Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5877–5880
Bioorganic & Medicinal Chemistry Letters

Farnesyl protein transferase inhibitors targeting the catalytic zinc for enhanced binding

F. George Njoroge,* Bancha Vibulbhan, Patrick Pinto, Corey Strickland, Paul Kirschmeier, W. Robert Bishop and V. Girijavallabhan

Schering–Plough Research Institute, 2015 Galloping Hill Road, K-15-3-3545, Kenilworth, NJ 07033, USA

Received 20 July 2004; revised 9 September 2004; accepted 10 September 2004
Available online 30 September 2004

Abstract—Successful efforts to make farnesyl transferase (FT) inhibitors with appropriately tethered ligands designed to interact with a catalytic zinc that exist in the enzyme have been realized. Thus, by introducing either a pyridylmethylamino or propylaminolimidazole amide moieties off the 2-position of the piperidine ring, FT inhibitors with activities in the picomolar range have been achieved as exemplified by compounds **12a** and **12b**. An X-ray structure of **11b** bound to FT shows the enhanced activity is a result of interacting with the active-site zinc.

It has become evident that intervention of the signal transduction process in tumor cells facilitated by farnesyl protein transferase (FT), can lead to discovery of novel noncytotoxics drugs some of which have been advanced into clinical trials.[1]

SARASAR® (SCH 66336), a drug that was discovered at Schering–Plough Research Institute is nonpeptidic trihalocycloheptabenzopyridine that inhibits the activity of FT at 2 nM concentration. Preclinical studies results established that **SARASAR**® was bioavailable in monkeys, dogs, and rodents and was thus advanced for clinical trials in humans[2] (Fig. 1).

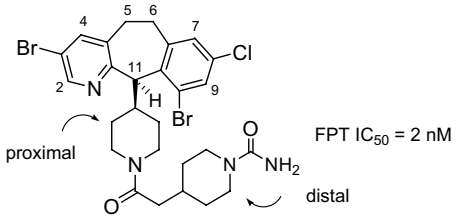


Figure 1. **SARASAR**®, SCH 66336.

Preliminary results from Phase I and II have demonstrated **SARASAR**® to be safe and tolerable in patients. In some cases efficacy has been observed and thus has prompted recommendation of **SARASAR**® to be advanced to Phase III clinical trials.[3]

Elucidation of the X-ray structure of **SARASAR**®, bound to FPTase revealed the following interesting observations: (1) the benzocycloheptapyridine tricyclic ring presented itself in a 'butterfly' conformation, with the ethano bridge puckered forward and the two aryl groups extending toward the convex phase of the molecule, (2) the proximal piperidinyl moiety extended upwards in a pseudoaxial orientation, firmly locked in position by the bulky bromine group at the 10 position, (3) the amide carbonyl interacted with structural water—an interaction that resulted in enhanced activity from the resulting inhibitor and was critical to the activity of this compound, and (4) the distal piperidinyl moiety extended out into the solvent providing a useful appendage for attaching a variety of moieties that would be important for enhancing solubility or improving pharmacokinetic profile.

From the bound structure of **SARASAR**® to FT (see Fig. 2), it was evident that there was a catalytic zinc in close proximity to the piperidyl group off the C-11 of the tricyclic ring system. The zinc is ~9 Å from the C-11 atom, allowing moderately sized groups to reach

Keyword: Farnesyl protein transferase bound to zinc.
* Corresponding author. Tel.: +1 9087403121; fax: +1 8087407152; e-mail: george.njoroge@spcorp.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.09.026

