Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1895–1899
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and structure–activity relationships of tetrahydroquinoline-based farnesyltransferase inhibitors

Louis J. Lombardo,* Amy Camuso, John Clark, Krista Fager, Johnni Gullo-Brown, John T. Hunt, Ivan Inigo, David Kan, Barry Koplowitz, Francis Lee, Kelly McGlinchey, Ligang Qian, Carolyn Ricca, George Rovnyak, Sarah Traeger, John Tokarski, David K. Williams, Laurence I. Wu, Yufen Zhao, Veeraswamy Manne and Rajeev S. Bhide

Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000, United States

Received 12 January 2005; revised 31 January 2005; accepted 1 February 2005

Abstract—Tetrahydroquinoline-based small molecule inhibitors of farnesyltransferase (FT) have been identified. Lead compounds were shown to have nanomolar to sub-nanomolar activity in biochemical assays with excellent potency in a Ras-mutated cellular reversion assay. BMS-316810 (**9e**), a 0.7 nM FT inhibitor, was orally-active in a nude mouse tumor allograft efficacy study.

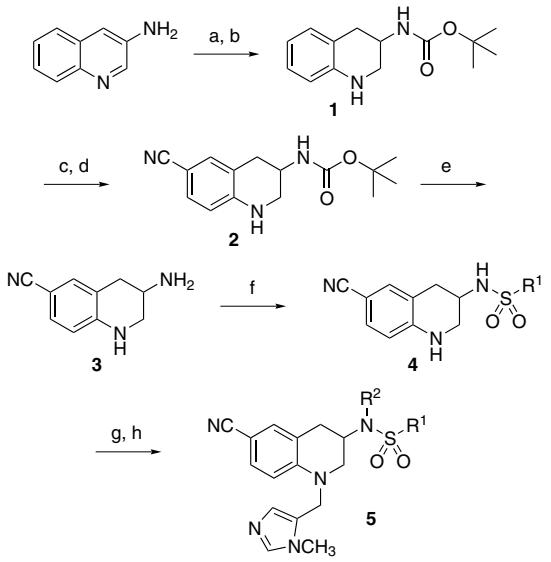
Ras proteins are a family of GTP-binding proteins involved in cellular signal transduction and cell proliferation. Oncogenic mutations of the Ras protein cause disruption of its GTPase activity and prevent interaction with GTPase activating protein, resulting in constitutive activation of the Ras signaling cascade.[1,2] Oncogenic *ras* thereby promotes unregulated cell growth and is estimated to occur in 30–50% of colon, 30% of lung, and 90% of pancreatic tumors in humans.[3] The signaling functions of both normal and oncogenic Ras are contingent upon their association with the cell membrane, which is accomplished by a series of post-translational modifications of the newly synthesized protein.[4] Farnesylation of the C-terminal cysteine residue of p21 ras protein, which is catalyzed by the zinc metalloenzyme farnesyltransferase (FT), is critical for its ability to associate with the membrane. Thus, disruption of farnesyltransferase enzymatic activity is an attractive pharmacological target for controlling *ras* dependent tumor growth.[5]

The activity of benzodiazepine-based farnesyltransferase inhibitors has been described.[6] Motivated by the potent in vitro activity and excellent in vivo efficacy of these leads, tetrahydroquinoline-based inhibitors were proposed as a second-generation series. Herein, we describe the synthesis, structure–activity relationships, and in vivo activity of novel tetrahydroquinoline farnesyltransferase inhibitors in mutant Ras cancer models.

