Journal of Medicinal Chemistry Letter

J. Med. Chem. **2009,** *52,* 6205–6208 **6205**
DOI: 10.1021/jm901280q

Discovery of a New Class of Protein Farnesyltransferase Inhibitors in the Arylthiophene Series

Sébastien Lethu, Maryon Ginisty, Damien Bosc, and Joëlle Dubois*

Institut de Chimie des Substances Naturelles, UPR2301 CNRS, Centre de Recherche de Gif-sur-Yvette, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France

Received August 26, 2009

Abstract: Screening of the ICSN chemical library led to the discovery of 3-(4-chlorophenyl)-4-cyano-5-thioalkylthiophene 2-carboxylic acids as potent farnesyltransferase inhibitors. Enzymatic kinetic studies showed that this original FTI series belongs to the CaaX competitive inhibitor class. Preliminary SAR studies allowed us to improve the IC_{50} from 110 to 7.5 nM.

Since its discovery in the late 1980s, protein farnesyltransferase (FTase[a]) has been the subject of numerous studies.[1−3] This heterodimeric metalloenzyme belongs to the protein prenyl transferase family. It catalyzes the transfer of a farnesyl group (C_{15}) from farnesylpyrophosphate (FPP) to the free thiol group of a cysteine residue embedded in the C-terminal CaaX motif of proteins where C is a cysteine, a an aliphatic amino acid, and X a serine, a methionine, an alanine, or a glutamine.[4,5] Protein farnesylation is an important posttranslational modification occurring on several cell signaling proteins like small GTPases, including the oncogenic Ras proteins. Therefore, farnesyltransferase inhibitors (FTIs) have been extensively developed for anticancer therapy, and diverse compounds with druglike properties are available.[6,7] Recently, FTase has also appeared as a potential target for the treatment of parasitic diseases, as it has been shown that protein farnesylation occurs in trypanosomatids and in the malaria parasite.[8] Development of FTIs has been realized by rational design focused on modifications of isoprenoid or CaaX substrates or by library screening. These studies led to the discovery of three potent FTIs that are currently under clinical trials for anticancer therapy: BMS214662 (rational design approach) and lonafarnib and tipifarnib (library screening approach).[9]

In the course of our search for new FTIs, we screened our Institut's chemical and natural product libraries on our automated fluorescence-based FTase assay.[10] Two arylthiophene derivatives among the 4500 compounds in the ICSN library held more specifically our attention because of their activity and structure (Figure 1). Initial screening showed a submicromolar activity for these compounds bearing an original structure in the FTI's field.

*To whom correspondence should be addressed. Phone: +33 1 69 82 30 58. Fax: +33 1 69 07 72 47. E-mail: joelle.dubois@icsn.cnrs-gif.fr.

[a] Abbreviations: FTase, protein farnesyltransferase; FPP, farnesyl pyrophosphate; FTI, farnesyltransferase inhibitor; ICSN, Institut de Chimie des Substances Naturelles; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; SAR, structure−activity relationship; rt, room temperature; IC_{50}, inhibitor concentration that inhibits 50% of protein farnesyltransferase enzyme activity.

Several biological activities have already been reported for 3-arylthiophene derivatives. Some of them display anthelmintic activity against *Haemonchus contortus*,[11] and others have antileishmanial and antifungal activities[12] or inhibit c-Jun-N-terminal kinases and other kinases.[13] Certain arylthiophenes have also been patented as herbicides[14] or for fungicidal[15] and HLA-DM activities.[16] Our compounds belong to the 3-aryl-4-cyano-5-substituted thiophene 2-carboxylic acid family, among which some are AMPA receptor allosteric modulators.[17,18]

In this paper we describe the determination of **1** binding mode on the enzyme along with preliminary structure−activity relationship (SAR) studies on this new FTI series.

To understand the mode of action of our hit compound **1**, enzymatic kinetic experiments were carried out to determine where it binds to FTase. These studies showed that **1** was a CaaX competitive inhibitor with K_i = 105 nM and a FPP noncompetitive inhibitor with K_i = 110 nM. Thus, this compound occupies the CaaX binding site without interacting with the FPP binding site. The CaaX peptide fills a large part of the FTase active site cavity, and the zinc ion directly coordinates the sulfur atom of the cysteine.[4] Our kinetic results suggest that these arylthiophene derivatives interact with the zinc ion of the catalytic site probably by means of the thioether function in conjunction with the sulfur atom of the thiophene ring. Preliminary SAR studies were therefore undertaken to determine the binding contribution of different parts of the molecule.

