Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 3069–3072

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Novel Class of Highly Potent, Selective, and Non-Peptidic Inhibitor of Ras Farnesyltransferase (FTase)

Hyunil Lee, Jinho Lee, Sangkyun Lee, Youseung Shin, Wonhee Jung, Jong-Hyun Kim, Kiwon Park, Kwihwa Kim, Heung Soo Cho, Seonggu Ro, Sunhwa Lee, ShinWu Jeong, Taesaeng Choi, Hyun-Ho Chung and Jong Sung Koh*

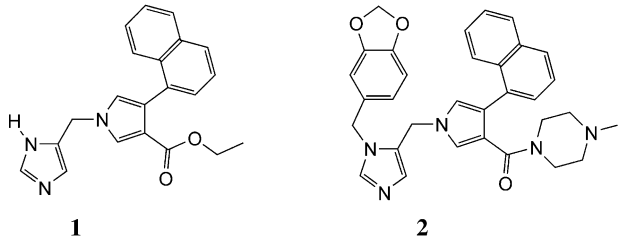
Life Science R&D, LGCI, Science Town, Taejon 305-380, Republic of Korea

Received 2 July 2001; accepted 17 September 2001

Abstract—Design, synthesis and structure–activity relationship of a class of aryl pyrroles as farnesyltransferase inhibitors are described. In vitro and in vivo evaluation of a panel of these inhibitors led to identification of **2** (**LB42908**) as a highly potent (IC_{50} = 0.9 nM against H-Ras and 2.4 nM against K-Ras) antitumor agent that is currently undergoing preclinical studies.

Mutated versions of three human *ras* genes are frequently found in many human cancers, most notably cancers of the pancreas, colon, and lung, implying an important role for aberrant Ras function in human tumor growth.[1–4] The frequency of mutations is not uniformed with three human *ras* genes. Thus, although mutated H-*ras* was the first identified, and consequently has been the most heavily studied, mutations in K- and N-*ras* are much more frequently seen in human tumors. The p21 Ras oncogenic products are synthesized as cytosolic proteins, which undergo post-translational modifications for attachment of the normal as well as mutated Ras proteins to the membrane.[5,6] A key step in a series of posttranslational modifications of the oncogenic product Ras is farnesylation of the thiol group of cystein located at the C-terminal CAAX sequence in Ras proteins. The enzyme farnesyltransferase (FTase) catalyzes this reaction.[7,8] Inhibitors of farnesylation (FTIs) would therefore have potential as anticancer agents for tumors in which a *ras* gene is oncogenically mutated.[9–11] In fact, the efficacy of FTIs in murine models has been demonstrated by their ability to inhibit H-, K-, and N-*ras*-dependent tumor growth in nude mice and to induce tumor regression in H- and N-*ras* transgenic mice.[12–14]

Numerous structurally diverse classes of FTIs that mimic tetrapeptide of Ras C-terminal CAAX motif have been reported in the literatures.[11,15–18] Avenues toward improving the biological properties of a peptidomimetic inhibitor have included the use of non-peptide surrogates for the central AA portion, deletion of carboxyl containing terminus, and replacement of cystein moiety. Notable examples have featured the substitution of 4-aminobenzoic acid for central hydrophobic dipeptide and the use of an imidazole ring as an alternative to the cystein thiol group, which is believed to be involved in an important binding interaction with Zn++ of FTase.[16,17] However, the peptidic nature of these inhibitors was considered as a liability for clinical application. Our efforts focused on the discovery of more stable and druglike molecules, with the specific aims of finding a surrogate for the thiol as well as non-peptidic template to which the putative zinc ligand could be attached. In our search for a suitable template, we have investigated pyrroles (Fig. 1). The hypothetical pharmacophore model of pyrrole series features an aromatic binding interaction, the spacer for the central



1 **2**

Figure 1. Structures of lead compound **1** and the potent FTase inhibitor **2** (**LB42908**).

