Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4383–4387
Bioorganic & Medicinal Chemistry Letters

A library synthesis of 4-hydroxy-3-methyl-6-phenylbenzofuran-2-carboxylic acid ethyl ester derivatives as anti-tumor agents

Ichiro Hayakawa,[a] Rieko Shioya,[a] Toshinori Agatsuma,[b] Hidehiko Furukawa,[b] Shunji Naruto[c] and Yuichi Sugano[a,*]

[a]*Lead Discovery Research Laboratories, Sankyo Co. Ltd, Shinagawa-ku, Tokyo 140-8710, Japan*
[b]*Biomedical Research Laboratories, Sankyo Co. Ltd, Shinagawa-ku, Tokyo 140-8710, Japan*
[c]*R&D Information Technology Department, Sankyo Co. Ltd, Shinagawa-ku, Tokyo 140-8710, Japan*

Received 2 June 2004; revised 17 June 2004; accepted 21 June 2004

Abstract—As a result of a hit-to-lead program using a technique of solution-phase parallel synthesis, a highly potent (2,4-dimethoxyphenyl)-[6-(3-fluorophenyl)-4-hydroxy-3-methylbenzofuran-2-yl]methanone (**15b**) was synthesized as an optimized derivative of 4-hydroxy-3-methyl-6-phenylbenzofuran-2-carboxylic acid ethyl ester (**1**), which was discovered as a screening hit from small-molecule libraries and exhibited selective cytotoxicity against a tumorigenic cell line.

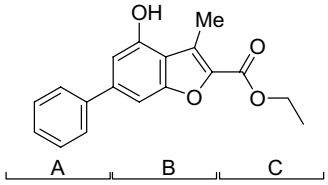
1. Introduction

As described in our preceding reports, a potential hit compound 4-hydroxy-3-methyl-6-phenylbenzofuran-2-carboxylic acid ethyl ester (**1**) was discovered from the screening of small-molecule libraries in search for novel anti-tumor drugs. The hit compound **1** exhibited potent and selective cytotoxicity against a tumorigenic cell line, WI-38 VA-13 subline 2RA (VA-13) (EC_{50} = 40 ng/mL), and no cytotoxicity was observed against the normal cell line, WI-38 (EC_{50} > 4000 ng/mL).[1–3]

Substructures B and C of compound **1** were already optimized to improve the biological activity and the metabolic tolerance. As substructure B, which consists of the central benzofuran ring and its substituents, was suggested to be responsible for the constitutive expression of biological activity, modification of substructure B was considered a challenge.[3] The biologically labile ethyl ester of substructure C was revealed to be replaceable by phenyl ketones, which are generally tolerant against hydration and metabolic decomposition.[1] The relationship between substructure C and cytotoxicity was investigated by the systematic modification of substructure C employing techniques of combinatorial chemistry. The study resulted in discovering a highly potent compound, derivative **2**, which was metabolically very stable and lasted much longer than the original ethyl ester (**1**) in mouse liver microsomes.[1] In this communication, we report the synthetic modification of substructure A, which was still left untouched, and the biological activity of the synthesized derivatives (Fig. 1).

2. Chemistry

The synthetic procedure of compound **1** is shown in Scheme 1. Starting from a cyclohexane-1,3-dione

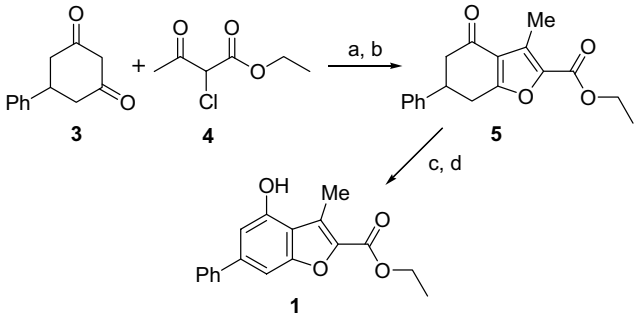


1: EC_{50} = 40 ng/mL

Figure 1. Structure of a hit compound of an anti-tumor drug screening.

