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Thienopyridine and benzofuran derivatives as potent anti-tumor agents possessing different structure–activity relationships

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Abstract—(3-Amino-6-thiophen-2-yl-thieno[2,3-*b*]pyridin-2-yl)phenylmethanone (**3**) was discovered as a new type of cytotoxic agent selective against a tumorigenic cell line. The molecular structure of a previously reported compound, (4-hydroxy-3-methyl-6-phenylbenzofuran-2-yl)phenylmethanone (**2**), had remarkably similar bioisosteric substructures to that of compound **3**. Although the relationship between the molecular structure and biological activity of each derivative synthesized from these two hit compounds (**2** and **3**) were studied, unexpectedly no correlation was observed. However, after further synthetic study from **3**, one of the most potent derivative (**10k**) having a different SAR profile from **2**, was discovered.

1. Introduction

As a part of program to develop new drugs, the structure optimization of lead compounds is the most important phase in the search for efficient derivatives, and undesired properties of the lead compounds should be resolved during this step. What is most problematic, here, is actually the generation of lead compounds from hit compounds that were selected by random screening. Not only must lead compounds have sufficient biological potential to be the backbone of a library, they must contain sufficient structure components for library construction (Fig. 1).

Very few hit compounds yield an excellent single lead compound that is suitable for library construction for structure optimization. Therefore the structural information of multiple hit compounds is often combined to form a lead compound for an accurate library design. The structures of the hit compounds are integrated, and the common substructures are determined to design a reliable scaffold. The hit compounds that are used in this approach should interact with the same domain of the same target molecule.

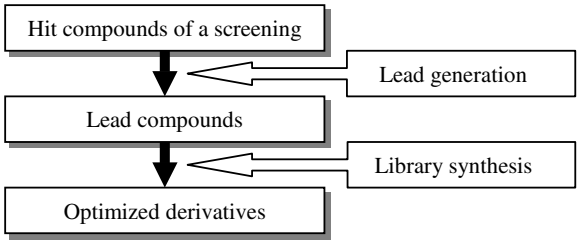

Figure 1. A flow chart from screening hit(s) to library design. If a screening hit contains sufficient information for the library design, the compound was simply used after the hit evaluation. Multiple hits were used to ensure the design of a more reliable library.

2. Screening for hit compounds

During our project to develop new anticancer drugs as small molecules, a cell-based screening system had been established in our laboratory. Since then, we have found and reported 4-hydroxy-3-methyl-6-phenylbenzofuran-2-carboxylic acid ethyl ester (**1**) to be the most promising in a series of hit compounds.[1,2] This hit compound **1** showed selective and potent cytotoxicity against a tumorigenic cell line, WI-38 VA-13 subline 2RA

Keywords: Lead compound; SAR; Anticancer.
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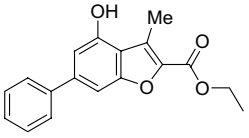
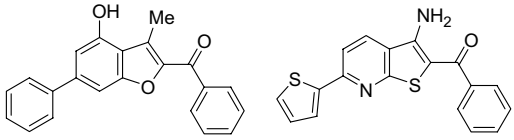


1: EC_{50} = 40 ng/mL

Figure 2. Hit compound **1**.



2: EC_{50} = 600 ng/mL **3**: EC_{50} = 90 ng/mL

Figure 3. Lead compound **2** derived from hit compound **1**, and the second hit compound **3** discovered in the same screening.

(VA-13) ($EC_{50} = 40$ ng/mL), despite no cytotoxicity was observed against the normal parental cell line, WI-38 ($EC_{50} > 4000$ ng/mL) (Fig. 2).

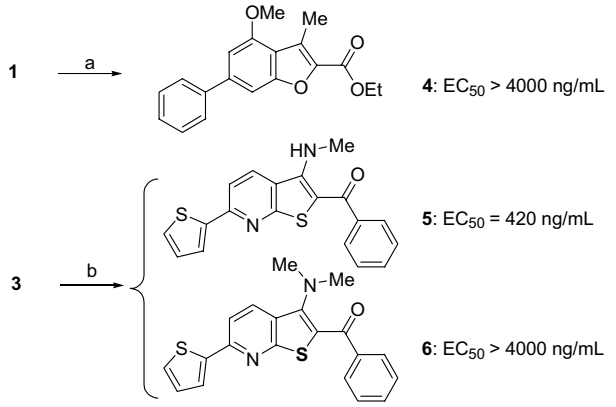
After further synthetic study, (4-hydroxy-3-methyl-6-phenylbenzofuran-2-yl)phenylmethanone (**2**) was derived as a lead compound that did not contain a biologically unstable ester group.[1,2] After further screening, (3-amino-6-thiophen-2-yl-thieno[2,3-*b*]pyridin-2-yl)-phenylmethanone (**3**) was also discovered as a hit compound in the same screening (Fig. 3).

