Brief Articles

Second Generation of Hydroxyethylamine BACE-1 Inhibitors: Optimizing Potency and Oral Bioavailability†

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Received January 8, 2008

BACE-1 inhibition has the potential to provide a disease-modifying therapy for the treatment of Alzheimer's disease. Optimization of a first generation of BACE-1 inhibitors led to the discovery of novel hydroxyethylamines (HEAs) bearing a tricyclic nonprime side. These derivatives have nanomolar cell potency and are orally bioavailable.

Introduction

Alzheimer's disease is a devastating neurodegenerative disorder for which no disease-modifying treatment is currently available.[1] The disease is characterized by the progressive formation of insoluble amyloid plaques[2] and neurofibrillary tangles[3] in the brain. These plaques mainly comprise a small 4 kDa amyloid-β (Aβ[a]) peptide generated by the proteolytic processing of a larger membrane-bound precursor protein, known as the amyloid precursor protein (APP). Cleavage of APP by BACE-1 (also known as β-secretase, memapsin-2, or Asp-2) generates a membrane-tethered C-terminal fragment, subsequently processed by γ-secretase to produce Aβ peptides predominantly of 40 or 42 amino acids in length (Aβ40, Aβ42).[4]

Inhibition of BACE-1 is expected to stop amyloid plaque formation and to be well tolerated. Indeed, mice genetically deficient in this enzyme do not produce Aβ[5a,b] and are healthy, viable, and fertile.[5a–c] Extensive efforts have therefore been followed in an attempt to discover inhibitors of this aspartyl protease.[6] In most cases, the design of inhibitors has been based on the transition state mimetic approach, which relies on replacement of the scissile amide bond of an appropriate substrate with a stable mimetic of the putative transition-state structure.[7]

Discussion

We have recently disclosed our first generation of BACE-1 inhibitors, based on an hydroxyethylamine (HEA) core, such as **1** (Figure 1).[8] The key interactions of such derivatives with the enzyme are depicted below and comprise (a) a network of H-bonds between the HEA core and the two aspartate residues present in the active site, (b) H-bonds between an amide and two protein residues, Gln-134 and Gly-291, (c) a lipophilic interaction of the central benzylic substituent with the S_1 pocket, (d) an H-bond from the 6-sultam oxygen to Asn-294 (backbone NH), (e) lipophilic interactions with the S_3 and S_2' pockets via the -NHC_2H_5 meta substituent of the benzamide nonprime side and the *m*-CF_3 of the benzyl prime side, respectively. The loss of any of these key interactions led to at least a 10-fold decrease of potency (interactions d and e above) if not complete loss of activity (interactions a−c).

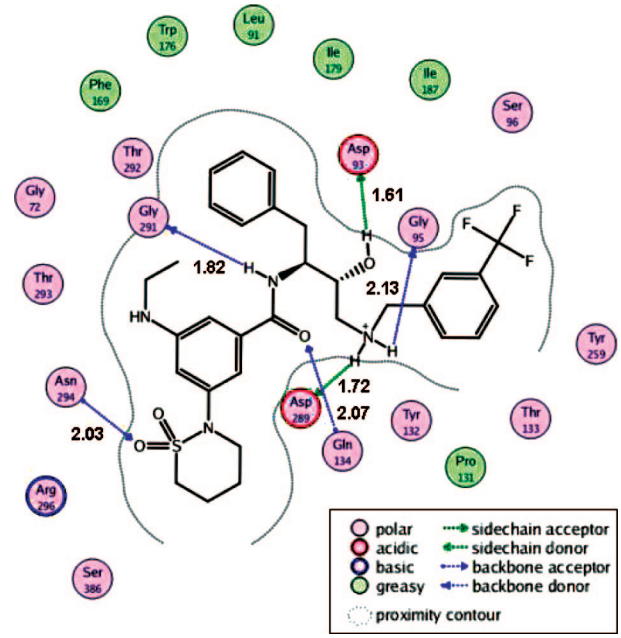

Figure 1. Key interactions of inhibitor **1** bound to BACE-1. Visualization is generated by MOE (Molecular Operating Environment, Chemical Computing Group).