*Corresponding author. Tel.: +82-42-866-2114; fax: +82-42-863-8010; e-mail: jskob@lgci.co.kr

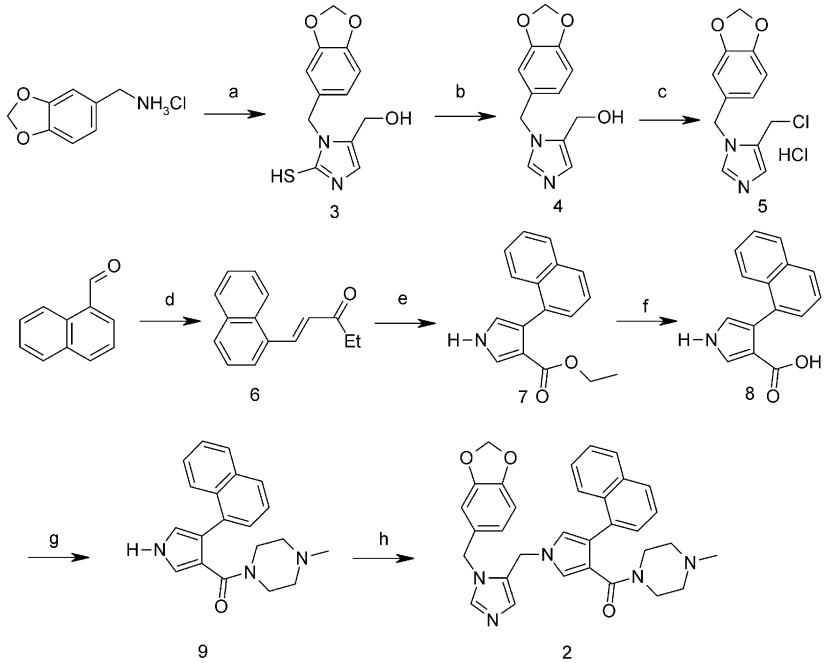
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00624-2

hydrophobic dipeptide, an H-bond acceptor, and zinc binding ligand. Initially, compound **1** was designed and synthesized. Pyrrole **1** inhibited human FTase against H-Ras with IC_{50} of 500 nM. We optimized this compound through the chemical modification along the pyrrole and imidazole rings.[19] In vitro and in vivo evaluation of a panel of these inhibitors has led to identification of [1-{[1-(1,3-benzodioxol-5-ylmethyl)-1H-imidazol-5-yl]-methyl}-4-(1-naphtyl)-1H-pyrrol-3-yl](4-methyl-1-piperazynyl)methanone (**2, LB42908**) as a highly potent antitumor agent that is currently undergoing preclinical studies. Herein, we report the structure–activity relationship (SAR) that has been observed for with this class.

The synthesis of **2** is illustrated in Scheme 1. 5-Hydroxymethyl imidazole **4** was prepared from piperonyl amine and dihydroxyacetone dimer by a literature procedure.[20] Compound **4** was chlorinated with thionyl chloride to give the chloromethyl hydride compound **5** in a quantitative yield. Pyrrole ester **7** was prepared from 1-naphtaldehyde as a starting material by a literature procedure.[21] Compound **7** was converted to the acid **8** with potassium hydroxide. Acid **8** was converted to the corresponding acid chloride with thionyl chloride; the acid chloride was subsequently reacted with 4-methylpiperazine to give pyrrole amide **9**. Compound **9** was coupled with chloromethyl imidazole **5**, to give the representative compound **2**. Compound **1** was synthesized by coupling the trityl protected 5-chloromethyl imidazole hydrochloride with pyrrole **7** and deprotection of the coupled product in the presence of trifluoroacetic acid and triethysilylhydride. **10**–**16** (Table 1) were synthesized analogously by coupling the corresponding 5-chloromethyl imidazole hydrochloride to the corresponding pyrroles.