The chemical structures of these two compounds (**2** and **3**) can be considered to have bioisoteric substructures except functional groups on central fused rings. A benzene ring is usually biologically equivalent to a thiophene or a pyridine ring, and the dimension of furan ring is almost equal to thiophene ring. Although these hits were discovered by a cell-based screening and the target molecules of these compounds were unidentified, lead generation based on these two hit compounds was attempted.

3. Evaluation of different functional groups

The major difference between hit compound **2** and **3** is the functional groups connected to the central fused ring and their positions. Compound **3** possesses an amino group instead of the methyl group in compound **2**. Since an amino group and not a hydroxy group was present in **3**, this may explain the hydrophilic properties of those functional groups are interchangeable.

In order to evaluate these functional groups in terms of biological activity, derivatives whose functional group was masked with methyl groups were synthesized and the cytotoxicity of the resulting compounds was tested (Scheme 1). Methyl ether **4**, which was prepared from the original hit compound **1**, completely lost its cytotoxicity. Monomethylamine **5** derived from **3** still showed weak activity, and the cytotoxicity of dimethylamine **6**, which had no hydrogen atom on the nitrogen, had completely disappeared. These results suggest that the functional groups contributed largely to the biological activity, and that one might replace the other if these two hit compounds bound to the same target molecule.

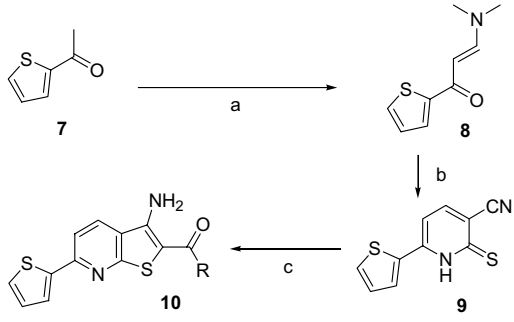


Scheme 1. Synthesis and cytotoxicity of methylated hit compounds. (a) 1.5 equiv NaH (55% dispersion in mineral oil), then 2.0 equiv MeI in DCM, 25 °C, 1.0 h (98%); (b) 1.1 equiv Bu^{t}OK and 1.1 equiv MeI in DMF, 25 °C, 1.0 h (**5**: 79%, **6**: trace).

Although a thorough investigation of the domain comprising the central fused ring and its substituents would be required for further structure exploitation of the hit compounds, the synthetic modification of this domain was considered challenging. The phenyl ketone moiety of compound **3** was substituted instead to investigate the relationship between the structure and cytotoxic activity of the derivatives. The biological activity was compared with that of benzofuran (**2**) derivatives, which have already been described in our previous report.[1,2] The derivatives of compound **3** were readily synthesized from acetylthiophene (**7**) as shown in Scheme 2.[3,4]

4. Structure–activity relationship

The cytotoxicities of the synthesized derivatives are listed in Table 1. While phenyl ketone (**11a** [**2**]) without a



Scheme 2. Synthesis of thieno[2,3-*b*]pyridine derivatives. (a) 4.0 equiv $Me_2NCH(OMe)_2$ neat, reflux, 7.0 h (60%); (b) 1.0 equiv $NCCH_2$-$CSNH_2$ and 2.2 equiv Bu^{t}OK in DMF, 50 °C, 3.0 h (93%); (c) 1.0 equiv $BrCH_2COR$, 1.0 equiv aqueous 10% KOH in DMF 25 °C, and then 1.0 equiv aqueous 10% KOH 25 °C, 1.0 h (**10b**:[8] 67%; **10c**: 61%; **10d**: 55%; **10f**: 78%; **10g**: 82%; **10h**: 77%; **10i**: 63%; see also Table 2).

substituent on the benzene ring showed relatively weak cytotoxicity, its derivatives, which had a methoxy group at the *ortho* position (**11b**), were moderately active. The methoxy group at the *para* position (**11c**) also improved the biological activity to some extent, and **11d**, which has two methoxy groups at the *ortho* and *para* positions, was the most potent benzofuran derivative. Ethyl ester (**11f** [**1**]) and methyl ketone (**11g**) were another class of highly potent derivatives.[1,2]

In addition, the simple phenyl ketone (**10a** [**3**]) was relatively potent. Although a derivative with a methoxy group at the *ortho* position (**10b**[8]) showed cytotoxicity, derivatives with a *para* methoxy group (**10c** and **d**) considerably lost their biological activity. Although the dimethoxy phenyl group was recognized to be the best substructure of benzofuran derivative (**11d**), it did not perform as a substructure of thienopyridine derivatives (**10d**). In contrast with **11f** [**1**] and **11g**, neither ethyl ester (**10f**) nor methyl ketone (**10g**) showed cytotoxicity.

