicator of compound cytotoxicity.

3-Methyl-4-oxo-6-phenyl-4,5,6,7-tetrahydrobenzofuran-2-carboxylic Acid Ethyl Ester (7a) To a solution of KOH (7.21 g, 110 mmol) in H_2O (20 ml), was added 5-phenyl-1,3-cyclohexanedione (20.8 g, 110 mmol), and the mixture was stirred at room temperature for 15 min. Ethyl 2-chloroacetoacetate (15.2 ml, 110 mmol) in MeOH (40 ml) was added, and the mixture was stirred for 20 h at room temperature. AcOH (62 ml) was added, and the reaction mixture was heated under reflux for 3 h and then the solvent was removed. The mixture was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane : EtOAc=2 : 1 as a solvent) to give 19.1 g (58%) of **7a** as a colorless solid. mp 101—102 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.45—7.36 (2H, m), 7.33—7.27 (3H, m), 4.40 (2H, q, J=7.1 Hz), 3.63—3.50 (1H, m), 3.24 (1H, dd, J=5.1, 17.6 Hz), 3.09 (1H, dd, J=10.8, 17.6 Hz), 2.80 (2H, d, J=8.3 Hz), 2.60 (3H, s), 1.41 (3H, t, J=7.1 Hz); IR (KBr) cm^{-1}: 1709, 1677, 1604, 1456, 1321, 1244; HR-MS (EI) Calcd for $C_{18}H_{18}O_4$ $(M)^+$: 298.1205, Found: 298.1207.

6-(3-Fluorophenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydrobenzofuran-2-carboxylic Acid Ethyl Ester (7b) Compound **7b** was prepared using a similar procedure as for **7a** (25%, colorless solid) from **6b** (503 mg, 2.44 mmol). mp 98—100 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.38—7.30 (1H, m), 7.06—6.96 (3H, m), 4.39 (2H, q, J=7.1 Hz), 3.60—3.50 (1H, m), 3.25 (1H, dd, J=5.0, 17.0 Hz), 3.06 (1H, dd, J=11.0, 17.0 Hz), 2.79—2.75 (2H, m), 2.59 (3H, s), 1.40 (3H, t, J=7.1 Hz); IR (KBr) cm^{-1}: 1710, 1673, 1589, 1450, 1244; HR-MS (ESI) Calcd for $C_{18}H_{18}FO_4$ $(M+H)^+$: 317.1189, Found: 317.1179.

3-Methyl-4-oxo-6-phenyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic Acid Ethyl Ester (8a) To a solution of ethyl acetoacetate (15.0 g, 93.8 mmol) in AcOH (30 ml), was added a solution of $NaNO_2$ (8.00 g, 116 mmol) in H_2O (25 ml) below 12 °C, and the mixture was stirred at such a rate that the temperature remained below 12 °C for 3 h and was left at room temperature overnight. A solution of 5-phenyl-1,3-cyclohexanedione (24.0 g, 128 mmol) in AcOH (60 ml) was added. Then the zinc dust (16.7 g, 255 mmol) was added at such a rate that the temperature did not rise above 60 °C. After stirring for 0.5 h and refluxing for 3 h, the solution was separated from the excess zinc dust and poured into iced water (500 ml) to give a yellow solid. The crude product was purified by recrystallization from ben-

zene to give 9.45 g (34%) of **8a** as a colorless solid. mp 207—208 °C (dec.); ^{1}H-NMR ($CDCl_3$) δ: 9.26 (1H, s), 7.38—7.26 (5H, m), 4.34 (2H, q, J=7.1 Hz), 3.56—3.44 (1H, m), 3.09 (1H, dd, J=5.0, 16.0 Hz), 3.00 (1H, dd, J=11.0, 16.0 Hz), 2.77—2.73 (2H, m), 2.63 (3H, s), 1.37 (3H, t, J=7.1 Hz); IR (KBr) cm^{-1}: 3194, 1692, 1635, 1488, 1282, 1184; HR-MS (ESI) Calcd for $C_{18}H_{20}NO_3$ $(M+H)^+$: 298.1443, Found: 298.1429.