We first looked at the importance of the thiophene central core. We used a described protocol allowing variation of the heteroaryl core of the molecule.[18] Ketene dithioacetal **4** was obtained by deprotonation of *p*-chlorobenzoylpropionitrile **3** followed by condensation with carbon disulfide and subsequent quenching with isopropyl iodide. Compound **1** was then obtained by cyclocondensation of **4** with ethyl thioglycolate and subsequent basic hydrolysis (Scheme 1). This synthetic pathway allowed us to prepare the corresponding furan **8** and methylpyrrole **9** by condensation of **4** with ethyl bromoacetate or sarcosine ethyl ester, respectively.

Thiazole **15** was also considered as an alternative heterocyclic derivative. Ethyl *p*-chlorobenzoylacetate **10**, obtained from *p*-chlorobenzoic chloride and ethyl acetate, was chlorinated and subjected to condensation with thiourea.[19] Sandmeyer substitution of the amino group by a bromide further substituted by propane-2-thiol allowed introduction of the thioether function. Saponification afforded thiazole **15** (Scheme 2).

The importance of the cyano group in FTase binding was evaluated by the synthesis of 3-(*p*-chlorophenyl)-5-(isopropylthio)thiophene-2-carboxylic acid **16** using the

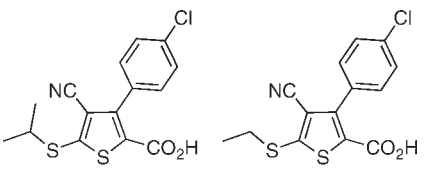
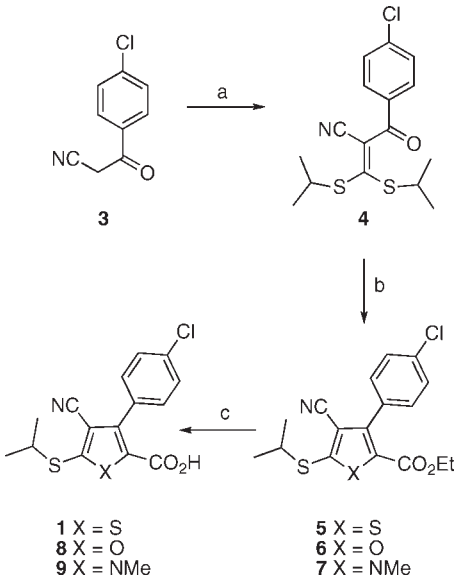


Figure 1. Structure of new FTIs.

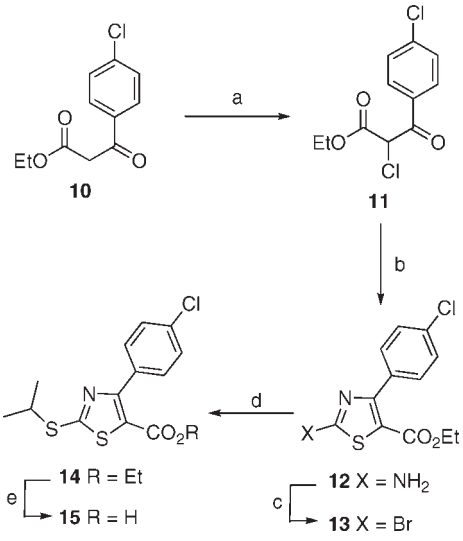
Published on Web 09/22/2009

Scheme 1. Synthesis of Compound **1** and of Its Heteroaromatic Analogues[a]



[a] Reagents: (a) (i) NaH, DMF, rt; (ii) CS_2, rt; (iii) *i*-PrI, 60 °C, (82%); (b) $HSCH_2CO_2Et$, Et_3N, EtOH, rt for **5** (92%), $BrCH_2CO_2Et$, LiHMDS, THF, −78 °C to rt for **6** (34%), $MeNHCH_2CO_2Et$, Et_3N, EtOH, reflux for **7** (46%); (c) NaOH, THF/H_2O/EtOH (4:3:2), rt for **1** (93%); NaOH, EtOH/H_2O (1:1), rt for **8** (87%); LiOH, THF/MeOH/H_2O (2:1:1), 60 °C for **9** (89%).