This first generation of compounds were potent inhibitors of BACE-1 but generally had suboptimal in vivo pharmacokinetics and low brain penetration. The lack of exposure following oral

† The PDB deposition codes for the BACE-1 complex crystal structures with inhibitors **1** and **8a** are 2vnm and 2vnn, respectively.

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[a] Abbreviations: Aβ, amyloid-β; APP, amyloid precursor protein; BACE, β-site amyloid precursor protein cleaving enzyme; HEA, hydroxyethylamine; Cat-D, cathepsin D.

10.1021/jm800138h CCC: $40.75
Published on Web 05/06/2008

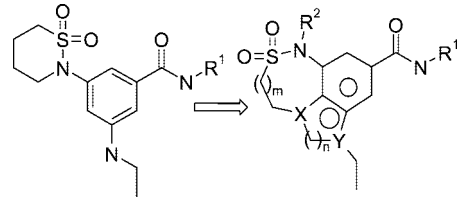


Figure 2. Toward a novel nonprime side: $n = 1, 2$; $m = 0-2$; X, Y = C or N.

administration could be attributed to low permeability and high blood clearance (up to liver blood flow in most cases), while the low brain penetration could be linked to the poor permeability and to efficient efflux by the Pgp transporter, most likely due to the high number of H-bond donors (HBD) and acceptors (HBA) present in these molecules.[9] A combination of the above factors meant that while lowering of amyloid production in animal models could be observed following oral administration of compounds from this series,[10] the effect was only observed at high doses (100 mg/kg).

Considering the number of lipophilic enzyme-binding interactions and the number of H-bonds required to achieve nanomolar potency in the first generation of inhibitors, it seemed likely that any novel series derived from these would also have substantial affinity for Pgp and hence would probably be relatively poorly brain-penetrant. Therefore, to obtain effective brain concentrations in a second generation of inhibitors, potency needed to be matched or improved while increasing effective blood concentrations at lower oral doses. To achieve this, it seemed reasonable to expect that the permeability of the compounds would need to be markedly improved while at the same time the clearance would need to be substantially reduced. It also seemed unlikely that this could be achieved without making substantial changes to the structures of these inhibitors.

Qualitative in vitro studies with liver microsomes suggested that the main metabolic pathways for compounds such as **1** were oxidation at the benzylic position on the prime side and dealkylation of the nonprime side aniline substituent. To resolve the latter issue, efforts focused initially on the discovery of a novel nonprime side in which the aniline functionality was absent. It was anticipated that if the ligand efficiency of such replacements, compared to the di-meta-substituted benzamide present in inhibitor **1**, could also be improved, then this might also allow further truncation of the prime side and elimination of the metabolically labile benzylic substituent.

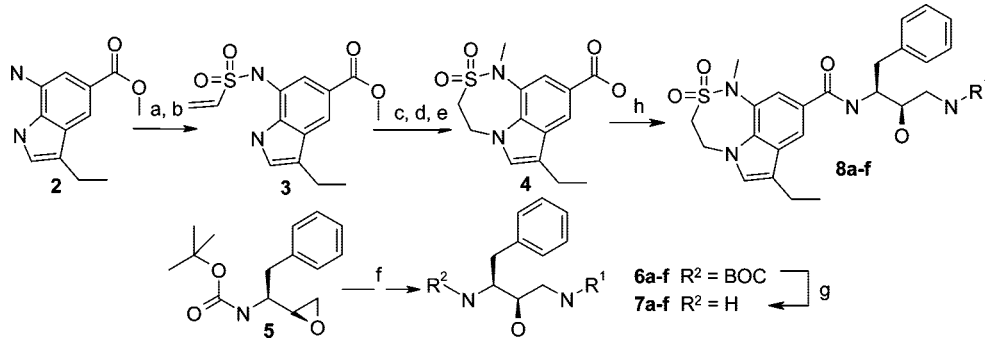
Molecular modeling suggested that the key nonprimed side interactions in the first generation of inhibitors could be mimicked by a tricyclic nonprime side such as that shown in Figure 2. It was reasoned that constraining the active conformation in this way would make binding to the protein much more efficient while also potentially reducing or eliminating (Y = N or C, respectively) the propensity for N-dealkylation.