Keyword: Anti-cancer.
* Corresponding author. Tel.: +81-3-3492-3131; fax: +81-3-5436-8578; e-mail: ysugan@sankyo.co.jp

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.06.067



Scheme 1. Reagents and conditions: (a) 1.5 equiv NaH (55% dispersion in mineral oil) in THF, 25 °C, 14.0 h (64%); (b) in AcOH, reflux, 1.0 h (82%); (c) 1.05 equiv $LiN(Pr^i)_2$ in THF, −78 °C, 1.0 h, and then 1.7 equiv Me_3SiCl, −78 to 25 °C, 1.0 h; (d) 0.5 equiv $Pd(OAc)_2$ and 0.5 equiv *p*-benzoquinone in MeCN, 25 °C, 1.5 h (42%, two steps from **5**).

with a phenyl group as a substituent at the fifth position (**3**), 3-methyl-4-oxo-6-phenyl-4,5,6,7-tetrahydrobenzofuran (**5**) was prepared, and the subsequent oxidation with palladium acetate converted the cyclohexenone **5** to phenol **1**.

For the construction of a combinatorial library focusing on compounds with a modified substructure C of compound **1**, a synthetic procedure employing solid-phase chemistry had been developed.[1] Although solid-phase chemistry produced numerous outputs for library synthesis, a solution-phase parallel synthesis was chosen for the library construction. Since the reaction steps were relatively short and the substructure to be modified was one substituent, it was difficult to take advantage of solid-phase chemistry. In addition, solid-phase synthesis, in general, requires extra reaction steps for the introduction of the starting molecule onto the solid support and for the cleavage and removal of the synthesized compound (Fig. 2).

The original synthetic procedure of compound **6** employed air and moisture sensitive reagents such as sodium hydride under strictly controlled reaction conditions, and this was the major issue to resolve for the parallel solution-phase synthesis. For the library synthesis, simple shape reaction vessels that were compact enough to carry out a large number of reactions in a limited workspace were desired. Labile reagents, which require space-consuming apparatuses and complex handling, were to be replaced.

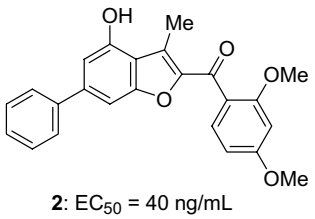
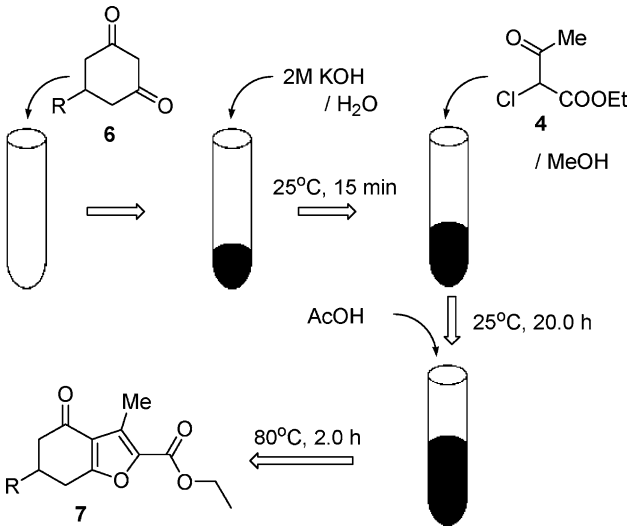


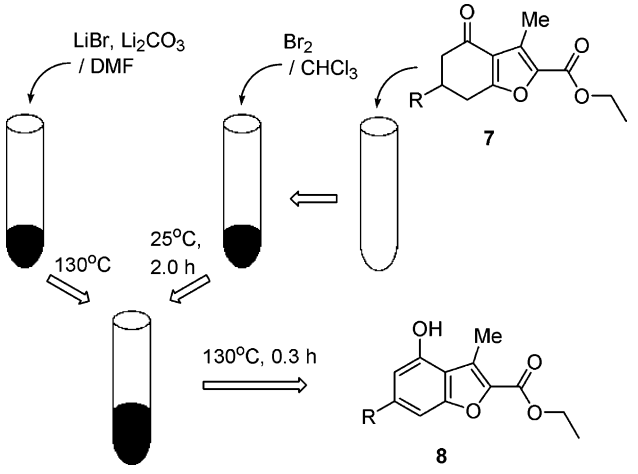
Figure 2. The structure of a potent and metabolically stable derivative.



Scheme 2. Using a simple reaction vessel, cyclohexane-1,3-dione was dissolved in aqueous 2 M potassium hydroxide (1.0 equiv), and then, a methanol solution of chloroketone (**4**) (1.0 equiv) was added. After the condensation–cyclization reaction was completed, the dehydration by adding acetic acid (large excess) and heating for 2 h was carried out to generate a cyclohexenone **7**.