Compounds were prepared following the synthetic sequence illustrated in Scheme 1. Boc-protected 3-amino-1,2,3,4-tetrahydroquinoline **1** was prepared by reaction of the sodium salt of commercially available 3-aminoquinoline with di-*tert*-butyldicarbonate, followed by catalytic hydrogenation over palladium in acidic methanol. Bromination with pyridinium tribromide proceeded regioselectively to afford the 6-bromo analog, which was converted to the corresponding 6-cyano derivative **2** by treatment with zinc cyanide and tetrakis(triphenylphosphine)palladium in dimethylformamide. Removal of the Boc group with trifluoroacetic acid in dichloromethane afforded the key intermediate 3-amino-6-cyano-1,2,3,4-tetrahydroquinoline **3** as a racemate in 27% overall yield. Completion of the target molecules (**5**) was accomplished following a three step sequence of sulfonamide formation, reductive amination, and alkylation. In general, reaction of amine **3** with a variety of sulfonyl chlorides under standard conditions gave the corresponding sulfonamides (**4**) in good to excellent yield. Installation of the imidazole using previously described reductive amination conditions,[7] followed by alkylation of the sulfonamide nitrogen with an

Keywords: Farnesyltransferase inhibitor; BMS-316810.
* Corresponding author. Tel.: +1 609 252 4265; fax: +1 609 252 4710; e-mail: louis.lombardo@bms.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.02.004

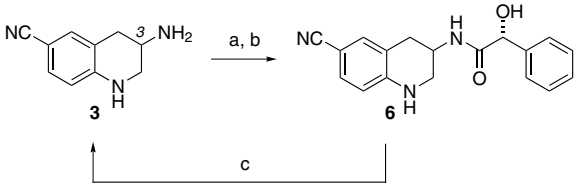


Scheme 1. Synthesis of tetrahydroquinoline-derived farnesyltransferase inhibitors. (a) NaHMDS, $(Boc)_2O$, THF, 100%; (b) H_2, Pd/C, HOAC, CH_3OH, 46%; (c) PBr_3, THF, 75%; (d) $ZnCN_2$, $Pd(PPh_3)_4$, DMF, 81%; (e) 50% CF_3CO_2H, CH_2Cl_2, 100%; (f) R^1SO_2Cl, Et_3N, CH_2Cl_2, 60–95%; (g) 1-methyl-5-formylimidazole, Et_3SiH, CF_3CO_2H, CH_2Cl_2, 75–90%; (h) R^2Br, base, DMF, 50–80%.

appropriate alkyl halide under basic conditions (NaHMDS or Cs_2CO_3) afforded final compounds in high yield.

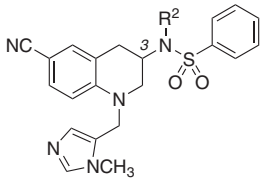
Multigram quantities of enantiomerically-pure **3** could be obtained using the covalent resolution process outlined in Scheme 2. Carbodiimide coupling of racemic **3** with *S*-(+)-mandelic acid proceeded in quantitative yield to afford diastereomeric amides (**6**), which could subsequently be separated by flash chromatography on silica gel or fractional crystallization. Hydrolysis of the diastereomerically-pure amides in refluxing acidic ethanol afforded the corresponding enantiomerically-pure amines (**3**) in high overall yield. Each enantiomer of **3** was converted to the target compounds as described in Scheme 1. The absolute configuration at the C-3 position of the tetrahydroquinoline ring was assigned by single crystal X-ray crystallography of one diastereomer of compound **6**, which was determined to be of the (*S*,*S*)-configuration.

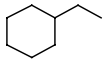
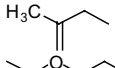
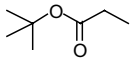
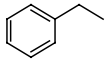
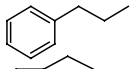
Initial structure–activity relationship (SAR) studies focused on assessing the effect of alkyl group substitution (R^2) on the C-3 nitrogen of a benzenesulfonamide-derivatized tetrahydroquinoline scaffold (Table 1). Consistent with the defined pharmacophore for benzodiazepine FT inhibitors, the 6-cyano and 1-(1-methylimidazolylmeth-5-yl) groups were held constant in the tetrahydroquinoline series of compounds.[6] Generally, the R^2 position was tolerant of a broad range of substituents (compounds **7b**; **7d–f**; **7h**), with lower alkyl-, alkenyl-, carboxymethyl-, and aralkyl-substituted analogues all demonstrating excellent potency in a human recombinant farnesyltransferase inhibition biochemical assay. Good enzyme inhibitory activity was also observed with the unsubstituted derivative **7a**, but a reduction in potency was measured with the bulkier cyclohexylmethyl (**7c**) and homologated phenethyl (**7g**) analogues.