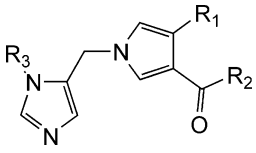
The compounds of this series were first tested for inhibition of the recombinant human FTase and geranylgeranylproteintransferase I (GGTase I). The IC_{50} values of **2** and its related compounds are presented in Table 1. Initial lead compound **1** inhibited FTase with modest activity with IC_{50} value of 500 nM. The dramatic boost of inhibitory activity from **12** to **11** is clearly noteworthy, indicating the hydrophobic aromatic substituent (R_1) at C-3 of the pyrrole is very important to the inhibitory potency of the imidazole-containing pyrrole. Other findings on the SARs of this series are as follows: the replacement of ethyl ester of pyrrole (R_2) by a either morpholinyl group or 4-methylpiperazinyl amide group enhanced potency to a great extent; the hydrogen part of the imidazole (R_3) was favorably replaced by methyl group; and noticeably, the replacement of hydrogen (R_3) by a benzyl group greatly enhanced the inhibitory activity of FTase against K-Ras (Table 1). The representative compound **2** exhibited a good selectivity (15,000-fold) for FTase inhibition versus GGTase I inhibition.

To determine the inhibition of anchorage-independent growth of human tumor cells, soft agar growth assays were performed using standard procedures.[13,23] A variety of human tumor cell lines containing activated H-*ras* and K-*ras* were both inhibited in their ability to form colonies in soft agar, an important finding in light of the prevalence of cells harboring mutations in K-*ras* in human carcinomas (Table 2). Finally, in vivo antitumor activity of compound **2**:2HCl was evaluated by testing its ability to inhibit the growth and to regress the size of tumors in nude mice.[24] Compound **2**:2HCl showed significant dose-dependent inhibition of the growth of tumors by both HCT116, human colon carcinoma (containing mutated K-*ras*) at 40 and 60 mg/kg



Scheme 1. Synthesis of **2**: (a) (1) 3-dihydroxyacetonedimer, KSCN, AcOH/*i*-PrOH; (b) HNO_3, $NaNO_2/H_2O$ (75%, two steps yield); (c) $SOCl_2/CHCl_3$ (90%); (d) triethylphosphonoacetate, DBU/CH_3CN (96%); (e) tosylmethylisocyanide, *t*-BuOK/THF (63.5%); (f) KOH/EtOH, reflux (84%); (g) (1) DMF (0.05 equiv), $SOCl_2/CH_2Cl_2$; (2) 4-methylpiperazine (85%); (h) 5, NaH (2.5 equiv)/DMF, 0 °C (80%).

Table 1. Inhibitory activity of **2** and related compound against human FTase and GGTase I[a]



Compd	R_1	R_2	R_3	FTASE IC_{50} (nM) H-Ras	FTASE IC_{50} (nM) K-Ras	GGTase I IC_{50} (nM)
1	Naph	OCH_2CH_2	H	500	ND	ND
10	Naph	Piperidine	H	720	ND	ND
11	Naph	Morpholine	H	30	1830	ND
12	Ph	Morpholine	H	2000	ND	ND
13	Naph	Morpholine	CH_3	3.0	100	>100,000
14	Naph	Morpholine	Benzyl	1.5	2.1	ND
15	Naph	Morpholine	Piperony	0.9	2.0	12,000
16	Naph	$N(CH_3)CH_2CH_2OCH_3$	Piperonyl	1.1	3.5	10,000
2	Naph	4-Methylpiperazine	Piperonyl	0.9	2.4	16,000

IC_{50} values are the means of three experiments.
[a]The farnesyltransferase and geranylgeranytransferase I inhibitory assays were performed as described in Ref 22.