Scheme 2. Synthesis of Thiazole **15**[a]



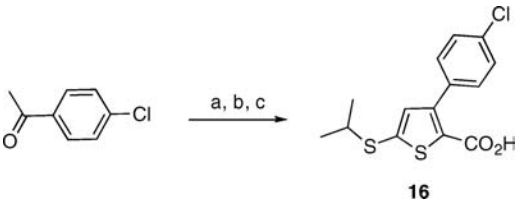
[a] Reagents: (a) SO_2Cl_2, toluene, rt (85%); (b) thiourea, EtOH, reflux (98%); (c) $CuBr_2$, *t*-BuONO, CH_3CN, 60 °C (89%); (d) *i*-PrSH, *t*-BuOK, THF, 10 °C to rt (76%); (e) NaOH, EtOH/H_2O (1:1), rt (95%).

same pathway as for thiophene **1** but starting from methyl *p*-chlorophenyl ketone (Scheme 3).

Influence of the thioether group was studied with 3-(*p*-chlorophenyl)-4-cyano thiophene 2-carboxylic acids available in commercial libraries. The alkyl group of the thioether varied from one to six carbons (**1**, **2**, and **17**−**21**), and a *N*-morpholine derivative **22** was also available to evaluate the importance of a sulfur atom at this position.

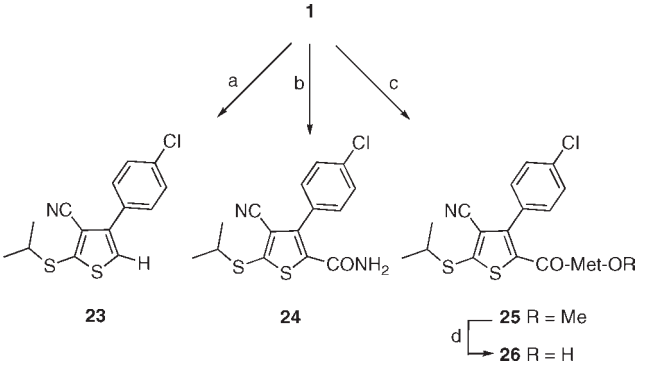
Carboxylic acid was the most considered part of this preliminary SAR study. The acid was decarboxylated at high temperature (**23**), transformed into a primary amide (**24**), or

Scheme 3. Synthesis of Compound **16**[a]



[a] Reagents: (a) (i) NaH, DMF, rt; (ii) CS_2, rt; (iii) *i*-PrI, TBAB, DMF, 60 °C (79%); (b) $HSCH_2CO_2Et$, K_2CO_3, EtOH, reflux (71%); (c) NaOH, THF/EtOH/H_2O (2:1:1), rt (64%).

Scheme 4. Modification of the Carboxylic Acid from Compound **1**[a]



[a] Reagents: (a) Cu, quinoline, 250 °C (81%); (b) (i) $SOCl_2$, CH_2Cl_2, rt; (ii) NH_4OH, rt (86%); (c) Met-OMe, HOBt, Et_3N, EDCI, CH_2Cl_2, rt (90%); (d) LiOH, MeOH/THF/H_2O (2:1:1), rt (50%).

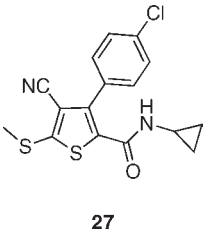


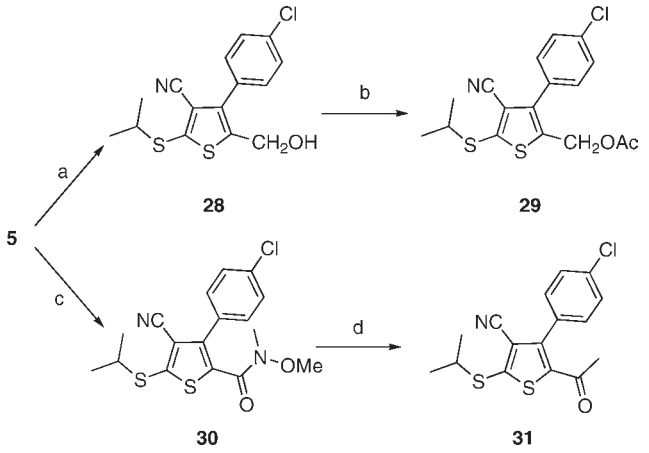
Figure 2. Commercial amide derivative.