4-Hydroxy-3-methyl-6-phenyl-4,5,6,7-tetrahydrobenzofuran-2-carboxylic Acid Ethyl Ester (10) To a solution of **7a** (100 mg, 0.335 mmol) and $CeCl_3 \cdot 7H_2O$ (150 mg, 0.402 mmol) in MeOH (2 ml), was added $NaBH_4$ (13 mg, 0.335 mmol), and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with EtOAc, and washed with aqueous 5% HCl, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by PTLC (hexane : EtOAc=3 : 1 as a solvent) to give 86 mg (85%) of **10** as a colorless amorphous foam. ^{1}H-NMR ($CDCl_3$) δ: 7.38—7.32 (2H, m), 7.29—7.19 (3H, m), 4.99—4.88 (1H, m), 4.37 (2H, q, J=7.3 Hz), 3.18—3.08 (1H, m), 2.98—2.75 (2H, m), 2.50—2.37 (1H, m), 2.46 (3H, s), 1.99—1.87 (1H, m), 1.53—1.50 (1H, m), 1.39 (3H, t, J=7.3 Hz); EI-MS m/z: 300 $(M)^+$.

4-(*tert*-Butyldimethylsilanyloxy)-3-methyl-6-phenyl-4,5,6,7-tetrahydrobenzofuran-2-carboxylic Acid Ethyl Ester (11) *tert*-Butyldimethylsilyl chloride (80 mg, 0.533 mmol) was added to a stirred solution of **10** (79 mg, 0.267 mmol) and imidazole (55 mg, 0.800 mmol) in DMF (1.0 ml) at room temperature. The mixture was stirred for 40 h at room temperature. The reaction mixture was diluted with EtOAc, and washed with 10% aqueous $KHSO_4$, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane : EtOAc=4 : 1 as a solvent) to give 104 mg (95%) of **11** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 7.39—7.34 (2H, m), 7.29—7.25 (3H, m), 5.04—4.96 (1H, m), 4.36 (2H, q, J=7.3 Hz), 3.09—2.92 (1H, m), 2.88—2.70 (2H, m), 2.42 (3H, s), 2.37—2.30 (1H, m), 2.00—1.92 (1H, m), 1.38 (3H, t, J=7.3 Hz), 0.92 (9H, s), 0.17 (3H, s), 0.13 (3H, s); FAB-MS m/z: 415 $(M+H)^+$.

4-(*tert*-Butyldimethylsilanyloxy)-3-methyl-6-phenyl-4,5,6,7-tetrahydrobenzofuran-2-carboxylic Acid *N*-Methoxy-*N*-methylamide (12) A mixture of **11** (103 mg, 0.251 mmol) and 1 N NaOH (1.0 ml) in EtOH (2.0 ml) was heated under reflux for 2 h. The mixture was acidified (pH=3.0) with 10% aqueous HCl, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. To a solution of residue, 1-hydroxybenzotriazole (34 mg, 0.251 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (49 mg, 0.502 mmol) and Et_3N (105 μl, 0.753 mmol) in DMF (1.5 ml), was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (96 mg, 0.502 mmol) under ice cooling. After stirring the mixture for 14 h at room temperature. The reaction mixture was diluted with EtOAc, and washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane : EtOAc=2 : 1 as a solvent) to give 84 mg (79%) of **12** as a colorless amorphous foam. ^{1}H-NMR ($CDCl_3$) δ: 7.39—7.34 (2H, m), 7.29—7.26 (3H, m), 5.05—4.99 (1H, m), 3.78 (3H, s), 3.29 (3H, s), 3.12—3.00 (1H, m), 2.90—2.71 (2H, m), 2.37 (3H, s), 2.37—2.31 (1H, m), 2.02—1.89 (1H, m), 0.92 (9H, s), 0.18 (3H, s), 0.13 (3H, s); FAB-MS m/z: 430 $(M+H)^+$.

