



## Synthesis and structure–activity relationships of novel benzofuran farnesyltransferase inhibitors

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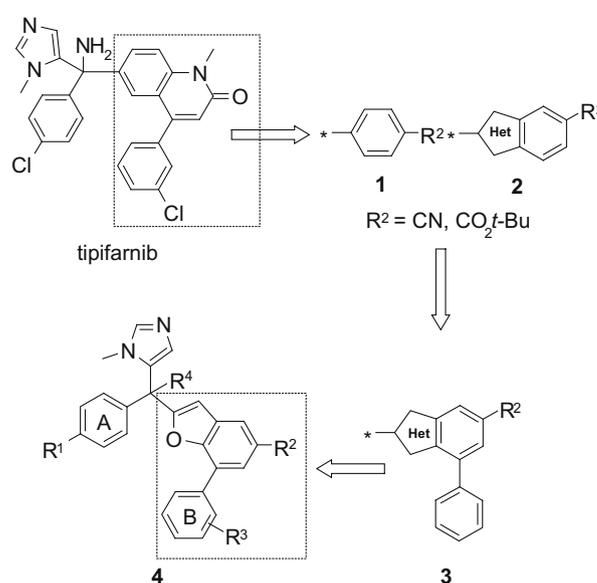
### ABSTRACT

A series of benzofuran-based farnesyltransferase inhibitors have been designed and synthesized as anti-tumor agents. Among them, **11f** showed the most potent enzyme inhibitory activity ( $IC_{50} = 1.1$  nM) and antitumor activity in human cancer xenografts in mice.

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Membrane-bound GTP binding proteins (G-proteins) act as molecular switches to regulate cell growth by cycling between the inactive GDP-bound state and the active GTP-bound state. In tumor cells, the constitutive activation of some G-proteins contributes to their malignant growth properties. In normal cells, this switching mechanism is highly regulated and G-proteins are found predominantly in their inactive GDP-binding state. All of these G-proteins originally have the CAAX tetrapeptide motif (C: Cys, A: an aliphatic amino acid, X: Ser, Met, Gln, Ala) at their C-terminal.<sup>1</sup> Farnesyltransferase (FTase) enzymes recognize this CAAX tetrapeptide motif and transfer the farnesyl group to the cysteine thiol. This farnesylation is critical for membrane binding and the biological function of G-proteins.<sup>2</sup> In the last decade, many classes of FTase inhibitors have been reported and discussed as antitumor agents.<sup>3</sup> Tipifarnib (R115777) is one of the most potent FTase inhibitors that is undergoing clinical trials.<sup>4</sup> In order to discover new potent FTase inhibitors, we searched for a novel core template in place of the quinolinone moiety of tipifarnib. The first templates, 4-substituted phenyl rings **1** and bicyclic structures **2** such as benzofuran and benzothiophene, displayed moderate FTase inhibition but no antiproliferative activity against tumor cells. Further modification studies resulted in the second templates, the biaryl series **3** which showed weak activity against both FTase and tumor cells. We report herein our design and synthesis of the series of benzo-