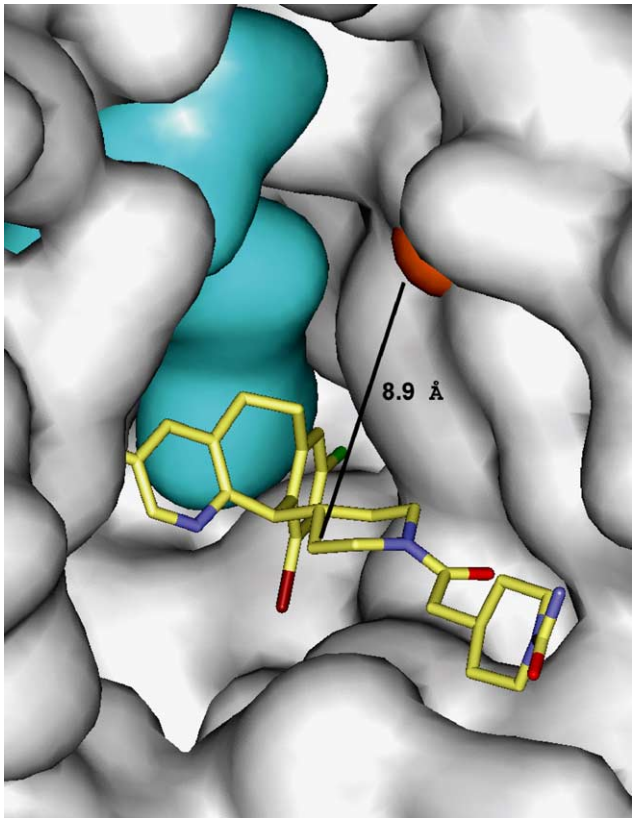


Figure 2. Structure of **SARASAR®** bound to FPT (blue surface—FPP, white surface—FT, orange sphere—zinc, yellow sticks—**SARASAR®**).[6]

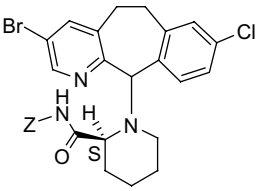
it. The major challenge, therefore, was to introduce an appropriately tethered functionality that would effectively interact with this zinc. This interaction was anticipated to provide potent compounds as demonstrated by various other groups.[4]

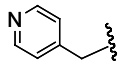
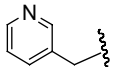
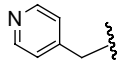
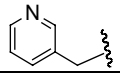
A study to investigate the effect of tethering a pyridyl or imidazole moieties via an amide linkage was carried out. Evaluation was done for both the di- and tri-halogenated tricyclic compounds.

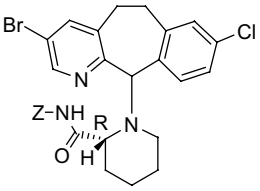
1. Chemistry

Compounds prepared in this study are shown in Tables 1 and 2. Starting from previously reported azaketone **1**,[2a] reduction with $NaBH_4$ gave the alcohol **2** that was subsequently converted to chloro derivative **3** using thionyl chloride in methylene chloride. Reaction of the chloride with either D-pipecolinic acid methyl esters in presence of triethylamine in CH_2Cl_2 gave the corresponding C-11 tricyclic pipecolinate **4** as diastereomeric mixtures that were readily separable on normal silica gel column using 1% ethyl acetate–dichloromethane as eluent. The less polar isomer **5** was arbitrarily designated as isomer I while the more polar isomer compound **6** were designated as isomer II. Hydrolysis of compounds **5** and **6** using LiOH afforded the corresponding carboxylic acids that were, without further purification, coupled to the appropriate amine to provide target compounds

Table 1. Inhibition of FT with compounds in the diahalotricyclic series



Compd #	Structure Z=	Hras FT inhibition
7a		0% @ 1.1 μM
7b		0.30 μM
8a		13% @ 1.1 μM
8b		0.16 μM