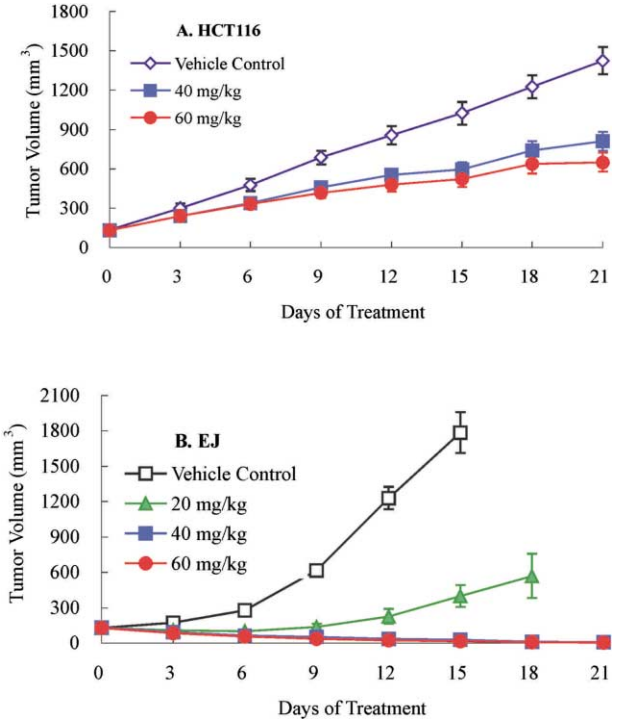


Figure 2. In vivo efficacy studies carried out in human tumor xenograft models in athymic nude mice. Nude mice bearing HCT116 or EJ tumor xenograft were prepared following the method described in ref 24. Drug treatment was initiated after tumor volume reached around 100 mm^3. Mean tumor volume for each group was plotted vs days of treatment. Standard errors of the mean (SES) are indicated as error bars. Panel A shows compound **2**-induced tumor growth inhibition in a HCT116, human colon carcinoma model. Panel B shows compound **2**-induced tumor growth inhibition and tumor regression in an EJ, human bladder carcinoma model.

and EJ, human bladder carcinoma (containing mutated H-*ras*) at 20, 40 and 60 mg/kg, when given to animals orally twice daily for 21 days (Fig. 2 and Table 3). In addition, compound **2**:2HCl regressed, in a dose-dependent manner, the size of tumors by EJ cells into 5.8 and 0.4% of each initial size at dose levels of 40 and 60 mg/kg, respectively (Fig. 2, Panel B).

Table 2. Inhibition of anchorage-independent growth of *ras*-transformed and human tumor cell lines by compound **2**:2HCl[10,23]

	Inhibition of cell growth in soft agar (GI_{50} nM)				
	HT29 K-*ras*	HCT116 K-*ras*	A549 K-*ras*	EJ H-*ras*	T24 H-*ras*
LB42908	4.5	17.6	1.2	0.56	0.45

Table 3. Inhibition (%) of tumor growth relative to control tumors in tumor xenograft nude mice by compound **2**:2HCl

Tumor model	20 mg/kg, bid	40 mg/kg, bid	60 mg/kg, bid
HCT116[a]	—[c]	43[d]	55[d]
EJ[b]	78[d]	Regression[d]	Regression[d]

[a]Determined on day 21.
[b]Determined on day 15.
[c]—not tested.
[d]$p < 0.001$ compared with control tumors by Student's *t*-test.

In summary, we have discovered a novel class of pyrrole based FTase inhibitors that inhibit FTase in the nanomolar range against H-Ras and K-Ras, and devoid of problematic thiol functional groups. Compound **2** (**LB42908**) showed strong activity against human FTase and very good selectivity for FTase over GGTase I. In addition, **LB42908** showed potent inhibitory effects in an anchorage-independent soft agar assays with several human tumor cell lines harboring mutations in K-*ras*. **LB42908** is orally active and shows statistically significant dose dependent inhibition on tumor growth in nude mouse xenograft models.

References and Notes

1. Casey, P. J.; Solski, P. A.; Der, C. J.; Buss, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8323.
2. Rodenhuis, S. *Semin. Cancer Biol.* **1992**, *3*, 241.
3. Bos, J. L. *Cancer Res.* **1989**, *49*, 4682.
4. Barbacid, M. *Ann. Rev. Biochem.* **1987**, *56*, 779.

