Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters 16 (2006) 984–988
Bioorganic & Medicinal Chemistry Letters

Enhanced FTase activity achieved via piperazine interaction with catalytic zinc

F. George Njoroge,* Bancha Vibulbhan, Patrick Pinto, Corey Strickland, W. Robert Bishop, Amin Nomeir and Vivyoor Girijavallabhan

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

Received 15 September 2005; revised 24 October 2005; accepted 26 October 2005
Available online 16 November 2005

Abstract—Benzocycloheptapyridine tricyclic compounds with piperazine or substituted piperidine moieties extending either from the 5- or 6-position of the tricyclic bridgehead exhibited enhanced FTase activity: this resulted from favorable binding of the ligand nitrogen with the catalytic zinc found in the FTase. A single isomer at C-11 with piperazine adduct extending from the 6-position, compound **24**, exhibited excellent FTase activity with IC_{50} = 0.007 μM, soft agar IC_{50} = 72 nM, and Rat AUC(PO, 10 mpk) = 4.0 μM · h. X-ray of (−)-[8-chloro-6-(1-piperazinyl)-1*H*-benzo[5,6]]cyclohepta[1,2-*b*]pyridine-11-yl]-1-(methylsulfonyl)piperidine **24** bound to Ftase revealed favorable interaction between piperazine nitrogen and catalytic zinc atom.

Inhibition of farnesyl protein transferase (FTase), an enzyme that plays a critical role in regulating tumor cell growth, remains a favorable target in pursuit of non-toxic antitumor agents.[1] Tremendous progress has been achieved in this area and several compounds have advanced to various stages of clinical trials.[2] These compounds have demonstrated good efficacy in pre-clinical studies in rodents and shown encouraging efficacy in some early phase trials in humans beings.[2] Clinical activity of FTase inhibitors remains to be demonstrated in Phase III trials.

A benzocycloheptapyridine tricyclic compound, lonafarnib® (SCH 66336, compound **1**, Fig. 1), has been advanced for clinical trials from our laboratories.[3] This compound was found to bind with high affinity to the FTase with IC_{50} = 2 nM. The binding of compound **1** was greatly enhanced by presence of halogens at 3, 8, and 10 positions of the tricyclic ring system. Through X-ray structural studies of **1** bound to the FTase, it was established that a great part of the activity was contributed by the existence of the bromide group at position 10 through the fact that this moiety locked the conformation of the tricyclic ring system in a pseudoaxial position and made significant hydrophobic contacts with FTase.

Recently, we sought for a way to enhance the binding of our FT inhibitors by introducing motifs that could interact with catalytic zinc that exists in the enzyme. This interaction was anticipated to provide potent compounds as demonstrated by various other groups.[4] We successfully prepared very potent inhibitors which possessed either a pyridyl or imidazole appropriately tethered from a piperidine ring system as shown by compound **2**[5] (Fig. 1). Although compound **2** lacked the amide carbonyl, a moiety present at the bottom of the 4-position of the piperidine ring attached to the tricyclic ring in lonafarnib®; nevertheless, it was still a very potent molecule with activity in single digit

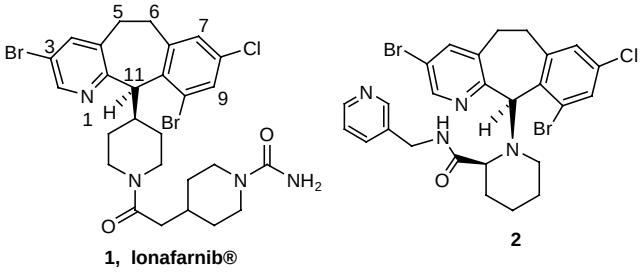


Figure 1.

Keywords: Farnesyl protein transferase; FTase inhibitors; Benzocycloheptapyridine; lonafarnib.
* Corresponding author. Tel.: +1 908 740 3121; fax: +1 908 740 7152; e-mail: george.njoroge@spcorp.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.10.090

nanomolar range (FTase IC_{50} = 4 nM).[5] After careful evaluation of how compounds **1** and **2** were bound to the enzyme (Fig. 2), it was clear that one could also reach the catalytic zinc via an appropriately appendaged moiety off the 5 or 6 position of the bridge of the tricyclic ring system.

It was envisioned that if a strong bonding was achieved between the zinc and the ligand, a more potent inhibitor could be obtained: optimum interaction with zinc could also make it possible to eliminate some of the halogens in our previous lead molecule **1** and to substantially simplify the nature of our inhibitors.