The synthesis of the first tricyclic acid used to test this hypothesis is depicted in Scheme 1 starting from readily available indole **2**.[11] Addition of 2-chloroethanesulfonyl chloride gave vinyl sulfonamide **3**. The sulfonamide **3** was then cyclized and alkylated with methyl iodide to remove the additional, and likely unnecessary, HBD. Subsequent saponification of the ester then led to the acid **4**, which could be coupled with amines **7a**−**f**, in turn obtained from commercially available epoxide **5** via intermediates **6a**−**f**, to give tricyclic HEA derivatives **8a**−**f**.

The activity of these novel derivatives is summarized in Table 1. BACE-2 and Cat-D, both structurally related to BACE-1, were chosen as representative aspartyl proteases for selectivity screens. Gratifyingly, inhibitor **8a** was actually slightly more potent in a cell based assay, albeit less selective, than the parent compound **1**. The modeling hypothesis was confirmed experimentally, and cocrystallization of these inhibitors with a BACE-1 construct showed an excellent superposition of the two nonprime sides and the same interaction with Asn-294 as seen in Figure 3.

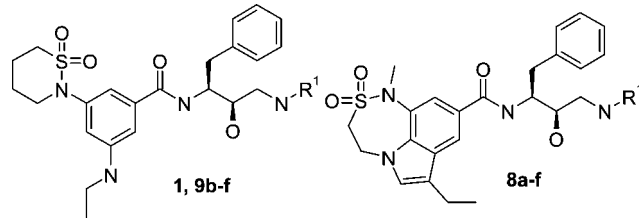
Subsequent removal of the meta substituent of the benzylic prime side in this series of compounds now resulted in only a small loss of potency in a cell-based assay when compared to the effect of similar changes to the 6-sultam series (compare **8b** and **9b**). This trend was continued with a number of "favored" prime sides from the sultam series[8] (compare **8c**,**d** with **9c**,**d**). Further truncation of the prime side then resulted in the identification of compounds with lower MW and low nanomolar potency (e.g., see **8e**,**f** vs **9e**,**f**). Excellent selectivity versus BACE-2 and especially Cat-D was also observed in this series of truncated inhibitors. Moreover, good oral exposure could now be achieved and inhibitor **8e**, for example, was shown to have nanomolar potency in a cellular assay and improved oral bioavailability in rats[12] and good bioavailability in dogs (Table 2). In particular, micromolar blood concentrations could now be achieved at relatively low doses. Interestingly, CNS penetration studies (Table 3) demonstrated that submicromolar blood concentrations resulted in brain concentrations well in

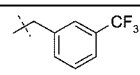
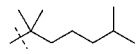
Scheme 1. Synthesis of BACE-1 Inhibitors with Tricyclic Nonprime Side[a]



[a] Reagents and conditions: (a) 2-chloroethanesulfonyl chloride, pyridine, DMAP, CH_2Cl_2, 25 °C; (b) NEt_3, CH_2Cl_2, 25 °C; (c) NaH, DMF, 25−100 °C, 85% (three steps); (d) NaH, MeI, DMF, 25 °C, 76%; (e) NaOH, EtOH/H_2O, 25 °C, 57%; (f) R^1NH_2, EtOH, reflux, 70−80%; (g) HCl, dioxane or *p*-CH_3PhSO_3H, CH_3CN, 25 °C, 75−85%; (h) **7a**−**f**, EDAC·HCl, HOBT, NEM, DMF or CH_2Cl_2, 25 °C, 60−80%.

Table 1. Comparison of Activity and Selectivity of HEAs with Dimetasubstituted and Tricyclic Nonprime Sides



Cpd	R^1	BACE-1 IC_{50} [a,b] (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	Aβ40 IC_{50} [b,c] (nM)	Aβ42 IC_{50} [b,c] (nM)
8a		2 (2)	59	1260	10.19 ± 0.76	8.45 ± 1.12
1		3 (2)	1430	3900	20.89 ± 3.92	13.15 ± 1.66
8b		34 (1)	3020	17180	33.09 ± 7.65	22.95 ± 0.51
9b		120 (1)	32360	26300	246.9 ± 20.72	101.38 ± 12.38
8c		5 (3)	114	1000	28.85 ± 4.08	19.19 ± 1.44
9c		30 (1)	7940	5250	75.11 ± 15.11	62.94 ± 33.95
8d		9 (2)	210	660	27.38 ± 1.74	21.31 ± 1.78
9d		69 (2)	4370	2340	149.03 ± 4.99	122.13 ± 12.65
8e		20 (4)	263	7933	16.32 ± 3.23	16.85 ± 1.99
9e		120 (1)	19500	30200	1324 ± 119	1262 ± 188
8f		15 (1)	1260	58880	48.90 ±16.35	57.03 ± 15.66
9f		140 (1)	47860	$> 10^5$	nd	nd