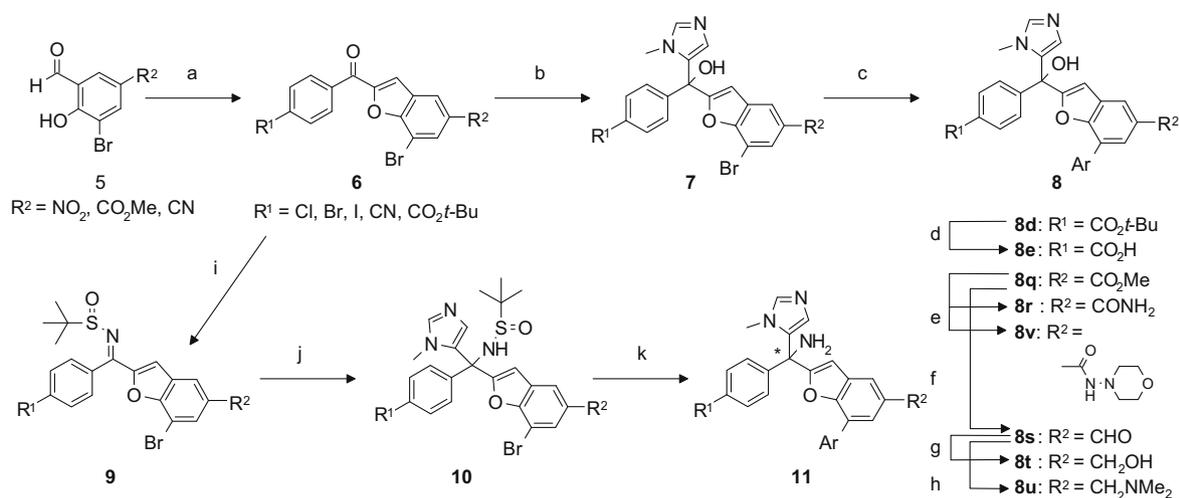
furan FTase inhibitors **4** using the X-ray structure of human FTase. (Fig. 1).



**Figure 1.** Modification of tipifarnib from core structures **1** through **3** to give benzofuran derivative (**4**).

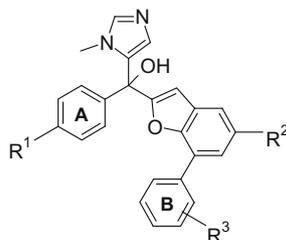
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**Scheme 1.** Reagents and conditions: (a) 4-substituted phenacyl bromide,  $\text{K}_2\text{CO}_3$ ,  $\text{CH}_3\text{CN}$ , reflux, 1 h, 36–80%; (b) 1-methyl-2-TES-imidazole,  $n\text{-BuLi}$ , THF,  $-78^\circ\text{C}$ , 55–81%; (c)  $\text{ArB(OH)}_2$ ,  $\text{Pd}_2(\text{dba})_3$ ,  $\text{PPh}_3$ ,  $\text{Na}_2\text{CO}_3$ , toluene/MeOH/ $\text{H}_2\text{O}$ ,  $80^\circ\text{C}$ , 17–97%; (d) TFA/ $\text{CH}_2\text{Cl}_2$ , rt, 1 h, 73%; (e) i— $\text{LiOH}$ , THF/ $\text{H}_2\text{O}$ , 46%; ii— $\text{WSCI}$ , HOBt, DIPEA, appropriate amine, THF, 51% for **8r**, 58% for **8v**; (f) i— $\text{LiOH}$ , THF, rt, ii— $\text{HNMeOMe}$ ,  $\text{WSCI}$ , HOBt, DIPEA, DMF, rt, 56%, (iii) LAH, THF,  $-78^\circ\text{C}$ , 96%; (g)  $\text{NaBH}_4$ , MeOH, rt, 1.5 h, 90%; (h) dimethylamine,  $\text{NaB(OAc)}_3\text{H}$ , AcOH, THF, rt, 72%; (i) 2-methyl-2-propanesulfonamide,  $\text{Ti(OEt)}_4$ , toluene,  $70^\circ\text{C}$ , 2 h, 52–70%, (j) i—1-methyl-2-TES-imidazole,  $n\text{-BuLi}$ , THF, rt, 3 h, 56–76%, ii—chiral resolution by chiral HPLC system; (k) i—2-(substituted phenyl)-[1,3,2]dioxaborinane,  $\text{Pd(PPh}_3)_4$ ,  $\text{K}_3\text{PO}_4$ , DMF,  $100^\circ\text{C}$ , 1 h, 81–98%, ii— $\text{HCl}$ , THF, rt, 60–93%.