This report describes successful efforts in introducing piperazine and piperidine adducts at the 5- and 6- position of the bridgehead of the benzocycloheptapyridine: a feature that allowed us to realize very potent FTase inhibitors that were devoid of the 3 and 10-bromo moieties.

Compounds prepared in this study were tested for their ability to inhibit the FT catalyzed transfer of [^{3}H]farnesyl moiety from farnesyl pyrophosphate to H-Ras-CVLS as previously described.[6] Initial efforts concentrated on screening for potential piperazine or substituted piperidine compounds that would interact with the catalytic zinc found in the active site of FTase. Studies with closely related compounds[4] established that possibilities existed of reaching the zinc atom either from the 5- or the 6-position of the bridgehead through an appropriate zinc coordinating ligand (Fig. 2). Since it was not clear which adducts, arising from either of these positions, would be the appropriate ones, we decided to assess the various mixtures that resulted from reactions outlined in Scheme 1. Thus, 5- bromo dehydro tricyclic compound of type **3**[3c] was treated with potassium *tert*-butoxide to give 'benzyne' type of intermediate **4** which, in presence of either

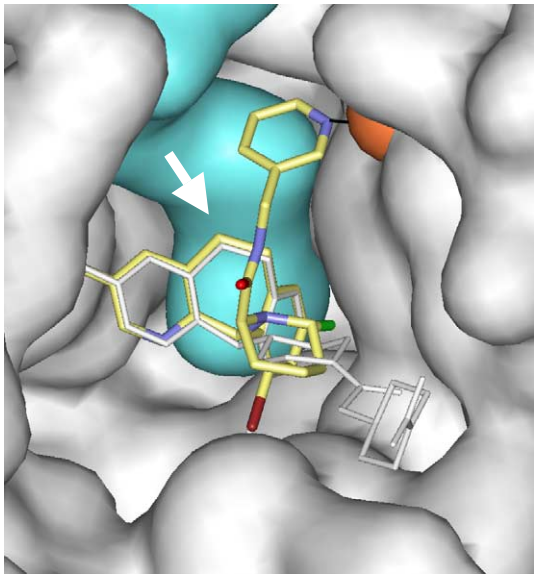
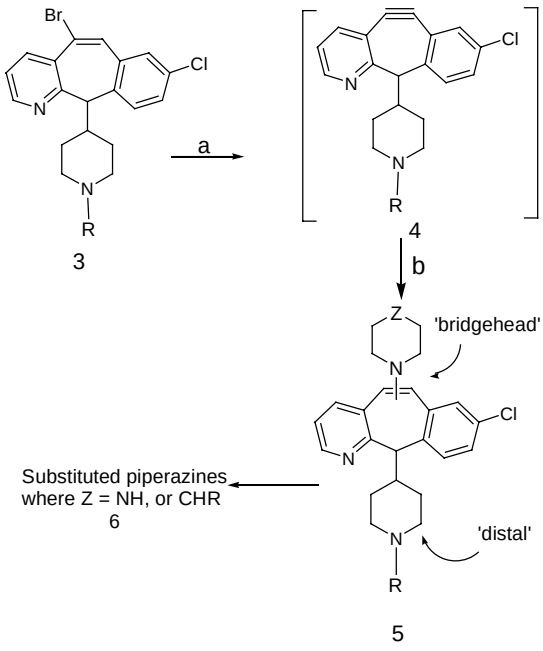


Figure 2. Structure of **2** bound to FPT (blue surface—FPP, white surface—FPT, orange sphere—zinc, white sticks—lonafarnib® yellow sticks—**2**).



Scheme 1. Reagents: (a) potassium *tert*-BuO, (b) piperazine or piperidine bases.

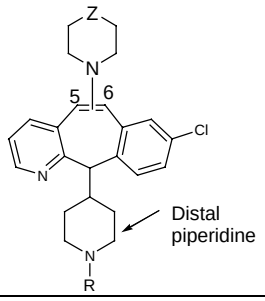
substituted piperidine or piperazine base, furnished enamines of type **5** with the nucleophilic amine, adding to both 5- and 6-position of the bridgehead in a ratio of approximately 20:60 as established by ^{1}H NMR. The resulting compounds were evaluated as mixtures with the aim of further resolving those mixtures that demonstrated superior activity. FTase activity of the various piperazine and piperidine derivatives are outlined in Table 1.