Scheme 2. Covalent resolution of the racemic 3-aminotetrahydroquinoline intermediate **3**. (a) *S*-(+)-mandelic acid, EDAC, HOBT, DMF, 100%; (b) flash chromatography (SiO_2; 2:1 EtOAc–hexanes) or fractional crystallization (acetone–hexanes), 66–78% of theoretical; (c) 15% H_2SO_4, EtOH, reflux, 72%.

Table 1. Effect of alkyl group substitution on the C-3 exocyclic nitrogen



Compd	R^2	FT[a,b] IC_{50}, nM	Cellular reversion[a] % @ 100 nM
7a	H	10	0% (30% @ 10 μM)
7b	CH_3	3.5	45%
7c		48	0% (90% @ 1 μM)
7d		0.9	95%
7e		4.0	75%
7f		3.0	85%
7g		>5000	0%
7h (racemic)		2.5	90%
7i (*S*)-		1.1	85%
7j (*R*)-		2.8	80%

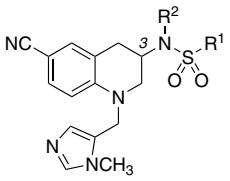
[a] Experimental details for all in vitro assays are contained in Ref. 8.
[b] IC_{50} values are the mean of at least two determinations. Standard deviations are typically within 30% of the IC_{50} value.

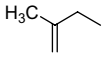
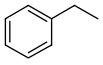
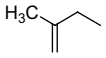
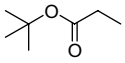
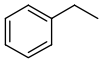
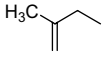
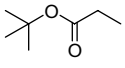
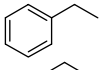
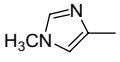
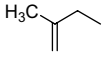
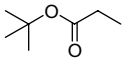
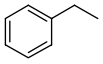
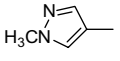
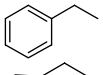
The cellular activity of tetrahydroquinoline FT inhibitors was evaluated in mutant H-*ras* transfected Rat-1 cells. This transformed cell line is characterized by unchecked proliferation in culture, with the cells no longer displaying contact-inhibited growth properties and possessing a distinctive, rounded and elongated phenotype. The activity of compounds under evaluation is reported as the percentage of cells no longer displaying this mutant Ras phenotype at the screening concentration of 100 nM, unless otherwise noted. Activity in the cellular reversion assay correlated well with biochemical potency through the series, with two notable exceptions. The

unsubstituted analogue **7a** was completely inactive at the 100 nM screening concentration, as was the cyclohexylmethyl compound **7c**. It is proposed that poor cellular permeability of **7a** was responsible for its low potency in this assay, but the weak activity of **7c** is not well understood. The reduced activity of the methyl-substituted derivative **7b** may also be due to poor cellular permeability, presumably because of the lower lipophilicity of this compound relative to others in the series.

A series of simple ester analogues related to the *tert*-butyl carboxymethyl compound **7e** were prepared, but their in vitro assessment was complicated by hydrolysis of the ester under assay conditions. The corresponding carboxylic acid was 3 times less active than compound **7e** as a farnesyltransferase inhibitor, but had no activity in cells at concentrations up to 1 μM. Amide analogues did not suffer from chemical instability in the assay media and possessed biochemical potencies similar to **7e**, but were considerably less potent in the cellular

Table 2. Effect of sulfonamide R^1 substitution and interaction with the R^2 alkyl group