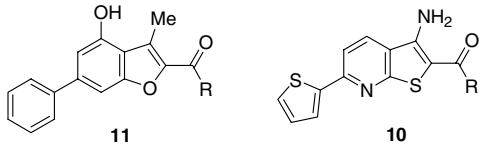
5. Conformational differences of the hit compounds

As the structure–activity relationship of the derivatives with two similar core structures (**10** and **11**) did not show the same profile, the core structures were further differentiated. The benzofuran derivatives (**11**) have a benzene substituent at the sixth position, and thienopyridine derivatives (**10**) were made to have a thiophene ring instead. Although these substituents are usually recognized to be biologically equivalent and interchangeable, the steric hindrance of the *ortho* hydrogen atoms in the thiophene ring and pyridine of **10** was estimated to be very small because of the lack of two hydrogen atoms (Fig. 4). The semi-empirical AM1 calculations for energy minimization indicated that the torsional angle of thienopyridine and thiophene rings of **10g** was almost flat, the corresponding angle of benzofuran derivative **11g**, however, was approximately 41.8°.[5] This result was consistent with that of simple biphenyl.[6]

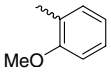
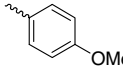
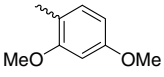
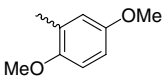
As this calculation suggests that the structure difference between thiophene and benzene may not be negligible as a bioisosteric structure in this case, the cytotoxicity of derivatives that have a benzene ring instead of a thiophene ring (**12** and **13**) was tested.[7] To our surprise, these compounds were revealed to have no biological activity at all, and this result suggested that the core structure of **10** and **11** were not bioisosteric (Fig. 5).

In order to discover the most potent substituent of the thienopyridine derivatives (**10**), another investigation was carried out independently from the structure–activity relationship studies of benzofuran derivatives (**11**) (Table 2). While the bioactivity of the benzofuran derivative was improved by placing a methoxy group in the *ortho* and/or a *para* position (**11b** and **c**), placing it in the *meta* position decreased activity.[1] In contrast to the benzofuran derivatives, some of the thienopyridine derivatives with a *meta* substituent (**10j**[10] and **k**[11]) showed increased cytotoxicity. The most potent derivative was the compound with a *meta* fluoro substituent (**10k**[11]), which was more potent than all the synthesized benzofuran derivatives. Introduction of a *para* substituent (**10n**, **o**, **p,** and **q**) weakened the biological activity.

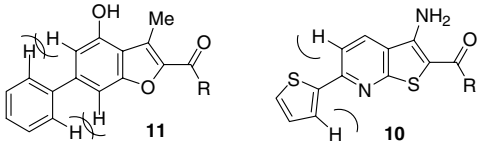
Table 1. The structure–activity relationship of benzofuran derivatives (**11**) and thienopyridine derivatives (**10**)



11 **10**

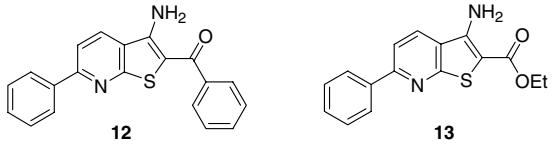
Compd	R	Cytotoxicity of **11** EC_{50}, ng/mL[a,b]	Cytotoxicity of **10** EC_{50}, ng/mL[a]
a		600 (compound **2**)	90 (compound **3**)
b[8]		90	59
c		280	770
d		40	520
e[9]		90	79
f	OEt	40 (compound **1**)	>4000
g	Me	80	2700
h	Et	270	890
i	*tert*-Bu	410	570

[a] Selectivity against a tumor cell line was confirmed.
[b] The compounds and corresponding biological data are reported in Ref. 1.



11 **10**

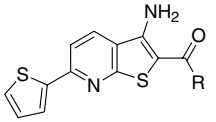
Figure 4. The steric hindrance of the protons at *ortho* position of two benzene rings (**11**) twists the connecting bond to an approximately 41.8° torsional angle. The thiophene and pyridine ring (**10**), however, produces almost no steric interaction.