[a] In all tables, IC_{50} values are the mean of the values of *n* different experiments, *n* being reported in parentheses and identical for BACE-1, BACE-2, and Cat-D. Each IC_{50} is within 3-fold of the mean value. [b] See Experimental Section for protocol. [c] Values are the mean of at least three separate experiments.

excess of the IC_{50} of inhibitor **8e**. The in vivo efficacy of inhibitors of this type is currently being investigated further.

Conclusion

In conclusion, further optimization of a first generation of orally active HEA inhibitors led to the discovery of compounds incorporating a tricyclic nonprime side and a truncated prime side residue, which showed nanomolar potency in a cell-based assay. This, combined with good oral bioavailability, is critical for the delivery of low dose orally active inhibitors with the potential to act as useful therapeutic agents for Alzheimer's patients. A full account of our findings within this series will be reported in due course.

Experimental Section

Methyl 7-Ethyl-1-methyl-3,4-dihydro-1*H*-[1,2,5]thiadiazepino[3,4,5-*hi*]indole-9-carboxylate 2,2-Dioxide. To a solution of methyl 7-amino-3-ethyl-1*H*-indole-5-carboxylate (**2**) (124 mg, 0.4 mmol, 1 equiv) in DMF (10 mL) at room temperature was added NaH (60% in mineral oil, 19 mg, 0.45 mmol, 1.2 equiv). After 5 min, the mixture was heated to 100 °C for 1 h and then cooled to room temperature. Ethanol (1 mL) was added and the solution dissolved in AcOEt. The organic phase was washed with a 2 N HCl aqueous solution, dried over $MgSO_4$, and concentrated in vacuo to give methyl 7-ethyl-3,4-dihydro-1*H*-[1,2,5]thiadiazepino[3,4,5-*hi*]indole-9-carboxylate 2,2-dioxide (95 mg, 77%), which was used in the next stage without further purification. To a solution of methyl 7-ethyl-3,4-dihydro-1*H*-[1,2,5]thiadiazepino[3,4,5-*hi*]indole-9-carboxylate 2,2-dioxide (191 mg, 0.62 mmol, 1 equiv) in DMF (10 mL) at room temperature were added NaH (60% in mineral oil, 50 mg, 1.24 mmol, 2 equiv) and, after 2 min, MeI (46 μL, 0.74 mmol, 1.2 equiv). The resulting mixture was stirred at room temperature for 30 min. Then EtOH (1 mL) was added and the solution concentrated in vacuo. The residue was dissolved in AcOEt, and the organic phase was washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (isohexane/AcOEt, 3/1 to 1/1) gave the title compound (152 mg, 76%). MS: $m/z = 323.0$ $[M + H]^+$. ^1H NMR (400 MHz, CD_6SO): $\delta = 1.26$ (t, $J = 7.6$ Hz, 3H), 2.73 (q,

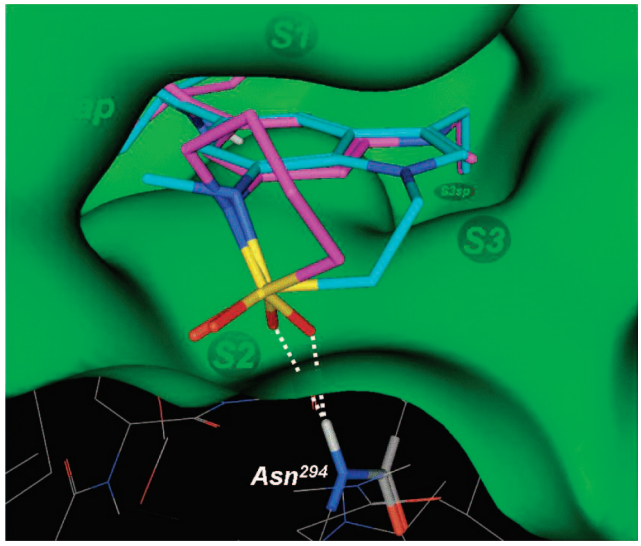


Figure 3. Superposition of the 6-sultam **1** (magenta) and tricyclic (light-blue) **8a** nonprime side of inhibitors bound to BACE-1.