Compd	R^1	R^2	FT IC_{50}, nM	Cellular reversion % @ 100 nM
8a	CH_3		0.8	90% (IC_{50} = 8 nM)
8b			2.8	0% (85% @ 1 μM)
9a		CH_3	3.1	0% (85% @ 1 μM)
9b			0.9	85% (IC_{50} = 60 nM)
9c			1.5	85% (IC_{50} = 10 nM)
9d			2.6	90% (IC_{50} < 20 nM)
9e BMS-316810			0.7	95% (IC_{50} = 13 nM)
10a		CH_3	17	0% (10% @ 1 μM)
10b			1.2	70% (IC_{50} = 100 nM)
10c			3.9	30% (IC_{50} = 225 nM)
10d			3.5	75% (IC_{50} = 70 nM)
10e			0.9	85%
11a			0.3	80% (IC_{50} = 50 nM)
11b			0.6	90% (IC_{50} = 30 nM)
11c			1.1	75%
11d			0.6	65%
12a		CH_3	7.6	0% (25% @ 1 μM)
12b			4.6	75%
12c			0.7	95%

reversion assay and were not pursued further (data not shown).

To assess the stereochemical preference for FT inhibition, the enantiomers of compound **7h** were evaluated in the biochemical and cellular reversion assays. The racemic 3-thienylmethyl analogue **7h** was determined to be virtually equipotent with both the (*S*)- and (*R*)-enantiomers **7i** (98.5% ee) and **7j** (98% ee), respectively. Consequently, all additional studies were performed on racemic mixtures.

Subsequent to characterizing the impact of the R^2 substituent and stereochemistry on the activity of the series, SAR studies were directed toward the sulfonamide (R^1) and the interaction of this group with the R^2 moiety (Table 2). A variety of R^1 substituents could be employed without degrading the excellent biochemical potency of the parent benzene sulfonamides. Thus, methyl (**8a**,**b**), pyridyl (**9a**–**e**; **10a**–**e**), imidazolyl (**11a**–**d**), and pyrazolyl (**12a**–**c**) sulfonamide analogues were found to be low nanomolar to subnanomolar farnesyltransferase inhibitors, regardless of the R^2 substituent appended to the C-3 nitrogen. The imidazolylsulfonamide **11a** was the most potent analogue of the series, with an IC_{50} of 0.3 nM.

Overall, compounds were highly effective in the cellular assay, with many examples demonstrating complete reversion of the Ras-transformed phenotype at the 100 nM screening concentration. Compounds could be further differentiated by titration in this assay to obtain IC_{50} values, which are included in the table when available. As a group, the 2-pyridylsulfonamides (**9b**–**e**) had the best activity profile, with IC_{50}s ranging from 60 to 10 nM, depending upon the R^2 substituent. In contrast, conservative modification to afford the regioisomeric 3-pyridylsulfonamide analogues (**10b**–**e**) gave compounds that were less effective in the reversion assay. Five-membered heterocyclic sulfonamides **11a**–**d** and **12b**,**c** were also generally less potent in cells than the 2-pyridylsulfonamide analogues.

Consistent with the benzenesulfonamide **7a**, methyl substitution at R^2 led to compounds with poor cellular activity (**9a**; **10a**; **12a**) regardless of the sulfonamide R^1 substituent. Less clear is the weak cellular activity of methanesulfonamide **8b**, especially in comparison to the excellent potency of the propenyl analogue **8a**.

A low-energy conformation of BMS-316810 (**9e**) was manually docked into the available crystal structure of farnesyltransferase (PDB ID 1sa5)[9] in the FT peptide binding site, which is a deep hydrophobic cleft formed at the interface of the α- and β-subunits (Fig. 1). The inhibitor and residues of FT within 4 Å were allowed to relax during 2000 cycles of conjugate gradient energy minimization.[10] BMS-316810 most likely binds as a ternary complex with farnesyl diphosphate (FPP) and the catalytic zinc ion in a conformation consistent with that reported for benzodiazepine-based inhibitors. FPP forms part of the inhibitor binding surface. The central tetrahydroquinoline ring stacks face-on-face with Tyr 361β, and the methylthiophene group binds in a hydrophobic pocket, stabilized by stacking against the FPP farnesyl moiety and end-on-face stacking with Trp 102β and Trp 106β. With the exception of the imidazole's coordination with the zinc ion, the compound forms only van der Waals contacts with the protein and the FPP farnesyl moiety. The sulfonamide pyridine ring is mostly solvent exposed, and stabilized primarily by stacking against the tetrahydroquinoline ring.