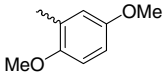
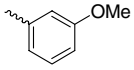
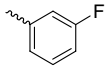
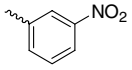
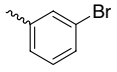
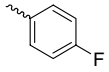
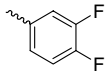
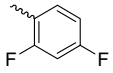
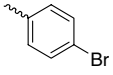
12 **13**

Figure 5. Derivatives of compound **10** whose thiophene ring is replaced with bioisosteric benzene ring.

Table 2. Biological activities of thienopyridine derivatives (**10**)



10

Compd	R	Yield (%) from **9** to **10**[a]	Cytotoxicity of **10** EC_{50}, ng/mL[b]
a		93	90
e[9]		59	79
j[10]		63	30
k[11]		78	25
l		68	150
m		75	500
n		64	1800
o		58	>4000
p		45	2300
q		46	2800

[a] Yields were calculated based on **9**.
[b] Selectivity against a tumor cell line was confirmed.

In conclusion, although the lead generation from two hit compounds **2** and **3** was unsuccessful, biologically potent derivatives from each hit compound were discovered. These compounds will be evaluated in the next stage of biological tests.

Acknowledgements

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5. SYBYL 6.6 (Tripos, Inc.) was used as a front-end.
6. Streitwieser, A.; Heathcook, C. H.; Kosower, E. M. *Introduction to Organic Chemistry*. 4th ed.; Macmillan Publishing Co.: New York, NY, 1992, pp 1051–1052.
7. The tested compounds were purchased from ChemBridge Corporation, San Diego, CA, USA.
8. Compound **10b**: orange powder. Mp 203–204 °C, ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.63 (d, 1H, $J = 8.1$ Hz), 8.21 (br s, 2H), 8.03 (d, 1H, $J = 8.4$ Hz), 7.97 (d, 1H, $J = 3.8$ Hz), 7.74 (d, 1H, $J = 5.1$ Hz), 7.49 (t, 1H, $J = 8.1$ Hz), 7.32 (d, 1H, $J = 7.6$ Hz), 7.23–7.15 (m, 2H), 7.05 (t, 1H, $J = 7.6$ Hz), 3.78 (s, 3H); MS(APCI, *m*/*z*) 367 $(M+1)^+$.
9. Compound **10e**: yellow powder. Mp 107–109 °C, ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.63 (d, 1H, $J = 8.6$ Hz), 8.22 (br s, 2H), 8.03 (d, 1H, $J = 8.9$ Hz), 7.97 (dd, 1H, $J_1 = 3.5$ Hz, $J_2 = 1.1$ Hz), 7.75 (dd, 1H, $J_1 = 5.1$ Hz, $J_2 = 1.1$ Hz), 7.22 (dd, 1H, $J_1 = 5.1$ Hz, $J_2 = 1.1$ Hz), 7.12–7.02 (m, 2H), 6.89 (d, 1H, $J = 2.7$ Hz), 3.75 (s, 3H), 3.73 (s, 3H); MS(APCI, *m*/*z*) 397 $(M+1)^+$.
10. Compound **10j**: yellow powder. Mp 222–223 °C, ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.67 (d, 1H, $J = 8.6$ Hz), 8.43 (br s, 2H), 8.06 (d, 1H, $J = 8.6$ Hz), 7.99 (d, 1H, $J = 3.2$ Hz), 7.77 (d, 1H, $J = 3.2$ Hz), 7.47 (t, 1H, $J = 7.6$ Hz), 7.37 (d, 1H, $J = 7.6$ Hz), 7.30 (s, 1H), 7.22 (dd, 1H, $J_1 = 4.9$ Hz, $J_2 = 3.8$ Hz), 7.16 (dd, 1H, $J_1 = 8.1$ Hz, $J_2 = 2.7$ Hz), 3.84 (s, 3H); MS(APCI, *m*/*z*) 367 $(M+1)^+$.
11. Compound **10k**: yellow solid. Mp 221–222 °C, ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.68 (d, 1H, $J = 8.6$ Hz), 8.49 (br s, 2H), 8.07 (d, 1H, $J = 8.6$ Hz), 7.99 (dd, 1H, $J_1 = 3.8$ Hz, $J_2 = 1.1$ Hz), 7.77 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 1.1$ Hz), 7.68–7.54 (m, 3H), 7.49–7.44 (m, 1H), 7.22 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 1.1$ Hz); MS(APCI, *m*/*z*) 355 $(M+1)^+$.