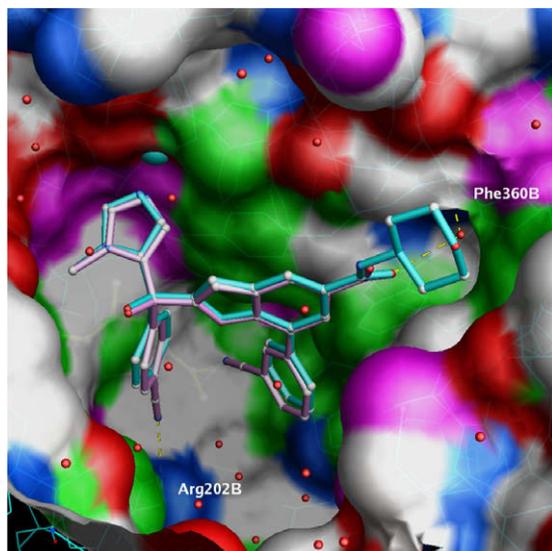
**Table 1**  
Activity of benzofuran ( $R = \text{OH}$ ) series



Compound	$R^1$	$R^2$	$R^3$	FTase/K-ras <sup>a</sup> IC <sub>50</sub> (nM)	QG56 IC <sub>50</sub> (nM)
<b>8a</b>	Cl	$\text{NO}_2$	H	170	1885
<b>8b</b>	Br	$\text{NO}_2$	H	360	1477
<b>8c</b>	I	$\text{NO}_2$	H	360	>10000
<b>8d</b>	$\text{CO}_2\text{t-Bu}$	$\text{NO}_2$	H	>1000	6248
<b>8e</b>	$\text{CO}_2\text{H}$	$\text{NO}_2$	H	850	>10000
<b>8f</b>	$\text{NO}_2$	$\text{NO}_2$	H	30	547
<b>8g</b>	OMe	$\text{NO}_2$	H	250	4967
<b>8h</b>	CN	$\text{NO}_2$	H	6.4	1477
<b>8i</b>	CN	$\text{NO}_2$	2-F	3.3	89.5
<b>8j</b>	CN	$\text{NO}_2$	3-OMe	8.5	38.8
<b>8k</b>	CN	$\text{NO}_2$	3-CN	2.8	22.9
<b>8l</b>	CN	$\text{NO}_2$	3-Me	11	23.2
<b>8m</b>	CN	$\text{NO}_2$	3-F	6.3	145
<b>8n</b>	CN	$\text{NO}_2$	4-OMe	4	36
<b>8o</b>	CN	$\text{NO}_2$	4-CN	3.4	32.6
<b>8p</b>	CN	$\text{NO}_2$	4-F	7.2	158
<b>8q</b>	CN	$\text{CO}_2\text{Me}$	3-OMe	3.2	36.3
<b>8r</b>	CN	CONH <sub>2</sub>	3-OMe	0.9	8.2
<b>8s</b>	CN	CHO	3-OMe	2	15.2
<b>8t</b>	CN	$\text{CH}_2\text{OH}$	3-OMe	1	16.8
<b>8u</b>	CN	$\text{CH}_2\text{NMe}_2$	3-OMe	11	142
<b>8v</b>	CN		3-OMe	2.6	nt <sup>b</sup>
<b>8w</b>	Cl		H	6.4	73.6
<b>8x</b>	CN	CN	H	2.4	14.5

<sup>a</sup> Human farnesyltransferase.

<sup>b</sup> Not tested.

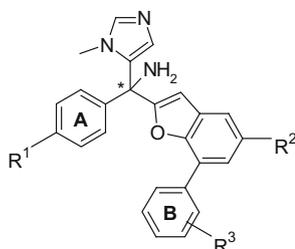


**Figure 2.** Crystal structure of FTase with **8k** (pink) and **8w** (cyan). Cyano group on A-ring of **8k** makes a hydrogen bond (3.1 Å) to Arg202B. There is a bridging water between the carbonyl oxygen on benzofuran of **8w** and amide of Phe360B.