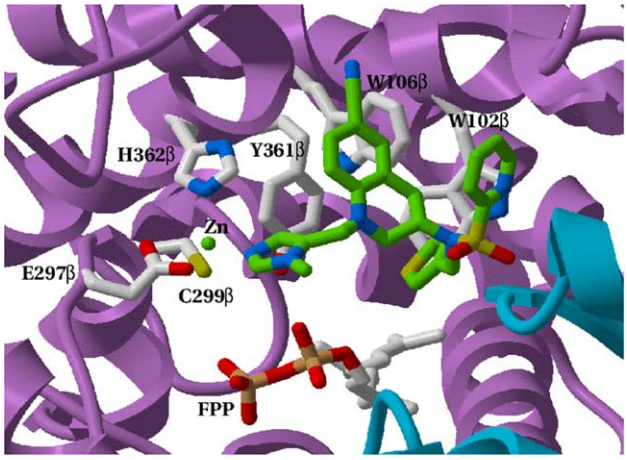


Figure 1. Model showing BMS-316810 (**9e**; carbon atoms in green) bound to the crystal structure of farnesyltransferase. The α- and β-subunits of farnesyltransferase are depicted in purple and blue, respectively. Graphics were produced using ICM.[11]

In a mouse exposure study, BMS-316810 (**9e**) was found to produce high systemic drug levels (AUC_{tot} = 61 μM h; C_{max} = 13 μM) following oral administration of a single 200 mg/kg dose formulated as a solution in 10% aqueous ethanol. The good oral absorption properties of BMS-316810 led to the selection of this compound for evaluation in in vivo anti-tumor efficacy studies.

The in vivo anti-tumor activity of BMS-316810 (**9e**) was assessed in nude mice implanted with subcutaneous San-1 tumor cells, which bear an activating mutation in the H-*ras* gene.[12] Following implantation, tumors were staged to approximately 125 mg before initiation of drug administration. At the high dose of 200 mg/kg twice daily for 14 days, BMS-316810 suppressed tumor growth for the duration of treatment with no observed toxicity (Fig. 2). Lower doses of 50 and 100 mg/kg, administered on the same schedule, were not effective. Expressed quantitatively, BMS-316810 delayed tumor growth to 500 mg by 15 days relative to untreated controls. Additionally, on day 21 following tumor implantation, BMS-316810 treated animals had tumors 21% the size of control animals. Taken together, these data confirm the efficacy and safety of tetrahydroquinoline FT inhibitors as anti-tumor agents.

In summary, tetrahydroquinoline-based small molecule inhibitors of farnesyltransferase have been identified. The series was shown to have excellent potency in biochemical and cellular assays, but no stereochemical preference for activity in either assay. BMS-316810 (**9e**), a

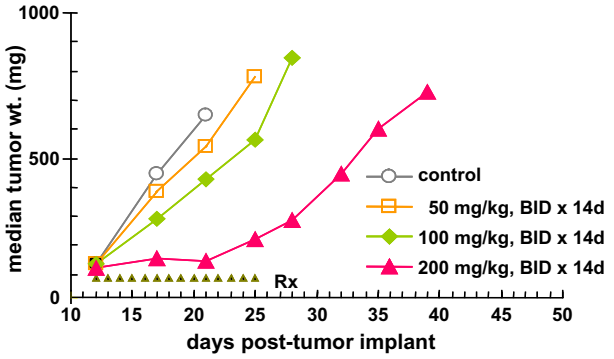


Figure 2. In vivo anti-tumor activity of BMS-316810 (**9e**) versus subcutaneous San-1 murine tumor implants in nude mice. Drug formulated as a solution in 10% aqueous ethanol.