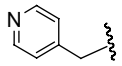
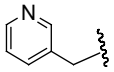
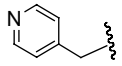
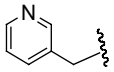
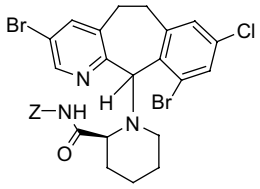
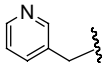
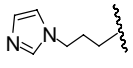
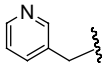
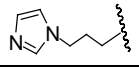
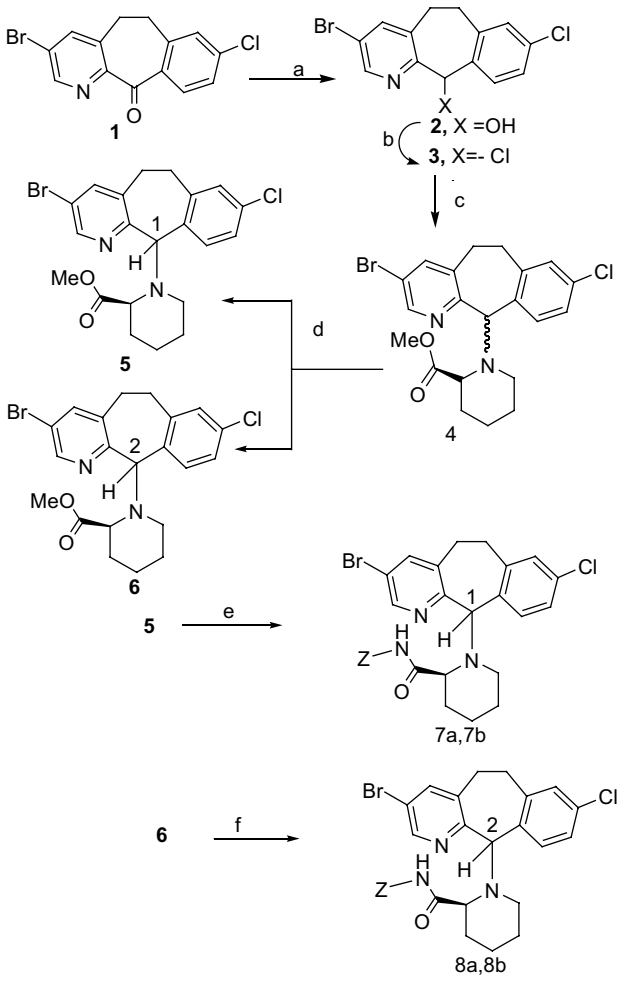
Compd #	Structure Z=	Hras FT inhibition
9a		18% @ 1.1 μM
9b		0% @ 0.4 μM
10a		27% @ 1.1 μM
10b		11% @ 0.4 μM

Table 2. Inhibition of FT with compounds in the trihalotricyclic series



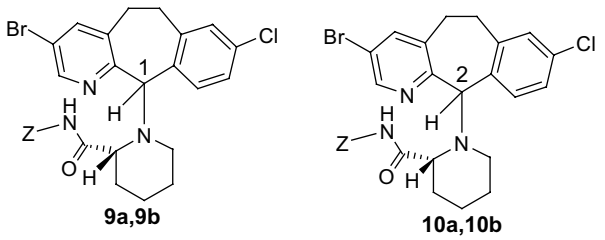
Compd #	Structure Z=	Hras FT inhibition IC_{50}, μM
11a		0.08
12a		0.0009
11b		0.004
12b		0.0004



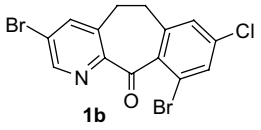
Scheme 1. Reagents and conditions: (a) $NaBH_4$–MeOH; (b) $SOCl_2$; (c) methyl pipecolinate; (d) separation of diastereomers using 1% EtOAc–CH_2Cl_2; (e) LiOH, then amine coupling.

7a,[7] and **b**, **8a**,[7] and **b**. Table 1 summarizes biological data obtained from the analysis of the dihalo analogs (Scheme 1).