The general synthesis of benzofuran derivatives **4** is outlined in Scheme 1. Intermediate **6** was provided from substituted benzaldehyde **5** by coupling with the appropriate phenacyl bromide under basic condition.<sup>5</sup>

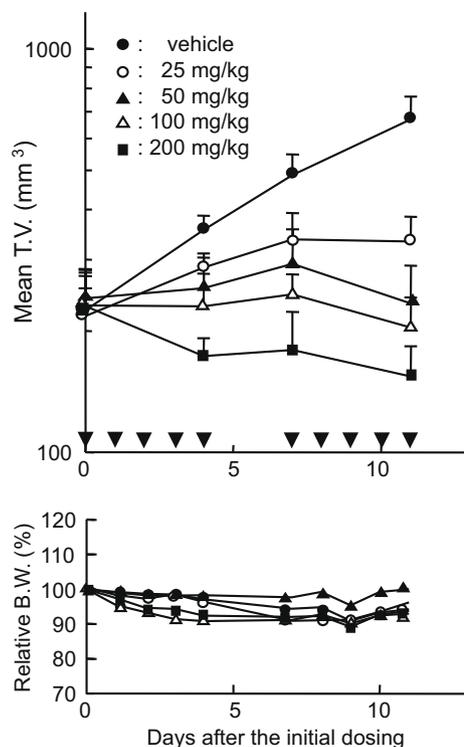
Compound **7** was prepared by treatment of 1-methyl-2-TES-imidazole with *n*-BuLi followed by the addition of the appropriate **6**. A variety of aryl substituents were introduced into **7** by Suzuki coupling reaction to give biaryl **8** as a racemate ( $R^4 = \text{OH}$ ). The *t*-butyl group in **8d** was hydrolyzed to carboxylic acid (**8e**). The methyl ester group in **8q** was converted to amides (**8r** and **8v**) and an aldehyde (**8s**) which was further converted to an alcohol (**8t**) and amino derivative (**8u**). The synthesis of the other benzofuran series **11** ( $R^4 = \text{NH}_2$ ) is also described in Scheme 1. Sulfineimine **9** was prepared by tetraethyl orthotitanate mediated condensation of **6** with sulfineamide.<sup>6</sup> Arylation of **9** with 1-methyl-2-TES-imidazole-5-lithium followed by chiral resolution using an HPLC system with a chiral column afforded **10**.<sup>7,8</sup> Suzuki coupling of **10** with 2-(substituted phenyl)-[1,3,2]dioxaborinane, followed by deprotection of the *t*-butylsulfine group under acidic condition gave the desired product **11**.

**Table 2**  
Activity of benzofuran ( $R^4 = \text{NH}_2$ ) series



Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Chiral	FTase/K-ras <sup>a</sup> IC <sub>50</sub> (nM)	QG56 IC <sub>50</sub> (nM)
<b>11a</b>	CN	NO <sub>2</sub>	H	S	1.5	5.9
<b>11b</b>	CN	NO <sub>2</sub>	H	R	49	206.1
<b>11c</b>	CN	NO <sub>2</sub>	3-OMe	S	0.8	1.1
<b>11d</b>	CN	CN	3-OMe	S	1.2	1.5
<b>11e</b>	CN	CN	3-CN	S	0.7	2
<b>11f</b>	CN	CN	3-F	S	1.1	2

<sup>a</sup> Human farnesyltransferase.