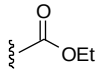
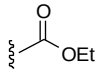
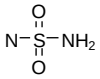
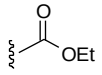
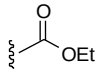
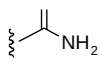
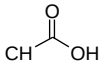
We initially investigated the effect of various substituents off the piperazine group at position 5 or 6 on the bridgehead, retaining the 'distal' piperidine (Scheme 1) as ethyl carbamate. Thus, having a free amino group as exemplified by compound **7** provided one of the best binding (IC_{50} = 0.04 μM). Capping the amino group of **7** with such groups as acetyl (compound **8**), sulfonylurea (compound **9**), led to loss of activity. However, when the piperazine was capped as urea, a weakly binding inhibitor (IC_{50} = 0.72 μM), compound **10** was obtained. Having uncapped bridgehead piperazine and modifying the bottom piperidine provided the following SAR; the uncapped piperazine, with unsubstituted distal piperidine, compound **11** was inactive at 1 μM range. Introducing a methyl group at this position gave compound **12** that was not active even at 1.5 μM range.

Introducing an acetyl group or urea moiety (compounds **13** and **14**) did not improve FTase activity. However, inhibitory activity was greatly enhanced by introduction of a sulfonyl group as exemplified by compound **15** that had FTase inhibitory activity with IC_{50} = 0.02 μM.

Retaining the sulfonamide capping at the distal piperidine and exploring some 4-substituted piperidines off the bridgehead provided limited improvement in FTase activity, thus, whereas the ethanol-substituted

Table 1. FTase activity of bridgehead modified analogs.

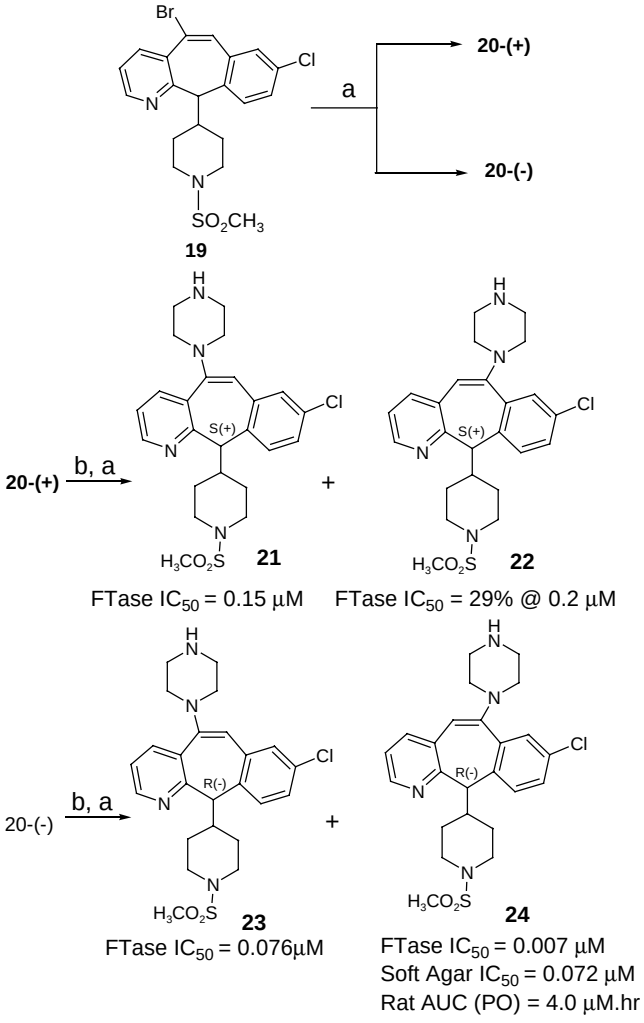


Compound	Z	R	FTase inhibitory activity (IC_{50}, μM)
7	NH		0.04
8			>1.0
9			>1.0
10			0.72
11	NH	H	>1.0
12	NH		>1.5
13	NH		>0.23
14	NH		>0.23
15	NH		0.02
16	CH–OH		0.10
17			>0.19
18			0.04

compound, **17**, was inactive the corresponding methanol adduct, compound **16**, was weakly active with IC_{50} = 0.10 μM in this series. Some enhancement in activity was observed with the 4-piperidinylacteic acid, compound **18**, which had an FTase activity IC_{50} = 0.04 μM.