Similarly, reaction of the chloro compound **3** with L-pipecolinic ethyl ester, followed by separation of the diastereomers on silica gel with subsequent ester hydrolysis, and amidation afforded compounds **9a** and **b** from isomer I and **10a** and **b** from isomer II.



Synthesis of 3,10-dibromo 8-chlorotrihalo analogs was carried out in a similar manner to that described for the dihalotricyclic series starting from ketone **1b**.[2a] However, analogs in this series were only prepared from D-pipecolinic acid as will become clear in the SAR discussion. In this series, compounds **11a** and **b** with a 3-pyridylmethylamino moiety and **12a** and **b** with propylamino imidazole moiety were prepared and are shown in Table 2.



2. Results and discussions

Compounds prepared in this study were tested for their ability to inhibit the FT catalyzed transfer of [3H]-farnesyl moiety from farnesyl pyrophosphate to H-Ras-CVLS as previously described.[5] We first investigated the effect of introduction of various pyridines groups, tethered off the amide functionality by a methylene spacer: modeling of tricyclic compounds bound to FT had previously shown that this type of tethering could lead to analogs that were within the interaction range with the existing catalytic zinc.

As shown in Table 1, the 4-pyridylmethylamino analogs prepared, compounds **7a**, **8a**, **9a**, and **10a** did not show any inhibitory activity even at 100 μM range in both the *S*- and *R*-pipecolinic acid series. In the case of 3-pyridylmethylamino substituted analogs, compounds in *R*-pipecolinic acid series, that is **9b** and **10b** were also found to be inactive at the concentration range tested. However, 3-pyridylmethylamino derivatives in the *S*-pipecolinic acid series exhibited weak FT inhibitory activity, thus, compound **7b** from isomer I had an $IC_{50} = 0.3\,\mu M$ while the corresponding derivative from isomer II, compound **8b**, was slightly more active with an $IC_{50} = 0.16\,\mu M$.

From results obtained from the dihalotricyclic series, it was clear that the *S*-pipecolinic moiety and 3-pyridine substitution were required for FT potency and this information was useful in determining compounds to be prepared in the trihalotricyclic series.

As expected, in the trihalotricyclic series, the 3-pyridyl derivative, compound **11a**, from isomer I showed an inhibitory activity of 0.08 μM against FT. The 3-pyridyl derivative from the corresponding isomer II, compound **11b**, was about 20 times more potent than **11a** with FT inhibitory $IC_{50} = 0.004\,\mu M$.

Further evaluation of other zinc biding ligands revealed that an imidazole ring tethered to the 2-position of the of the piperidyl ring by a three carbon spacer, gave very potent compounds. Thus, compound **12a**, the analog derived from isomer I had an $IC_{50} = 0.0009\,\mu M$ while the corresponding derivative from isomer II, compound **12b**, was slightly more potent with an $IC_{50} = 0.0004\,\mu M$.

To better understand the mode of binding of these inhibitors to FT, the X-ray crystal structure of compound **11b** bound to FT was solved.[6] As shown in Fig-