5. Kato, K.; Cox, A. D.; Hisaka, M. M.; Graham, S. M.; Buss, J. E.; Der, C. J. J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6403.
6. Der, C. J.; Cox, A. D. *Cancer Cells* **1991**, *3*, 331.
7. Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. L.; Brown, M. C. *Cell* **1990**, *62*, 81.
8. Clarke, S.; Vogel, J. P.; Deshenes, R. J.; Stock, J. J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4643.
9. Gibbs, J. B. *Cell* **1991**, *65*, 1.
10. Kohl, N. E.; Mosser, S. D.; deSolms, J.; Giuliani, E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scolinick, E. M.; Oliff, A.; Gibbs, J. B. *Science* **1993**, *260*, 1934.
11. James, J. L.; Goldstein, J. L.; Brown, M. S.; Rawsen, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C., Jr. *Science* **1993**, *260*, 1937.
12. Kohl, N. E.; Wilson, F. R.; Mosser, S. D.; Giuliani, E. A.; deSolms, S. J.; Conner, M. W.; Anthony, N. J.; Holtz, W. J.; Gomez, R. P.; Lee, T.-J.; Smith, R. L.; Graham, S. L.; Hartman, G. D.; Gibbs, J. B.; Oliff, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9141.
13. Kohl, N. E.; Omer, C. A.; Conner, M. W.; Anthony, N. J.; Davide, J. P.; deSolms, S. J.; Giuliani, E. A.; Gomez, R. P.; Graham, S. L.; Hamilton, K.; Handt, L. K.; Hartman, G. D.; Koblan, K. S.; Kral, A. M.; Miller, P. J.; Mosser, S. D.; O'Neill, T. J.; Shaber, M. D.; Gibbs, J. B.; Oliff, A. *Nat. Med.* **1995**, *1*, 792.
14. Mangues, R.; Corral, T.; Kohl, N. E.; Symmans, W. F.; Lu, S.; Malumbres, M.; Gibbs, J. B.; Oliff, A.; Pellicer, A. *Cancer Res.* **1998**, *58*, 1253.
15. Nigam, M.; Seong, C. M.; Qian, Y.; Hamilton, A.; Sebti, S. M. *J. Biol. Chem.* **1993**, *39*, 353.
16. Vasudevan, A.; Qian, Y.; Vogt, A.; Blackovich, M. A.; Ohkanda, J.; Sebti, S. M.; Hamilton, A. D. *J. Med. Chem.* **1999**, *42*, 1333.
17. Sun, J.; Blaskovich, M. A.; Knowels, D.; Qian, Y.; Ohkanda, J.; Bailey, R. D.; Hamilton, A. D.; Sebti, S. M. *Cancer Res.* **1999**, *59*, 4919.
18. Ciccarone, T. M.; MacTough, S. C.; Williams, T. M.; Dinsmore, C. J.; O'Neill, T. J.; Shah, D.; Culberson, J. C.; Koblan, K. S.; Kohl, N. E.; Gibbs, J. B.; Oliff, A.; Graham, S. L.; Hartman, G. D. *Biorg. Med. Chem. Lett.* **1999**, *9*, 1991.
19. Lee, H. I.; Koh, J. S.; Lee, J. H.; Jung, W. H., Shin, Y. S.; Chung, H. H.; Kim, J. H.; Ro, S. G.; Choi, T. S.; Jeong, S. W.; Lee, S. H.; Kim, K. W. WO9928315, 9938862, 9905117, 1999 and 0064891, 2000.
20. Artico, M.; Di Santo, R.; Costi, R.; Massa, S.; Retico, A.; Artico, M.; Apuzzo, G.; Simonetti, G.; Strippoli, V. *J. Med. Chem.* **1995**, *38*, 4223.
21. van Leusen, A. M.; Siderius, H.; Hoogenboom, B. E.; van Leusen, D. *Tetrahedron Lett.* **1972**, 5337.
22. The enzyme inhibitory assay was done by a modification of the procedure described by: Moores, S. L., Schaber, M. D., Mosser, S. D., Rands, E., O'Hara, M. B., Garsky, V. M., Marchall, M. S., Pompliano, D. L., Gibbs, J. B. *J. Biol. Chem.* **1991** *266*, 14603. Briefly, ras proteins (20 μM of H-Ras, 10 μM of K-Ras) were incubated for 30 min at 37°C with FTase enzyme (16.6 nM), [^{3}H]-farnesylpyrophosphate (FPP) (0.3 μM, 20Ci/mmol, Dupont NEN) and serially diluted test compounds at given concentration in the buffer containing 50 mM HEPES, pH 7.4, 250 mM $MgCl_2$, 25 mM KCl, 0.5% *N*-octylglucoside, 50 μM $ZnCl_2$, and 10 mM DTT. The enzyme reactions were then quenched by adding the solution containing 10% HCl in absolute ethanol. The [^{3}H] FPP-incorporated ras was collected by filter binding (25 mm Glass fiber filter, Whatman) and quantitated by scintillation counter. Similarly, inhibitory activity of GGTase I enzyme was determined using Rac protein (40 nM), GGTase I (133 nM) and [^{3}H]-geranylgeranyl pyrophosphate (GGPP, 15 Ci/mmol, Amersham).