Table 2. Mean ± SD of Pharmacokinetic Parameters of Tricyclic BACE-1 Inhibitor **8e**

species	Cl_{blood}[a] ((mL/min)/kg)	V_d[a] (L/kg)	$T^a_{1/2}$ (h)	C_{max}[b] (μM)	F (%)
rat, $n = 2-3$	72, 62	11, 8	3, 2.8	0.184 ± 0.082	17, 22
dog, $n = 3$	23 ± 5	4.9 ± 0.9	4.2 ± 0.2	2.969 ± 0.787	79 ± 50[c]

[a] 1 mg of free base (fb)/kg/h iv dose (solution of mesylate salt in 0.9% w/v saline containing 10% w/v Kleptose). [b] 10 mg fb/kg (solution of mesylate salt in 1% v/v Tween-80 and 1% w/v methylcellulose aqueous). [c] Two dogs had $F_{po} \approx 50\%$, while the third dog had F_{po} markedly higher (>100%) than would have been predicted from the blood clearance.

Table 3. CNS Penetration Study in Rat for Inhibitor **8e**

parameter	
C_{ss}, blood (μM)[a]	0.604 ± 0.050
CL_{blood} ((mL/min)/kg)	55 ± 5
C_{ss}, brain (μM)	0.223 ± 0.038
brain/blood ratio	0.37 ± 0.09: 1

[a] 24 h iv infusion of inhibitor **8e** to four rats (mesylate salt in 0.9% w/v saline containing 10% w/v Kleptose) at 1 (mg fb/kg)/h.

$J = 7.6$ Hz, 2H), 3.44 (s, 3H), 3.87 (s, 3H), 4.01−4.04 (m, 2H), 4.52−4.55 (m, 2H), 7.3 (s, 1H), 7.62 (d, $J = 1.2$ Hz, 1H), 8.10 (d, $J = 1.2$ Hz, 1H).

7-Ethyl-1-methyl-3,4-dihydro-1*H*-[1,2,5]thiadiazepino[3,4,5-*hi*]indole-9-carboxylic Acid 2,2-Dioxide (4). To a solution of methyl 7-ethyl-1-methyl-3,4-dihydro-1*H*-[1,2,5]thiadiazepino[3,4,5-*hi*]indole-9-carboxylate 2,2-dioxide (135 mg, 0.42 mmol, 1 equiv) in EtOH (20 mL) was added 2 N aqueous NaOH solution (20 mL, 40 mmol, 95 equiv). The resulting mixture was stirred for 14 h at room temperature. Then most of EtOH was removed in vacuo. The residue was extracted with Et_2O. The aqueous layer was acidified using a 2 N HCl aqueous solution, and the white precipitate formed was extracted twice with AcOEt. The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to give the title compound (74 mg, 57%), which was used in the next step without further purification. MS: $m/z = 308.9$ $[M + H]^+$. ^{1}H NMR (400 MHz, CD_6SO): $\delta = 1.26$ (t, $J = 7.6$ Hz, 3H), 2.73 (q, $J = 7.6$ Hz, 2H), 3.43 (s, 3H), 4.00−4.03 (m, 2H), 4.51−4.54 (m, 2H), 7.3 (s, 1H), 7.62 (d, $J = 1.6$ Hz, 1H), 8.10 (d, $J = 1.6$ Hz, 1H), 12.5 (bs, 1H).