The moisture labile sodium hydride of the first step was replaced with an aqueous solution of potassium hydroxide. The subsequent dehydration was carried out by simply adding acetic acid to the reaction mixture without further work after the preceding condensation reaction to obtain cyclohexenone **7** in good yield (Scheme 2).

The following oxidation of cyclohexenone **5** using palladium acetate via trimethylsilyl enol ether was simplified by utilizing bromine under basic conditions (Scheme 3). As the developed procedure required no moisture-labile reaction steps, our original 'reaction station', which is arrays of reaction vessels placed in a heat block combined with an orbital shaker, was available for the library synthesis. This optimized procedure for a library



Scheme 3. Cyclohexenone **7** was dissolved in a chloroform solution of bromine (1.1 equiv). The solution was mixed with a dimethylformamide solution of lithium bromide (12 equiv) and lithium carbonate (12 equiv).

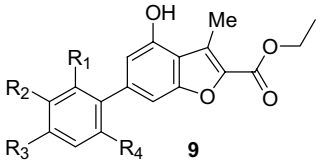
synthesis did not demand any highly sophisticated library synthesizers, which have capability of operating complicated reaction procedures.

3. Structure–activity relationship

Derivatives of compound **1**, which had various substituents on the benzene ring at the sixth position of benzofuran, were synthesized, and their cytotoxicity were evaluated. As shown in Table 1, most of the compounds that had a substituent at the *para*-position of the benzene ring had completely lost their biological activity (**9s** through **9B**) except when the substituent was a hydroxy group (**9q**) or fluoride (**9r**). While bulky substituents placed at the *ortho*-position also weakened the activity (**9a**,**c** through **9f**,**C**, and **9D**), the effect of introducing substituents at the *meta*-position was relatively smaller. Most of the *meta*-substituted derivatives (**9h** through **9m**, and **9o**) showed almost the same or a slightly weaker cytotoxicity than the unsubstituted compound **1**.

The synthesis of derivatives that have heterocycles or aliphatic substituents instead of the benzene ring was problematic because of the instability of the intermediates. A few examples, however, shown in Table 2, indicated that derivatives with heterocycles had moderate cytotoxicity and those with aliphatic substituents had lost their activity.

Table 1. Cytotoxicity of ester derivatives possessing a substituted benzene ring

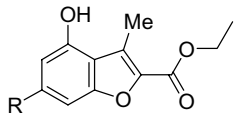


9

Compds	R_1	R_2	R_3	R_4	Yield		Cytotoxicity EC_{50} (ng/mL)[c]
					To **7**[a]	To **9**[b]	
1	H	H	H	H	58	91	40
9a	HO	H	H	H	46[d]	60	310
9b	F	H	H	H	14	50	48
9c	Cl	H	H	H	33	72	350
9d	Br	H	H	H	26	19	390
9e	MeO	H	H	H	11	27	380
9f	BnO	H	H	H	22	28	>4000
9g	H	HO	H	H	—	70[f]	520
9h	H	NH_2	H	H	—	99[g]	90
9i	H	NO_2	H	H	57	49	75
9j	H	F	H	H	25	40	17
9k	H	Cl	H	H	25	39	80
9l	H	Br	H	H	22	24	85
9m	H	Me	H	H	13	62	80
9n	H	CF_3	H	H	38	63	700
9o	H	MeO	H	H	20	18	80
9p	H	BnO	H	H	44	63	850
9q	H	H	HO	H	46	60	75
9r	H	H	F	H	53	52	370
9s	H	H	Cl	H	45	54	>4000
9t	H	H	Br	H	34	61	>4000
9u	H	H	Me	H	47	56	>4000
9v	H	H	MeO	H	72[e]	9	>4000
9w	H	H	EtO	H	76[e]	76	>4000
9x	H	H	Pn^iO	H	42[e]	54	>4000
9y	H	H	BnO	H	58	38	>4000
9z	H	H	$PhCH_2CH_2O$	H	16[e]	67	>4000
9A	Cl	H	Cl	H	38	53	>4000
9B	H	MeO	MeO	H	28	12	>4000
9C	F	H	H	Cl	38	55	>4000
9D	Cl	H	H	Cl	38	40	>4000

[a] Yields from diketone **6** after purification by chromatography.
[b] Yields from **7** after purification by chromatography.
[c] Selectivity against tumor cells was confirmed.
[d] Yield was calculated after the removal of benzyl protecting group for the hydroxy group.
[e] Yield from **7** corresponding to **9e**.
[f] Synthesized from **9p**.
[g] Synthesized from **9i**.