coupled with an amino acid under usual conditions. Because our hit compound **1** is a CaaX competitive inhibitor, we thought that introduction of an amino acid at this position would improve binding affinity and solubility. In the CaaX motifs recognized by FTase, X is often a methionine. Therefore, this amino acid was chosen for this preliminary study. Classical peptide formation with methionine methyl ester afforded **25** that was further hydrolyzed by lithine to afford the free acid **26** (Scheme 4).

To complete the 2-amide-3-(*p*-chlorophenyl)thiophene series, the cyclopropylamide **27**, commercially available as the methyl thioether, was also evaluated (Figure 2).

Synthesis of thiophene **1** went through its ester **5**, allowing the evaluation of the importance of a free carboxylic acid in this part of the molecule. Some modifications were realized on this ester such as reduction by sodium borohydride yielding the primary alcohol **28** followed by its acetylation (**29**). Compound **5** was also transformed into the Weinreb amide **30** that was alkylated by methyllithium to afford the methyl ketone **31** (Scheme 5).

Scheme 5. Modification of the Carboxylic Acid from Ester **5**[a]



[a] Reagents: (a) $NaBH_4$, LiCl, THF/EtOH (1:2), rt (78%); (b) Ac_2O, DMAP, CH_2Cl_2, rt (76%); (c) NHMe(OMe), *i*-PrMgCl, THF, −15 °C (80%); (d) MeLi, THF, −78 °C (74%).

Table 1. Inhibitory Activities of Various Heterocycles[a]

compd	Het	IC_{50} (μM)
1	thiophene	0.11 ± 0.008
8	furan	2.0 ± 0.17
9	NMe-pyrrole	47 ± 3
15	thiazole	51 ± 5.5

[a] IC_{50}(FTase inhibitor I) = 0.032 μM in our assay conditions.

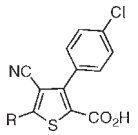
All these derivatives were evaluated on FTase and compared with commercial FTase inhibitor I.[20] Results are summarized in Tables 1−3.

The thiophene ring is the best heterocycle in this series. Furan **8** retains a relatively good inhibitory activity, 20-fold less than that of thiophene **1**. NMe-pyrrole **9** and thiazole **15** are weak inhibitors probably because **9** bears a methyl group at position 1 that can hamper binding to FTase and **15** lacks the cyano group at position 4 (Table 1). The importance of this latter group is confirmed by the weak activity of **16** where the cyano moiety has been replaced by hydrogen. Though **16** is more active than thiazole **15**, it exhibited IC_{50} = 15 ± 1 μM, 2 orders of magnitude lower than **1**.

All the thioether derivatives show close activities within the micromolar range (Table 2). Hit compound **1** is the most active derivative. Inhibitory activity decreases when the alkyl group is smaller (**2** and **17**) or larger (**18**−**21**) than isopropyl and varies with the alkyl chain length. Branched alkyl groups are better than linear ones especially when the branched carbon is adjacent to the sulfur atom (**20** vs **19**). The thioether at the C-5 position may contribute to FTase binding, since the *N*-morpholine analogue **22** is much less active with IC_{50} = 28 μM, compared to IC_{50} = 1.45 μM for cyclohexyl derivative **21**.

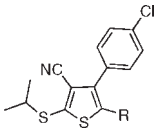
In general, removal or modification of the carboxylic function is detrimental to the activity (Table 3). When the carboxylic acid is removed (**23**), no activity is seen any more. Its reduction (**28**) abolishes the inhibition though acetylation of the primary alcohol (**29**) restores it slightly. Therefore, it appears that the presence of a carbonyl group is important for binding to FTase. The absence of a free carboxyl group lowers the activity as seen by the 30-fold decrease for ester derivative **5**. Predictably, furan, pyrrole, and thiazole esters **6**, **7**, and **14** are completely inactive (data not shown). When the carbonyl group is still present, some inhibition is retained. Methyl ketone **31** retains a good activity, though 8-fold lower than that of thiophene **1**, like the amide derivative **24**. The inhibitory activity drops when the amide is substituted by an alkyl group (**27**) and disappears when two substituents are present on the amide as in the Weinreb amide **30**. However, when methionine is added to **1**, the activity remains the same for the ester derivative **25** as for **5** but greatly increases for the acid form **26** with an IC_{50} in the low nM range. This is in good agreement with our general observation that an acidic derivative is a better inhibitor than its corresponding ester in the enzymatic assay. This result also supports our kinetic findings that these arylthiophene derivatives occupy the CaaX binding site.