(2*R*,3*S*)-3-Amino-1-(cyclopropylamino)-4-phenyl-2-butanol Bis(4-methylbenzenesulfonate) (7e). The title compound was obtained as a white solid in 51% from epoxide (**5**) using the following general procedure. To a solution of 1,1-dimethylethyl {(1*S*)-1-[(2*S*)-2-oxiranyl]-2-phenylethyl}carbamate (**5**) (2.63 g, 10 mmol, 1 equiv) in EtOH (40 mL) was added amine NH_2R^1 (30 mmol, 3 equiv), and the resulting mixture was refluxed for 4 h and then cooled to room temperature and concentrated in vacuo. The residue was dissolved in AcOEt, and the organic phase was washed twice with a saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and concentrated in vacuo to give the corresponding intermediate (**6x**), which was dissolved in CH_3CN (0.2 M). To the resulting solution was added at room temperature tosic acid monohydrate (3 equiv), and the resulting mixture was stirred for 5 h. The formed precipitate was filtered off and washed with Et_2O to give amine (**7x**) as a bis(4-methylbenzenesulfonate) salt. MS: $m/z = 221.1$ $[M + H]^+$. ^{1}H NMR (400 MHz, CD_6SO): $\delta =$ 0.71−0.79 (m, 3H), 0.83−0.87 (m, 1H), 2.29 (s, 6H), 2.69 (bs, 1H), 2.84 (dd, $J = 14.0$ and 7.6 Hz, 1H), 2.91 (dd, $J = 14.0$ and 7.6 Hz, 1H), 3.00 (t, $J = 11.6$ Hz, 1H), 3.29 (d, $J = 13.2$ Hz, 1H), 3.53 (bs, 1H), 4.03 (bs, 1H), 6.10 (d, $J = 5.6$ Hz, 1H), 7.13 (d, $J = 7.6$ Hz, 4H), 7.28−7.31 (m, 1H), 7.32−7.38 (m, 4H), 7.50 (d, $J = 7.6$ Hz, 4H), 7.97 (bs, 3H), 8.74 (bs, 2H).

***N*-[(1*S*,2*R*)-1-Benzyl-3-(cyclopropylamino)-2-hydroxypropyl]-7-ethyl-1-methyl-3,4-dihydro-1*H*-[1,2,5]thiadiazepino[3,4,5-*hi*]indole-9-carboxamide 2,2-Dioxide (8e).** The title compound was obtained in 66% yield using the following general procedure. To a solution of acid (0.3 mmol, 1 equiv) in CH_2Cl_2 or DMF (4 mL) were added EDAC·HCl (69 mg, 0.36 mmol, 1.2 equiv), HOBT (49 mg, 0.36 mmol, 1.2 equiv), then *N*-ethylmorpholine (150 μL, 1.2 mmol, 4 equiv) followed by the appropriate amine (0.3 mmol, 1 equiv). The resulting mixture was stirred at room temperature for 16 h and then diluted with CH_2Cl_2. The organic phase was washed with a saturated $NaHCO_3$ aqueous solution and with H_2O, then dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by mass-directed autoprep gave the corresponding formate salt amide (>99% purity) as a foam. MS: $m/z = 511.2$ $[M + H]^+$. ^{1}H NMR (400 MHz, CD_6SO): $\delta = 0.20-0.23$ (m, 2H), 0.32−0.35 (m, 2H), 1.27 (t, $J = 7.6$ Hz, 3H), 2.07−2.10 (m, 1H), 2.30 (bs, 1H), 2.62−2.79 (m, 4H), 2.83 (dd, $J = 13.6$, 10.4 Hz, 1H), 3.11 (dd, $J = 13.6$, 3.6 Hz, 1H), 3.41 (s, 3H), 3.57−3.64 (m, 1H), 3.97−4.01 (m, 2H), 4.11−4.18 (m, 1H), 4.45−4.49 (m, 2H), 4.91 (d, $J = 5.6$ Hz, 1H), 7.12 (t, $J = 6.8$ Hz, 1H), 7.18−7.25 (m, 5H), 7.49 (s, 1H), 7.92 (s, 1H), 8.26 (d, $J = 8.8$ Hz, 1H). HRMS (EI): m/z calcd for $C_{27}H_{35}N_4O_4S$, 511.2373; found, 511.2371.

Acknowledgment. The authors thank Christopher Seaman and Mark Vine for NMR support, Dr. Julie Quayle and Martin Read for analytical and purification support, and Dr. Bill Leavens for recording HRMS spectra.

Supporting Information Available: Experimental procedures for the synthesis of **1**, **8a**−**f**, and **9b**−**f**, in vitro assay protocols for the determination of IC_{50}, and X-ray crystallographic details. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM800138H