Now that it was evident that piperazine adduct with a free amino group extending from the 5- or the 6-positions of the bridgehead was desirable for FTase activity, we set out to investigate in greater detail, the residency of FTase activity of the sulfonamide, compound **15**. This entailed separation of all the possible regio- and stereo isomers of mixture **15** as outlined in Scheme 2.[8] Thus, vinyl bromide **19** was separated using a chiral column to give the two enantiomers **20**-(+) and **20**-(−) in equal ratio. As in the previous cases, **20**-(+) was treated with potassium *tert*-butoxide in the presence of piperazine to give the addition products at both 5 and 6 position in a ratio of ~20:60, respectively. The two regioisomers were separated on a chiral AD® column to give the 5-isomer as compound **21** and the 6-isomer as compound **22**. Similarly, **20**-(−)-isomer under identical conditions, provided the 5-piperazine adduct, compound **23**, and the 6-substituted analog, compound **24**.

Whereas compound **22** inhibited 29% of FTase at 0.2 μM, the corresponding 5-isomer, compound **21**, was more potent with IC_{50} = 0.15 μM, suggesting that with C-11 *R*-isomers, the molecule had to flip in such a way that allowed the bridgehead piperazine to effectively interact with catalytic zinc. These activities were essentially lower than those derived from the original sulfonamide **15** mixture (IC_{50} = 0.02 μM).



Scheme 2. Reagents: (a) chiral AD® separation; (b) potassium *tert*-butoxide, piperazine.

Evaluations of compounds derived from **20**-(−)-isomer revealed that these inhibitors were substantially more active than their **20**-(+) counterparts. However, unlike the (+)-isomer case, where the 5-substituted piperazine derivative **21** was more active than the 6-substituted piperazine **22**, in this case, the 6-isomer analogue, compound **24**, was the more potent with $IC_{50} = 0.007$ μM. The corresponding 5-isomer, compound **23**, was 10 times less active with FTase activity of 0.076 μM. Compound **24** was orally administered to rats and found to have good exposure with AUC = 4.0 μM · h. It also had good cellular activity with soft agar[6] $IC_{50} = 0.070$ μM, which was comparable to that of lonafarnib®, the clinical candidate.

To better understand the mode of binding of these bridgehead modified inhibitors to FTase, X-ray crystal structure of compound **24**[9] bound to the enzyme was solved.[7] As shown in Figure 3, **24** was bound to the same FTase site as lonafarnib® but was flipped in such a way that the 7-chloro on the tricyclic ring interacted with tyrosine-166α and the nitrogen of the piperazine formed a favorable interaction with catalytic zinc which was 3.4 Å away. The binding of the piperizinyl ligand to the zinc resulted in enhanced activity that was demonstrated by compound **24**.

From the X-ray structure, the stereochemistry of the C-11 was established to be 'S' with the distal piperidine assuming a pseudoaxial orientation.

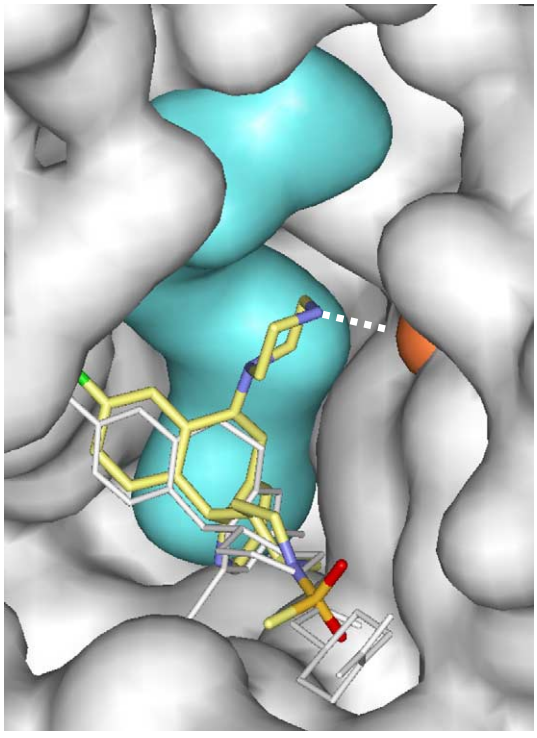


Figure 3. Structure of **24** bound to FPT (blue surface—FPP, white surface—FPT, orange sphere—zinc, and white sticks—lonafarnib® yellow sticks—**24**).

In summary, we have successfully introduced a piperazine adduct extended from the 6-position of the benzocycloheptapyridine core that resulted in enhanced binding to FTase through a favorable binding to catalytic zinc in the enzyme. Unlike our previously reported trihalogenated inhibitors, compound **24** was monohalogenated but acquired the extra binding through the favorable interaction of the piperazine moiety with zinc as demonstrated by its X-ray structure bound to the FTase. Compound **24** was orally bioavailable and had potency profile similar to that of our clinical compound lonafarnib®. Future work directed toward exploiting discoveries in this report is currently in progress.