Table 2. Cytotoxicity of ester derivatives possessing an aromatic heteroring or saturated substituent



10

Compds	R	Yield		Cytotoxicity EC_{50} (ng/mL)[c]
		To **7**[a]	To **10**[b]	
10a	2-Furanyl	44	2[d]	180
10b	2-Thiophenyl	37	5[d]	160
10c	*cyclo*-Hex	47	31	>4000
10d	Me_3CCH_2	35	6	>4000

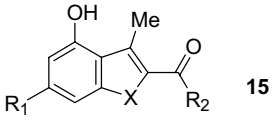
[a] Yields from diketone **6** after purification by chromatography.
[b] Yields from **7** after purification by chromatography.
[c] Selectivity against tumor cell was confirmed.
[d] Heterocycles were unstable under oxidative condition.

We have already reported that the ethyl ester group of compound **1** was readily hydrolyzed by esterases especially in the serum and liver, and the resultant carboxylic acid had completely lost its activity to kill tumor cells.[1] Substituted phenyl ketone derivatives corresponding to compound **1** were synthesized and dimethoxyphenyl ketone **2** was found to be a metabolically stable derivative. The most potent ethyl ester **9j** was converted to the corresponding ketone by the same procedure, and its cytotoxicity was evaluated.

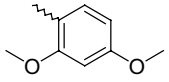
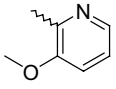
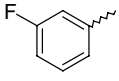
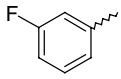
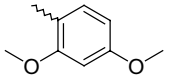
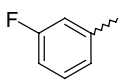
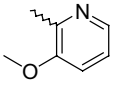
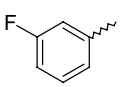
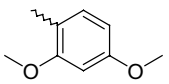
The results of the cytotoxicity test of ketone derivatives and related compounds are shown in Table 3. The original hit compound **1** was converted to dimethoxyphenyl ketone **2** without losing its biological activity.[1] The biological activity of dimethoxyphenyl ketone **15b** corresponding to ethyl ester **9j** was slightly weaker than the original ester **9j** but was still more potent than compound **2**. Considering the gastrointestinal absorption, 3-methoxypyridin-2-yl ketone was recognized as another promising structure for its effect of reducing the M log*P* (Moriguchi log*P*) value and providing remarkable cytotoxicity to compound **15a**.[4] Integrated compound **15c** with fluorophenyl group and dimethoxyphenyl ketone had almost the same potency as the dimethoxyphenyl ketone **15b**.

Finally the oxygen atom in benzofuran was replaced with nitrogen to confirm the biological activity of this core structure. Indole derivative **14** was synthesized as shown in Scheme 4 starting from the same substituted cyclohexane-1,3-dione **3** used to synthesize benzofuran derivatives. Phenyl ketone **15d** was synthesized via the corresponding ester using the same procedure as that mentioned above. Although the biological activity of the indole derivatives was, unfortunately, relatively weak,

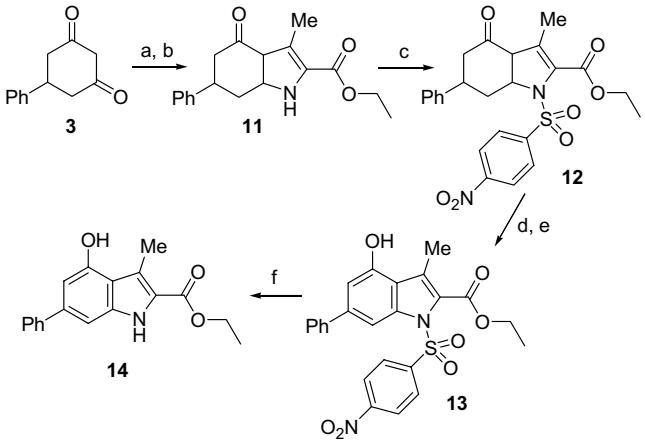
Table 3. Cytotoxicity against VA13 cell assay for ketone derivatives and related compounds



15

Compds	X	R_1	R_2	M log*P*[a]	Cytotoxicity EC_{50} (ng/mL)[b]
1	O		OEt	2.6	40
2	O			2.6	40
15a[6]	O			2.2	28
9j	O		OEt	2.7	17
15b[7]	O			2.7	30
15c[8]	O			2.3	27
14[9]	NH		OEt	2.6	470
15d[10]	NH			2.7	120

[a] Selectivity against tumor cells was confirmed.
[b] Moriguchi log*P*.