IC_{50}: the concentration of compound required to reduce the enzyme-catalyzed incorporation of [^{3}H]FPP or [^{3}H]GGPP into the corresponding substrate proteins by 50%.

For anchorage-independent soft-agar assay, human tumor cell lines were adapted in DMEM media (DMEM, GibcoBRL) containing 10% fetal bovine serum (FBS, GibcoBRL) in advance for 2–3 days after thawing. 0.5% bottom agar containing 5 mL of 1% agar (Sigma), 5 mL of 2×concn of DMEM having 20% FBS and 50 μL of 200× concd of test compounds was dispensed 1 mL/well into 6 well plate and solidified for 1 h at room temperature. Subsequently, 0.33% top agar containing 1.5 mL of 2×concn of DMEM and 23 μL of 200×concn of test compounds was prepared and cooled in sol state to 30 °C. Then, 1.5 mL of human tumor cells (2–5×10^3 cells/mL) was added to the top agar and mixed well. The top agar mixture was dispensed by 1.2 mL/well onto the solidified bottom agar. After two weeks incubation in humidified CO_2 incubator, the number of colonies formed were counted under the microscope. GI_{50} values were then quantitated from the dose–response curves by plotting the percentage of growth of colonies against the $\log_{10}$ of the corresponding concentration for each cell line.
23. Fukazawa, H.; Nakano, S.; Mizuno, S.; Uehara, Y. *Int. J. Cancer* **1996**, *67*, 876.
24. In vivo efficacy studies were carried out in xenograft models. Animals (athymic female nude mice; BALB/c AnNCrj-nu/nu; Charles River Laboratories, Japan; 5–7 weeks old) were subcutaneously inoculated with either HCT116 or EJ tumor cells on the intrascapular region at least 1 week of acclimation period after arrival from vendor. The size of inoculum for both tumor cell lines was 10^7 cells per animal. Once palpable, the size of tumor was measured in three dimensions every three days and the volume was calculated by using the equation, $V=1/6\times\pi\times L\times W\times H$ (V, volume; L, length; W, width; H, height). When the tumor reached a volume of around 100 mm^3, animals were randomly divided into control or treatment groups (8 animals/group) and it was assigned as Day 0. Treatment with compound **2** at the dose level of 20, 40 or 60 mg/kg was initiated on Day 1 and continued twice a day (bid) by gastric gavage for 21 days. Vehicle control received 0.9% saline. Data from tumor with ulcer were not included in the calculation of group mean. Student's *t*-test was used for statistical analysis.