Table 2. Inhibitory Activities of 5-Modified 3-(*p*-Chlorophenyl)-thiophenes



compd	R	IC_{50} (μM)
1	S-*i*-Pr	0.110 ± 0.008
2	S-Et	0.325 ± 0.01
17	S-Me	0.61 ± 0.03
18	S-*n*-Bu	1.24 ± 0.1
19	S-*i*-Bu	0.89 ± 0.06
20	S-*s*-Bu	0.345 ± 0.02
21	S-*c*-Hex	1.45 ± 0.15
22	*N*-morpholine	28 ± 2.4

Table 3. Inhibitory Activities of 2-Modified 3-(*p*-Chlorophenyl)-thiophenes



compd	R	IC_{50} (μM)
1	CO_2H	0.11 ± 0.008
5	CO_2Et	3.8 ± 0.25
23	H	inactive
24	$CONH_2$	1.1 ± 0.05
25	CO-Met-OMe	5.7 ± 0.4
26	CO-Met-OH	0.0075 ± 0.0007
27[a]	CONH-*c*-Pr	22 ± 2.4
28	CH_2OH	inactive
29	CH_2OAc	45 ± 5
30	CON(Me)OMe	inactive
31	COMe	0.79 ± 0.08

[a] Methylthioether derivative.

In conclusion, this new FTI series belongs to the nonpeptidic CaaX inhibitors, the most active FTI class. Our preliminary SAR study has shown that the presence of the two sulfur atoms and of the cyano function contributes to the good affinity of these derivatives. A carbonyl group at the 2-position is necessary to inhibit FTase, and compounds bearing a carboxylic acid or an amino acid are the best inhibitors of this series. Through the addition of a methionine on the acidic function we have succeeded in increasing the hit compound **1** activity by 1.5 orders of magnitude. Variations around this amino acid are currently under investigation. Many other modifications are also conceivable such as the

p-chlorophenyl moiety that has not been modified in this preliminary study. As well, other Zn-chelating functions could be introduced in the C-5 position of the thiophene ring to improve the activity and solubility of these derivatives. The aim of our research is to find new antiparasitic derivatives through FTase inhibition. Therefore, we will focus our efforts to modulate these arylthiophenes to make them selectively active on parasitic FTase.

Acknowledgment. The authors thank J.-B. Créchet for the generous gift of the *E. coli* strain expressing the yeast FTase, Dr. J. Ouazzani and P. Lopes for the production and purification of recombinant yeast FTase, N. Maroteau for his help in library screening, Dr. F. Guéritte, responsible for the ICSN compound library, and E. Galmiche and O. Thoison for HPLC analyses. Pr. J. Y. Lallemand is acknowledged for his interest in this work, and ICSN and CNRS are acknowledged for providing financial support.