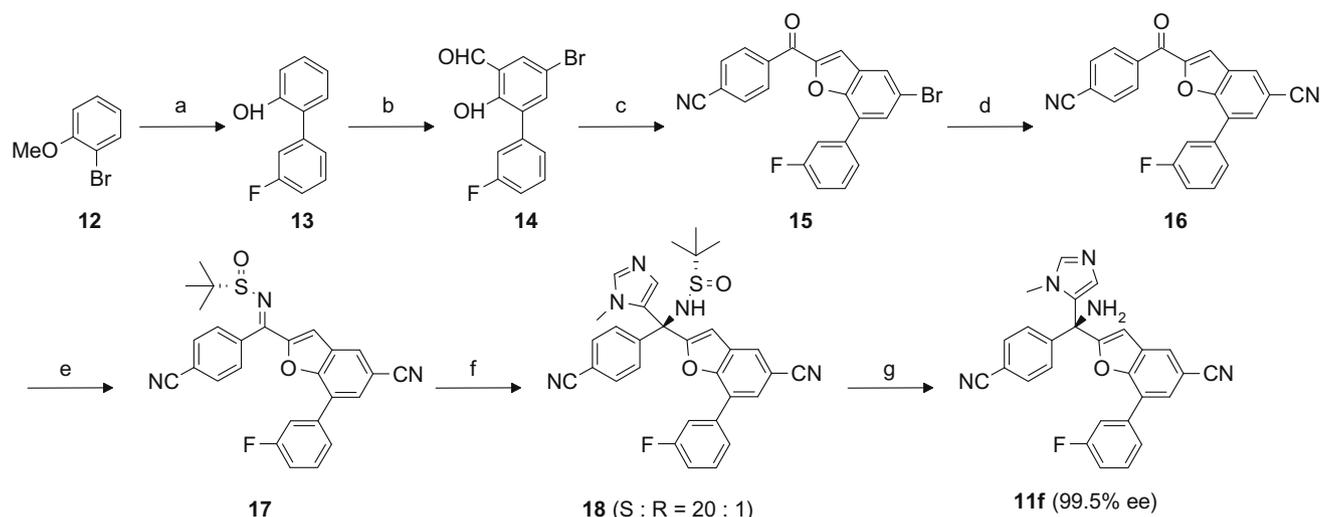
**Figure 3.** Effect of **11f** on QG56 tumor growth in mouse xenograft models. Tumor volume (TV) was measured twice/week (upper graph). Body weight (BW) was measured 5 times/week and relative BW calculated (lower graph).

The enzyme inhibitory activity (FTase/K-ras) and antiproliferative activity against human non-small cell lung carcinoma (QG56) of compounds (**8**) were evaluated.<sup>9</sup> The results from compound **8** having a hydroxyl group at the chiral center are shown in Table 1. Our first lead compound (**8a**) showed weak FTase inhibition ( $\text{IC}_{50} = 170 \text{ nM}$ ) and low antiproliferative activity ( $\text{IC}_{50} = 1885 \text{ nM}$ ). Introduction of a cyano group (**8h**) or a nitro group (**8f**) on the A-ring resulted in significant increase of enzyme inhibitory activities (6.4 and 30 nM respectively). Replacement of the chlorine atom with another halogen (**8b** and **8c**), *t*-butylester (**8d**), carboxylic acid (**8e**), or methoxy (**8g**) resulted in reduction of enzyme inhibitory activity (from 250 to 1000 nM). Further modification of the substituent R<sup>3</sup> in the compound (**8h**) was carried out. The compound having a 3-CN group (**8k**) showed potent enzyme inhibitory activity (2.8 nM) and antiproliferative activity (23 nM). The X-ray crystal structure of FTase complexed with **8k** shown in Figure 2 suggests that the hydrogen bonding between the cyano group on the A-ring and Arg202B improves the enzyme inhibitory activity.<sup>10</sup> No obvious interaction of the cyano group on the B-ring with the enzyme was seen. In fact, all the compounds having small substituents on the B-ring (**8i–p**) showed similar FTase inhibition (2.8–11 nM) compared with a non-substituted phenyl (**8h**, 6.4 nM) but exhibited improved antiproliferative activity against QG56 cells (23–158 nM). The effects of the substituent R<sup>2</sup> on the benzofuran ring of compound **8** are summarized in Table 1 (note **8q–v**). Interestingly, replacement of the nitro group