[4-(*tert*-Butyldimethylsilanyloxy)-3-methyl-6-phenyl-4,5,6,7-tetrahydrobenzofuran-2-yl]-(2,4-dimethoxyphenyl)methanone (13) *n*-Butyllithium (0.360 ml, 0.548 mmol, 1.53 M in hexane) was added dropwise to the stirred solution of the 1-bromo-2,4-dimethoxybenzene (123 mg, 0.566 mmol) in THF (1.0 ml) at −78 °C under N_2. The mixture was stirred for 30 min at the same temperature. After **12** (81 mg, 0.189 mmol) in THF (1.0 ml) was added to the reaction mixture over 5 min at −78 °C, the reaction mixture was stirred for 30 min at the same temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by PTLC (hexane : EtOAc=3 : 1 as a solvent) to give 64 mg (67%) of **13** as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 7.38—7.33 (3H, m), 7.28—7.23 (3H, m), 6.54—6.49 (2H, m), 5.06—5.00 (1H, m), 3.85 (3H, s), 3.79 (3H, s), 3.10—3.03 (1H, m), 2.90—2.69 (2H, m), 2.38—2.34 (1H, m), 2.29 (3H, s), 2.02—1.89 (1H, m), 0.92 (9H, s), 0.17 (3H, s), 0.12 (3H, s); FAB-MS m/z: 507 $(M+H)^+$.

(2,4-Dimethoxyphenyl)-(4-hydroxy-3-methyl-6-phenyl-4,5,6,7-tetrahydrobenzofuran-2-yl)methanone (14) To a stirred solution of **13** (62 mg, 0.122 mmol) in THF (1.0 ml) was added *n*-Bu_4NF (0.244 ml, 1.0 M solution in THF) and AcOH (14 μl, 0.244 mmol) at room temperature. The mixture was stirred for 14 h at room temperature. The *n*-Bu_4NF (0.488 ml, 1.0 M solution in THF) and AcOH (28 μl, 0.488 mmol) were added to the reaction mixture, and stirred for 1 h at 60 °C. The reaction mixture was diluted with EtOAc, and washed with H_2O, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane : EtOAc=1 : 1 as a solvent) to give 45 mg (94%) of **14** as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 7.39—7.32 (3H, m), 7.28—7.24 (3H, m), 6.55—6.50 (2H, m), 4.98—4.95 (1H, m), 3.86 (3H, s), 3.80 (3H, s), 3.17—3.10 (1H, m), 2.94—2.73 (2H, m), 2.51—2.45 (1H, m), 2.33 (3H, s), 2.00—1.88 (1H, m), 1.60—1.57 (1H, m); HR-MS (ESI) Calcd for $C_{24}H_{25}O_5$ $(M+H)^+$: 393.1702, Found: 393.1700.

2-(2,4-Dimethoxybenzoyl)-3-methyl-6-phenyl-4,5,6,7-tetrahydrobenzofuran-4-one (15) To a solution of **14** (42 mg, 0.107 mmol) in CH_2Cl_2 (1.0 ml) was added Dess–Martin periodinane (68 mg, 0.160 mmol), and the mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane : EtOAc=2 : 1 as a solvent) to give 33 mg (79%) of **15** as a colorless amorphous foam. ^{1}H-NMR ($CDCl_3$) δ: 7.43—7.35 (3H, m), 7.32—7.26 (3H, m), 6.56 (1H, dd, J=2.2, 8.6 Hz), 6.51 (1H, d, J=2.2 Hz), 3.87 (3H, s), 3.77 (3H, s), 3.62—3.52 (1H, m), 3.21 (1H, dd, J=5.0, 17.8 Hz), 3.07 (1H, dd, J=10.9, 17.8 Hz), 2.79 (2H, d, J=8.2 Hz), 2.42 (3H, s); IR (KBr) cm^{-1}: 1682, 1604, 1456, 1211, 1029, 944; HR-MS (ESI) Calcd for $C_{24}H_{23}O_5$ $(M+H)^+$: 391.1546, Found: 391.1544.

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