0.7 nM biochemical inhibitor of the FT enzyme, was orally-active versus the San-1 murine tumor cell line in a nude mouse tumor allograft efficacy study. The interaction of BMS-316810 with the farnesyltransferase enzyme was modeled through docking experiments with a reported crystal structure, and is predicted to be consistent with the binding of benzodiazepine-based inhibitors.

References and notes

1. (a) Barbacid, M. *Annu. Rev. Biochem.* **1987**, *56*, 779; (b) Kato, K.; Cox, A. D.; Hisaka, M. M.; Graham, S. M.; Buss, J. E.; Der, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6403; (c) Rowinsky, E. K.; Windle, J. J.; Von Hoff, D. D. *J. Clin. Oncol.* **1999**, *17*, 3631.
2. (a) McCormick, F. *Cell* **1989**, *56*, 5; (b) McCormick, F. *Curr. Opin. Cell Biol.* **1990**, *2*, 181.
3. Bos, J. L. *Cancer Res.* **1989**, *49*, 4682.
4. (a) Casey, P. J.; Solski, P. J.; Der, C. J.; Buss, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8323; (b) Hancock, J. F.; Magee, A. I.; Childs, J. E.; Marshall, C. J. *Cell* **1989**, *57*, 1167.
5. (a) Gibbs, J. B. *Cell* **1991**, *65*, 1; (b) Gibbs, J. B.; Oliff, A. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 143; (c) Rowinsky, E. K.; Windle, J. J.; Von Hoff, D. D. *J. Clin. Oncol.* **1999**, *17*, 3631.
6. (a) Ding, C. Z.; Batorsky, R.; Bhide, R.; Chao, H. J.; Cho, Y.; Chong, S.; Gullo-Brown, J.; Guo, P.; Kim, S. H.; Patel, M.; Penhallow, B. A.; Ricca, C.; Rose, W. C.; Schmidt, R.; Slusarchyk, W. A.; Vite, G.; Yan, N.; Manne, V.; Hunt, J. T. *J. Med. Chem.* **1999**, *42*, 5241; (b) Hunt, J. T.; Ding, C. Z.; Batorsky, R.; Bednarz, M.; Bhide, R.; Cho, Y.; Chong, S.; Chao, S.; Gullo-Brown, J.; Guo, P.; Kim, S.-H.; Lee, F. Y. F.; Leftheris, K.; Miller, A.; Mitt, T.; Patel, M.; Penhallow, B. A.; Ricca, C.; Rose, W. C.; Schmidt, R.; Slusarchyk, W. A.; Vite, G.; Manne, V. *J. Med. Chem.* **2000**, *43*, 3587.
7. Chen, B.-C.; Sundeen, J. E.; Guo, P.; Bednarz, M. S.; Zhao, R. *Tetrahedron Lett.* **2001**, *42*, 1245.
8. (a) Manne, V.; Yan, N.; Carboni, J. M., et al. *Oncogene* **1995**, *10*, 1763; (b) Carboni, J. M.; Yan, N.; Cox, A. D., et al. *Oncogene* **1995**, *10*, 1905.
9. Reid, T. S.; Beese, L. S. *Biochemistry* **2004**, *22*, 6877.
10. Hagler, A. T.; Ewig, C. S. *Comput. Phys. Commun.* **1994**, *84*, 131–155.
11. Molsoft LLC, 3366 No. Torrey Pines Ct., Ste. 300, La Jolla, CA 92037.
12. Manne, V.; Lee, F. Y. F.; Bol, D. K.; Gullo-Brown, J.; Fairchild, C. R.; Lombardo, L. J.; Smykla, R. A.; Vite, G. D.; Wen, M.-L.; Yu, C.; Wong, T. W.; Hunt, J. T. *Cancer Res.* **2004**, *64*, 3974.