Scheme 4. Synthesis of indole derivatives. Reagents and conditions: (a) 1.0 equiv ethyl acetylacetate, 1.1 equiv $NaNO_2$ in AcOH, 10 °C, 3.0 h; (b) 2.2 equiv zinc powder in AcOH, 60 °C, 3.0 h, 20% from **3**; (c) 1.2 equiv Bu′OK and then 2.5 equiv *p*-NO_2PhSO_2Cl in THF, 0 °C, 1.0 h; (d) 1.0 equiv Br_2 in $CHCl_3$, 25 °C, 2.0 h, 48% from **11**; (e) 4.0 equiv LiBr, 4.0 equiv Li_2CO_3 in DMF, 130 °C, 1.0 h; (f) 1.2 equiv PhSH, 3.0 equiv K_2CO_3 in DMF, 25 °C, 1.0 h, 65% from **13** (see Ref. 5).

their cytotoxicity was maintained. The indole derivatives will be applied to a higher level of biological tests as compounds different from benzofuran derivatives.

In conclusion, using a technique of solution-phase library synthesis, various substituents were introduced on the phenyl ring at the sixth position of the benzofuran core structure of compound **1**. By further introducing substituent at the ester moiety of **1**, highly potent derivatives **15b** and **15c** were discovered in addition to phenyl ketones **2** and **15a**.

Acknowledgements

The authors are grateful to Ms. Youko Oda for her technical support in the cytotoxicity test.

References and notes

1. Hayakawa, I.; Shioya, R.; Agatsuma, T.; Furukawa, H.; Naruto, S.; Sugano, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 455.
2. US Patent Application US60/306116, July 17 2001.
3. Hayakawa, I.; Shioya, R.; Agatsuma, T.; Furukawa, H.; Sugano, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3411.
4. Lipinski, C. A. JSSX Symposium, Tokyo, April 2003.
5. Kan, T.; Fukuyama, T. *Chem. Commun.* **2004**, 353.
6. Compound **15a**: two steps from **1** (46%). Yellow powder. Mp 243 °C, ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.35 (d, 1H, J = 3.0 Hz), 7.91 (s, 1H), 7.49–7.26 (m, 7H), 6.91 (s, 1H), 6.69 (s, 1H), 3.90 (s, 3H), 2.53 (s, 3H); MS (APCI, *m*/*z*) 360 $(M+1)^+$.
7. Compound **15b**: two steps from **9j** (59%). Yellow powder. Mp 194 °C, ^{1}H NMR (270 MHz, acetone-d_6): δ 7.89 (s, 1H), 7.39–7.27 (m, 4H), 7.15 (d, 1H, J = 1.4 Hz), 7.05–6.97 (m, 1H), 6.88 (d, 1H, J = 1.4 Hz), 6.59–6.53 (m, 2H), 3.79 (s, 3H), 3.65 (s, 3H), 2.55 (s, 3H); MS (APCI, *m*/*z*) 407 $(M+1)^+$.
8. Compound **15c**: two steps from **9j** (49%). Yellow powder. Mp 226 °C, ^{1}H NMR (270 MHz, acetone-d_6): δ 8.13 (d, 1H, J = 4.6 Hz), 7.89 (s, 1H), 7.43 (d, 1H, J = 4.6 Hz), 7.40 (d, 1H, J = 4.6 Hz), 7.38–7.29 (m, 4H), 7.12 (d, 1H, J = 1.4 Hz), 7.16–6.98 (m, 1H), 6.90 (d, 1H, J = 1.4 Hz), 3.75 (s, 3H), 2.49 (s, 3H); MS (APCI, *m*/*z*) 378 $(M+1)^+$.
9. Compound **14**: yellow powder. Mp 160 °C, ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.69 (s, 1H), 7.61–7.31 (m, 5H), 7.11 (d, 1H, J = 1.4 Hz), 6.64 (d, 1H, J = 1.1 Hz), 4.42 (q, 2H, J = 7.1 Hz), 2.84 (s, 3H), 1.43 (t, 3H, J = 7.0 Hz); MS (APCI, *m*/*z*) 296 $(M+1)^+$.
10. Compound **15d**: two steps from **14** (36%). Yellow powder. ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.93 (s, 1H), 7.39–7.26 (m, 5H), 7.10 (s, 1H), 7.06–7.03 (m, 1H), 6.59 (s, 1H), 3.88 (s, 3H), 3.79 (s, 3H), 2.38 (s, 3H); MS (APCI, *m*/*z*) 406 $(M+1)^+$.