**Table 3**  
Pharmacokinetics of **11f** in mice<sup>a</sup>

	Cmax (μg/mL)	AUC <sub>0–24 h</sub> (μg h/mL)	CL/F (mL/min/kg)	t <sub>1/2</sub> (h)	BA (%)
IV 25 mg/kg	—	15.4	27.0	1.2	—
PO 25 mg/kg	1.57	10.2	40.8	2.4	66
PO 100 mg/kg	5.01	31.3	41.0	12.3	51

<sup>a</sup> Vehicle: 0.06 M HCl, 0.44% CMC distilled water.



**Scheme 2.** Reagents and conditions: (a) *i*-4-fluoroboronic acid, Pd(OAc)<sub>2</sub>, PPh<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub>, MeOH, reflux, 14 h, ii-BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C to rt, 87%; (b) *i*-paraformaldehyde, MgCl<sub>2</sub>, Et<sub>3</sub>N, THF, reflux, 2 h, ii-NBS, CHCl<sub>3</sub>, reflux, 2 h; (c) 4-cyanophenacyl bromide, K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN, reflux, 1 h, 78% from 13; (d) Zn(CN)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, DMF, 100 °C, 4 h, 90%; (e) (*S*)-2-methyl-2-propanesulfonamide, Ti(OEt)<sub>4</sub>, toluene, 70 °C, 2 h, 90%; (f) 5-bromo-*N*-methyl-imidazole, EtMgBr, CH<sub>2</sub>Cl<sub>2</sub>, THF, rt, 3 h, 80%; (g) *i*-4-M HCl in THF, rt, 1 h; ii-crystallization from H<sub>2</sub>O/MeOH/EtOH, 70%.

with a carbonyl function such as a methylester (**8q**) amide (**8r**, **8v**), or CHO (**8s**), showed 3- to 8-fold higher enzyme inhibitory activity (0.9–3.2 nM) than **8j** (8.5 nM). X-ray crystal structure of FTase with **8w** (Fig. 2) revealed a bridging water molecule forming a hydrogen bonding between the oxygen atom of the carbonyl in the substituent R<sup>2</sup> on the benzofuran and the amide backbone of the Phe360B.<sup>10</sup> Thus, the compounds with a carbonyl moiety have strong activity against FTase (**8a** vs. **8w**). Compounds **8t** and **8x** also showed strong enzyme inhibitory activity (1.0 and 2.4 nM, respectively, with FTase). These results indicate that not only a carbonyl moiety but also a hydrogen bond accepting (HBA) group is essential to increase the enzyme inhibitory activity. **8x** has strikingly strong cellular activity (14.5 nM) even though it has no substituent (R<sup>3</sup> = H) on the B-ring.

The results from *in vitro* evaluation of compound **11** (R<sup>4</sup> = NH<sub>2</sub>) are shown in Table 2. With regard to the stereochemistry of the chiral amino group, the nitro derivative with an (*S*)-configuration (**11a**) showed 33 times higher enzyme inhibitory activity than the corresponding (*R*)-isomer (**11b**). Furthermore, in the (*S*)-isomer series, replacement of the nitro group (R<sup>2</sup>) with a cyano group and the introduction of favorable substituents (R<sup>3</sup>) on the B-ring led us to identify compounds (**11d–f**) showing potent inhibitory activities against both FTase (0.7–1.2 nM) and QG56 cells (1.5–2.0 nM) (Table 2).

The antitumor activity of compounds **11c–f** was evaluated with a human cancer xenograft model showing potent antitumor activity *in vivo*. The *in vivo* efficacy of **11f** in human non-small cell lung carcinoma (QG56) xenograft in mice is shown in Figure 3.<sup>11</sup> **11f** was administered 5 times a week for 2 weeks orally at doses of 25–200 mg/kg per day. **11f** showed strong tumor regression with no noticeable body weight loss at the dose of 200 mg/kg.