Supporting Information Available: Synthesis details and spectral data for all new compounds, experimental details for FTase inhibition, Lineweaver−Burk plots of the kinetic data, and K_i determination. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; Brown, M. S. Inhibition of purified p21ras farnesyl:protein transferase by Cys-AAX tetrapeptides. *Cell* **1990**, *62*, 81–88.
(2) Gibbs, J. B.; Oliff, A.; Kohl, N. E. Farnesyltransferase inhibitors: Ras research yields a potential cancer therapeutic. *Cell* **1994**, *77*, 175–178.
(3) Tamanoi, F.; Kato-Stankiewicz, J.; Jiang, C.; Machado, I.; Thapar, N. Farnesylated proteins and cell cycle progression. *J. Cell. Biochem., Suppl.* **2001**, No. Suppl. 37, 64–70.
(4) Zhang, H. Protein prenyltransferases. *Handb. Metalloproteins* **2004**, *3*, 37–48.
(5) Park, H. W.; Beese, L. S. Protein farnesyltransferase. *Curr. Opin. Struct. Biol.* **1997**, *7*, 873–880.
(6) Sebti, S. M.; Hamilton, A. D. Farnesyltransferase and geranylgeranyltransferase I inhibitors in cancer therapy: important mechanistic and bench to bedside issues. *Expert Opin. Invest. Drugs* **2000**, *9*, 2767–2782.
(7) Zhu, K.; Hamilton, A. D.; Sebti, S. M. Farnesyltransferase inhibitors as anticancer agents: current status. *Curr. Opin. Invest. Drugs* **2003**, *4*, 1428–1435.
(8) Eastman, R. T.; Buckner, F. S.; Yokoyama, K.; Gelb, M. H.; Van Voorhis, W. C. Fighting parasitic disease by blocking protein farnesylation. *J. Lipid Res.* **2006**, *47*, 233–240.
(9) Banerjee, S.; McGeady, P. Inhibitors of protein prenylation. *Curr. Enzyme Inhib.* **2005**, *1*, 183–206.
(10) Our enzymatic assay is the adaptation to 96-well plate format of the fluorescence-based assay described in the following references: (a) Pompliano, D. L.; Gomez, R. P.; Anthony, N. J. Intramolecular fluorescence enhancement: a continuous assay of Ras farnesyl: protein transferase. *J. Am. Chem. Soc.* **1992**, *114*, 7946–7946. (b) Cassidy, P. B.; Dolence, J. M.; Poulter, C. D. Continuous fluorescence assay for prenyltransferases. *Methods Enzymol.* **1995**, *250*, 30–43.
(11) González, I. C.; Davis, L. N.; Smith, C. K., II. Novel thiophenes and analogues with anthelmintic activity against *Haemonchus contortus*. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4037–4043.
(12) Ram, V. J.; Goel, A.; Shukla, P. K.; Kapil, A. Synthesis of thiophenes and thieno[3,2-*c*]pyran-4-ones as antileishmanial and antifungal agents. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3101–3106.
(13) Cao, J.; Green, J.; Moon, Y.-C.; Wang, J.; Ledeboer, M.; Harrington, E.; Gao, H. Preparation of Thienyl-Substituted Pyrimidinyl, Pyridinyl and Triazinyl Amines as Inhibitors of c-Jun N-Terminal Kinases (JNK) and Other Protein Kinases. WO Patent 2002083667, October 24, **2002**.
(14) Abdulla, R. F.; Morris, K. W.; Williams, J. C., Jr. 3-Cyano-4-arylthiophenes. Eur. Patent 0273602, December 2, **1987**.
(15) Dolman, H.; Kuipers, J. Preparation of Thio Compounds Having Fungicidal Activity. Eur. Patent 0234622, February 2, **1987**.
(16) Nicholson, M.; Wucherpfennig, K.; Stein, R. L.; Yeh, L.-A.; Cuny, G. D. Thiophene Derivatives for Up-Regulating HLA-DM Activity. WO Patent 2005066152, July 21, **2005**.
(17) Castano Mansanet, A. M.; Dominguez-Manzanares, E.; Escribano, A. M.; Fernandez, M. C.; Hornback, W. J.; Jimenez-Aguado, A. M.; Tromiczak, E. G.; Wu, Z.; Zarrinmayeh, H.; Zimmerman, D. M. Preparation of Thiophene and Furan Compounds for Potentiating Glutamate Receptor Function. WO Patent 2005070916, August 04, **2005**.
(18) Fernandez, M.-C.; Castaño, A.; Dominguez, E.; Escribano, A.; Jiang, D.; Jimenez, A.; Hong, E.; Hornback, W. J.; Nisenbaum, E. S.; Rankl, N.; Tromiczak, E.; Vaught, G.; Zarrinmayeh, H.; Zimmerman, D. M. A novel class of AMPA receptor allosteric modulators. Part 1: Design, synthesis, and SAR of 3-aryl-4-cyano-5-substituted-heteroaryl-2-carboxylic acid derivatives. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5057–5061.
(19) Ladouceur, G. H.; Schoen, W. R.; Burke, M. J. Benzofuran and Dihydrobenzofuran Derivatives Useful as Beta-3 Adrenoreceptor Agonists. WO Patent 03024948, March 27, **2003**.
(20) *N*-[2(*S*)-[2(*R*)-Amino-3-mercaptopropylamino]-3-methylbutyl]-Phe-Met-OH from Calbiochem.