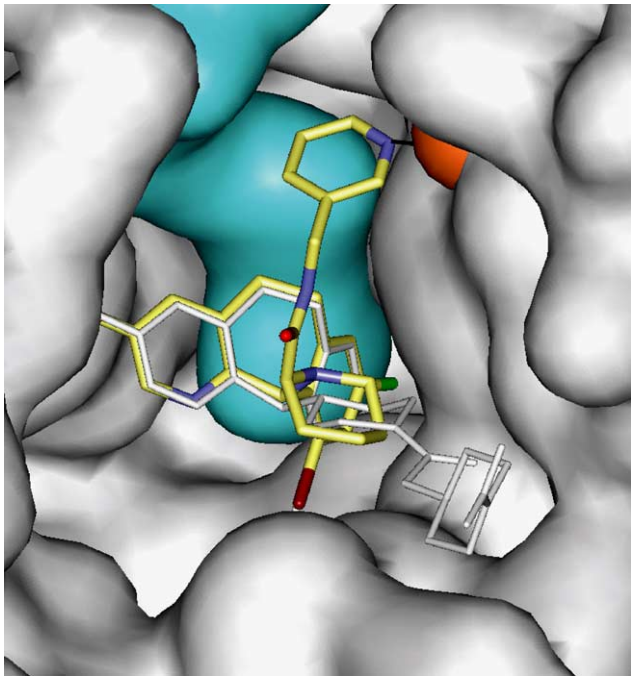


Figure 3. Structure of **11b** bound to FPT (blue surface—FPP, white surface—FPT, orange sphere—zinc, white sticks—**SARASAR®**, yellow sticks—**11b**).

ure 3, **11b** binds to the same FT site as **SARASAR®**, even though it lacks the distal piperidinyl group. The benzocycloheptapyridine tricyclic rings are virtually superimposable. The 3-pyridylmethylamino group reaches over the benzocycloheptapyridine tricyclic rings and interacts with the zinc. It is 2.2 Å away from the catalytic zinc, a relationship that results in a very favorable interaction between the inhibitor and the zinc atom. The rest of the tricyclic ring system orients in the same way as **SARASAR®** with the piperidine ring assuming a pseudo axial geometry. This binding of the pyridyl ligand to the zinc results in the enhanced activity that is demonstrated by these series of compounds.