After evaluation of several parameters including solubility, biological stability, and pharmacokinetics we identified **11f** as a clinical candidate. The pharmacokinetics data in mice of **11f** is presented in Table 3. **11f** was administered intravenously at dose of 25 mg/kg and orally at doses of 25 and 100 mg/kg. Oral bioavailability of **11f** was more than 50% and AUC was increased dose-dependently. It has been confirmed that there was a good correlation between AUC and tumor growth inhibition *in vivo*.

The chiral synthesis of **11f** was established by modification of the synthetic route shown in Scheme 1 and outlined in Scheme 2. The most dramatic improvement was made in the diastereose-

lective addition of an imidazole moiety into chiral sulfineimine **17** to give **18**. Although the addition of 1-methyl-2-TES-imidazole treated with *n*-BuLi resulted in low diastereoselectivity (S:R = 2:1), the Grignard condition, treated by EtMgBr, showed dramatically improved selectivity (S:R = 20:1). This new synthetic process has significant advantage for further scale-up synthesis because all the intermediates can be purified by crystallization without using column chromatography.<sup>12</sup>

In summary, we discovered a series of benzofuran compounds showing potent FTase inhibitory activity. A cyano group at the para-position of the A-ring (R<sup>1</sup> position) showed excellent FTase inhibition. Substituent of the functional group at the B-ring (R<sup>3</sup> position) increased antiproliferative activity against human cancer cell lines. Introduction of an HBA group at the R<sup>2</sup> position resulted in improved inhibitory activities both in enzyme and cellular assays. X-ray crystal structure of FTase with **8k** and **8w** revealed important hydrogen bonding of (1) the cyano group at the R<sup>1</sup> position of **8k** with Arg202 and (2) the carbonyl group at the R<sup>2</sup> position of **8w** with a water molecule bound to the protein. Compound **11f**, a clinical candidate, showed strong tumor regression in the QG56 human NSCLC xenograft model with no noticeable body weight loss. **11f** also showed good pharmacokinetics profile in mice. Furthermore, we established diastereoselective synthesis of **11f** applicable to large scale synthesis.

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8. Prepared diastereomers were separated and purified by chiral column chromatography over DAICEL CHIRACEL OD (eluent: hexane/2-propanol 1:1).
9. Assay methods described in: Sakaitani, M.; Masubuchi, K.; Kohchi, M.; Hyoudoh, I.; Asoh, K.; Asai, M. WO Patent 04/037816, 2004.
10. Crystals of rat FTase with compounds were obtained within a week under the crystallization condition of 10% (w/v) PEG 6000, 0.2 M Mg acetate, 0.1 M Na acetate buffer (pH4.5) at 4 °C. The structures in complex with compounds **8k** and **8w** were solved at 2.6 and 2.4 Å, respectively, and their coordinates have been deposited in the Protein Data Bank under the access codes **2ZIS** and **2ZIR**.
11. Materials and methods human tumor cell line: The QG56 human non-small cell lung cancer cell line was cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum. Animals: 5-week-old male athymic nude mice were subjected to the experiment. Compound **11f** was dissolved in 0.06 M HCl, 0.44% CMC distilled water. Determination of antitumor activities: The in vivo evaluation procedure for anticancer drugs was based on the National Cancer Institute (NCI) guidelines. A single cell suspension of QG56 ( $5.0 \times 10^6$  cells per mouse) was inoculated subcutaneously into the right flank of each mouse. The tumor volume was estimated from two-dimensional measurements using the equation  $ab^2/2$ , where  $a$  and  $b$  represent tumor length and width, respectively. Drug administration was initiated on the day of grouping (day 0). **11f** was administered orally once a day from days 0 to 4 and days 7 to 11.
12. **11f** was analyzed by chiral column chromatography over DAICEL CHIRALPAK® AS (eluent: hexane/ethanol, 4:1) to determine enantiomeric excess.