References and notes

1. (a) Njoroge, F. G.; Girijavallabhan, V. M. *Curr. Med. Chem.- Imm. Endo. Metab. Agents* **2001**, *1*, 185; (b) End, D. W. *Investig. New Drugs* **1999**, *17*, 241; (c) Gibbs, R. A. *Curr. Opin. Drug Discov. Dev.* **2000**, *3*(5), 585; (d) Caonigro, F.; Casale, M.; Bryce, J. *Expert Opin. Investig. Drugs* **2003**, *12*, 943; (e) Kelland, L. R. *Expert Opin. Investig. Drugs* **2003**, *12*, 413.
2. (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. Topics Med. Chem.* **2003**, *3*, 1103; (d) Li, Q.; Wang, G. T.; Li, T.; Gwaltney, S. L., II; Woods, K. W.; Claiborne, A.; Wang, X.; Gu, W.; Cohen, J.; Stoll, V. S.; Hutchins, C.; Frost, D.; Rosenberg, S. H.; Sham, H. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5371; (e) Li, Q.; Wang, G. T.; Claiborne, A.; Hasvold, L.; Stoll, V. S.; Muchmore, S.; Cohen, J.; Jakob, C. G.; Gu, W.; Wang, X.; Gu, W.; Hutchins, C.; Frost, D.; Rosenberg, S. H.; Sham, H. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5367.
3. (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; (c) Njoroge, F. G.; Vibulbhan, B.; Pinto, P.; Bishop, W. R.; Bryant, M. S.; Nomeir, A. A.; Doll, R. J.; Girijavallabhan, V. M.; Ganguly, A. K. *J. Med. Chem.* **1998**, *41*, 1561.
4. (a) Rokosz, L. L.; Huang, C.-Y.; Reader, J. C.; Stauffer, T. M.; Southwick, E. B.; Li, G.; Baldwin, J. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5537; (b) Huang, C.-Y.; Rokosz, L. L.; Satuffer, T. M; Reader, J. C.; Li, G.; Huang, H.; Strickland, C.; Cooper, A.; Doll, R.; Ganguly, A. K.; Baldwin, J. J. in press.; (c) Hucke, O.; Gelb, M. H.; Verlinde, C. L. M.; Buckner, F. S. *J. Med Chem.* **2005**, *48*, 5418; (d) 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; (e) 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; (f) Angelovski, G.; Costisella, B.; Kolaric, B.; Engelhard, M.; Eibracht, P. *Biochem.* **2005**, *44*, 11214.

5. Njoroge, F. G.; Vibulbhan, B.; Pinto, P.; Strickland, C.; Kirschmeier, P.; Bishop, W. R.; Girijavallabhan, V. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5877.
6. 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.
7. Strickland, C. L.; Weber, P. T.; Windsor, W. T.; Wu, Z.; Le, H. V.; Albanese, M. W.; Alvarez, C. S.; Cesarz, D.; delRosario, 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.
8. *Preparation of compound **24** as a representative compound.* Racemic [5-bromo-8-chloro-1*H*-benzo[5,6]]cyclohepta[1,2-*b*]pyridine-11-yl]-1-(methylsulfonyl)piperidine (9.52 g, 20.3 mmol) was separated by HPLC on Chiral AD® column eluting with 40–50% isopropanol-hexanes (with 0.2% diethyl amine) to give 1.7 g of sulfonamide **20**-(+), and 1.1 g of **20**-(−). Sulfonamide **20**-(−) (0.31, 0.66 mmol) was dissolved in 5 mL THF and stirred over N_2 at rt. To this solution were added potassium *tert*-butoxide (0.11, 0.99 mmol), piperazine (0.29, 3.31 mmol) and the reaction mixture was stirred for 2 h. THF was stripped off the reaction mixture, H_2O was added and extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried over $MgSO_4$, filtered, and concentrated. The crude reaction mixture was purified using HPLC on Chiral AD® column eluting with 30% isopropanol-hexanes (with 0.2% DEA) to give 5-substituted piperazine sulfone **23** (0.04 g, retention time = 14.2 min) and 6-substituted sulfone **24** (0.12 g, retention time = 57.6 min), $MH^+ = 473$, $[\alpha]_D^{25}$ −30.5° (9.5 mg/2 mL CH_2Cl_2).
9. Crystallographic data for the inhibitor **24**-FTase complex shown in Figure 3 have been deposited with the RCSB (code rcsb034995) Protein Data Bank as PDB ID 2BED. The structural details can be viewed at www.rscb.org using the ID number above.