References and notes

1. (a) Njoroge, F. G.; Girijavallabhan, V. M. *Curr. Med. Chem. Immunol. Endo. Metab. Agents* **2001**, *1*, 185; (b) End, D. W. *Invest. New Drugs* **1999**, *17*, 241; (c) Gibbs, R. A. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*(5), 585; (d) Caonigro, F.; Casale, M.; Bryce, J. *Expert Opin. Invest. Drugs* **2003**, *12*, 943; (e) Kelland, L. R. *Expert Opin. Invest. Drugs* **2003**, *12*, 413.
2. (a) Njoroge, F. G.; Taveras, A. G.; Kelly, K.; Remiszewski, S.; Mallams, A. K.; Wolin, R.; Afonso, A.; Cooper, A. C.; Rane, D. F.; Liu, Y.-T.; Wong, J.; Vibulbhan, B.; Pinto, P.; Deskus, J.; Alvarez, C. S.; del Rosario, J.; Connolly, M.; Wang, J.; Desai, J.; Rossman, R. R.; Bishop, W. R.; Patton, R.; Wang, L.; Kirschmeier, P.; Bryant, M. S.; Nomeir, A. A.; Lin, C.-C.; Liu, M.; McPhail, A. T.; Doll, R. J.; Girijavallabhan, V. M.; Ganguly, A. K. *J. Med. Chem.* **1998**, *41*, 4890; (b) Liu, M.; Bryant, M. S.; Chen, J.; Lee, S.; Yaremko, B.; Lipari, P.; Malkowski, M.; Ferrari, E.; Nielsen, L.; Prioloi, N.; Dell, J.; Sinha, D.; Syed, J.; Korfmacher, W. A.; Nomeir, A. A.; Lin, C.-C.; Wang, L.; Taveras, A. G.; Doll, R. J.; Njoroge, F. G.; Mallams, A. K.; Remiszewski, S.; Catino, J. J.; Girijavallabhan, V. M.; Kirschmeir, P.; Bishop, W. R. *Cancer Res.* **1998**, *58*, 4947.
3. (a) Adjei, A. A.; Erlichman, C.; Davis, J. N.; Cutler, D. L.; Sloan, J. A.; Marks, R. S.; Hanson, L. J.; Svingen, P. A.; Atherton, P.; Bishop, W. R.; Kirschmeier, P.; Kaufmann, S. H. *Cancer Res.* **2000**, *60*, 1871; (b) Adjei, A. A. *Drugs Future* **2000**, *25*, 1069; (c) Taveras, A. G.; Kirschemeir, P.; Baum, C. *Curr. Top. Med. Chem.* **2003**, *3*, 1103.
4. (a) Reid, T. S.; Beese, L. S. *Biochemistry* **2004**, *43*, 6877; (b) Hunt, J. T.; Ding, C. Z.; Batorsky, R.; Bednarz, M.; Bhide, R.; Cho, Y.; Chong, S.; Chao, S.; Gullo-Brown, J.; Gou, G.; 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; (c) Williams, T. M.; Bergman, J. M.; Brashear, K.; Breslin, M. J.; Dinsmore, C. J.; Hutchinson, J. H.; MacTough, S. C.; Stump, C. A.; Wei, D. D.; Zartman, C. B.; Bogusky, M. J.; Cubeberson, J. C.; Buse-Doepner, C.; Davide, J.; Greenberg, I. B.; Hamilton, K. A.; Koblan, K. S.; Kohl, N. E.; Liu, D.; Lobell, R. B.; Mosser, S. D.; O'neill, T. J.; Rands, E.; Schaber, M. D.; Wilson, F.; Senderak, E.; Motzel, S. L.; Gibbs, J. B.; Graham, S. L.; Heimbrook, D. C.; Hartman, G. D.; Oliff, A. I.; Huff, J. R. *J. Med. Chem.* **1999**, *42*, 3779.
5. Bishop, W. R.; Bond, R.; Petrin, J.; Wang, L.; Patton, R.; Doll, R.; Njoroge, G.; Catino, J.; Shwartz, J.; Carr, D.; James, L.; Kirschmeier, P. *J. Biol. Chem.* **1995**, *270*, 30611.
6. Strickland, C. L.; Weber, P. T.; Windsor, W. T.; Wu, Z.; Le, H. V.; Albanese, M. W.; Alvarez, C. S.; Cesarz, D.; del Rosario, J.; Deskus, J.; Mallams, A. K.; Njoroge, F. G.; Piwinski, J. J.; Remiszewski, S.; Rossman, R. R.; Taveras, A. G.; Vibulbahn, B. V.; Doll, R. J.; Girijavallabhan, V. M.; Ganguly, A. K. *J. Med. Chem.* **1999**, *42*, 2125.
7. Preparation of compounds **7a** and **8a**: 3-Bromo-8-dichloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (1.10 g, 3.0 mmol) and L-pipecolinic acid ethyl ester hydrochloride (1.34 g, 6.98 mmol), triethylamine (2.91 μL, 21 mmol) were dissolved in 20 mL of dry CH_2Cl_2 and the mixture stored at 25 °C under nitrogen for 72 h. The reaction mixture was washed with $NaHCO_3$, H_2O, brine and then filtered, and dried. The product was chromatographed on silica gel column using 1% ethyl acetate–dichloromethane eluent to give diastereomeric isomers **5** and **6**. Ester 5 (0.26 g, 0.6 mmol) was dissolved in 6 mL of ethanol and 1.4 mL of 1 M LiOH (1.4 mmol) was added. The reaction mixture was heated in an oil bath at 80 °C for 10 h, then cooled and 1.5 mL of HCl was added to adjust pH to ~4.5. Solvents were evaporated and the resulting acid was dissolved in 3 mL DMF and NMM (184 mL, 1.6 mmol), 4-(aminoethyl)pyridine (74 μM, 0.078 g, 0.73 mmol) HOBT (0.098 g, 0.72 mmol), DEC (0.139 g, 0.72 mmol) were then added. The reaction mixture was stirred at room temperature for 16 h and then worked up. Purification using flash chromatography eluting with 3% (10% NH_4OH–MeOH)–CH_2Cl_2 solvent system gave compound **7a**. Compound **8a** was obtained from isomer **6** using a similar method. Other compounds reported in this paper were prepared in a similar manner.