Journal of Medicinal Chemistry

Article

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Design, Synthesis, and Biological Activity of a Novel Series of Human Sirtuin-2-Selective Inhibitors

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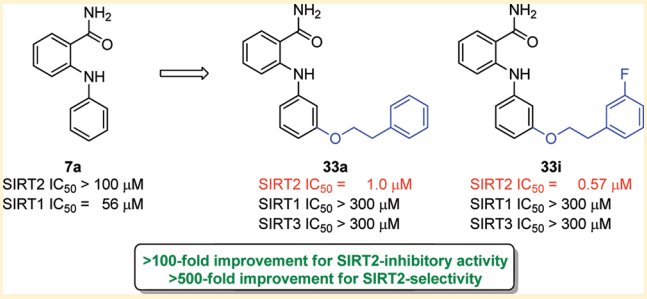
Supporting Information

ABSTRACT: Selective inhibitors of human sirtuin 2 (SIRT2), a deacetylase, are candidate therapeutic agents for neurodegenerative diseases such as Parkinson's disease and Huntington's disease as well as potential tools for elucidating the biological functions of SIRT2. On the basis of homology models of SIRT1 and SIRT2, we designed and prepared a series of 2-anilinobenzamide analogues. Enzyme assays using recombinant SIRT1 and SIRT2 revealed that 3′-phenethyloxy-2-anilinobenzamide analogues such as **33a** and **33i** are potent and selective SIRT2 inhibitors, showing more than 3.5-fold greater SIRT2-inhibitory activity and more than 35-fold greater SIRT2-selectivity compared with AGK2 (**3**), a previously reported SIRT2-selective inhibitor. Compound **33a** also induced a dose-dependent selective increase of α-tubulin acetylation in human colon cancer HCT116 cells, indicating selective inhibition of SIRT2 in the cells. These 3′-phenethyloxy-2-anilinobenzamide derivatives represent an entry into a new class of SIRT2-selective inhibitors.



INTRODUCTION

Reversible acetylation of protein lysine residues, which is tightly controlled by histone acetyltransferases and histone deacetylases (HDACs), is important in the regulation of various fundamental life processes such as gene expression and cell cycle progression.[1] Thus far, 18 HDAC family members have been identified and they can be divided into two categories, i.e., zinc-dependent enzymes (HDAC1−11) and NAD^+-dependent enzymes (SIRT1−7).[2] SIRT2 is unique in that it deacetylates histone in nucleus and nonhistone proteins such as α-tubulin, p65, and FOXO1 in cytoplasm, and it is involved in microtubule stabilization, gene expression, and cell cycle progression.[3] Furthermore, recent studies have indicated that changes of SIRT2 are associated with several diseases, especially neurodegenerative diseases. Green et al. reported that nicotinamide, a SIRT2 inhibitor, increased acetylated α-tubulin, reduced Thr 231-phosphorylated tau, and restored cognition in Alzheimer's disease transgenic mice.[4] Outeiro et al. reported that inhibition of SIRT2 rescued α-synuclein toxicity and modified inclusion morphology in a cellular model of Parkinson's disease.[5] In addition, SIRT2 inhibition has been reported to reduce neuronal cholesterol reduction and to be neuroprotective in cellular models of Huntington's disease.[6] Therefore, SIRT2-selective inhibitors are of great interest not only as tools for probing the biological functions of SIRT2 but also as candidate therapeutic agents with potentially few side effects.

Although many SIRT inhibitors have been found so far by us and other groups,[7] including salermide (**1**)[8] and EX-527 (**2**) (Chart 1),[9] most lack SIRT2 selectivity. It has been reported that SIRT2 is selectively inhibited by AGK2 (**3**)[5] and compounds **4**,[10] **5**,[11] and **6** (Chart 1).[12] Among these compounds, AGK2 (**3**) is the most widely used and most potent SIRT2-selective inhibitor.

We recently described a series of 2-anilinobenzamide analogues, including compounds **7a** and **7b** (Chart 2), which act as SIRT1 inhibitors.[13] Following these findings, we performed further investigation of 2-anilinobenzamide analogues, seeking to find SIRT2-selective inhibitors more potent and selective than AGK2 (**3**). We describe here the design, synthesis, and biological activity of a series of 2-anilinobenzamide derivatives.

Received: January 26, 2012
Published: May 29, 2012

   dx.doi.org/10.1021/jm3002108 | J. Med. Chem. 2012, 55, 5760−5773

Chart 1. Examples of SIRT Inhibitors

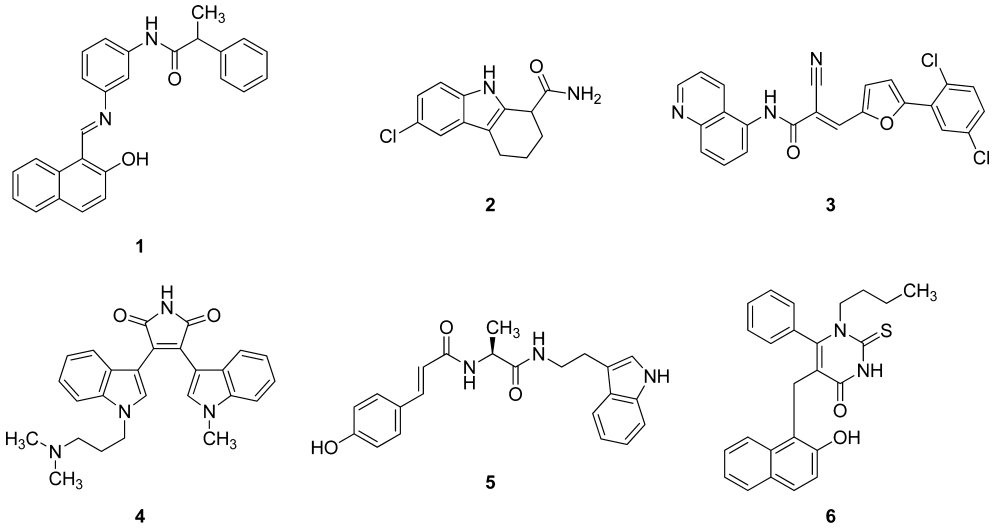
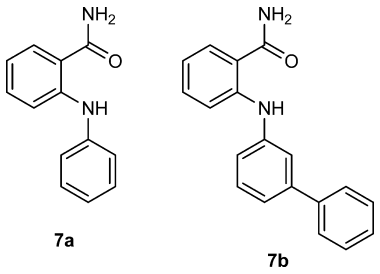


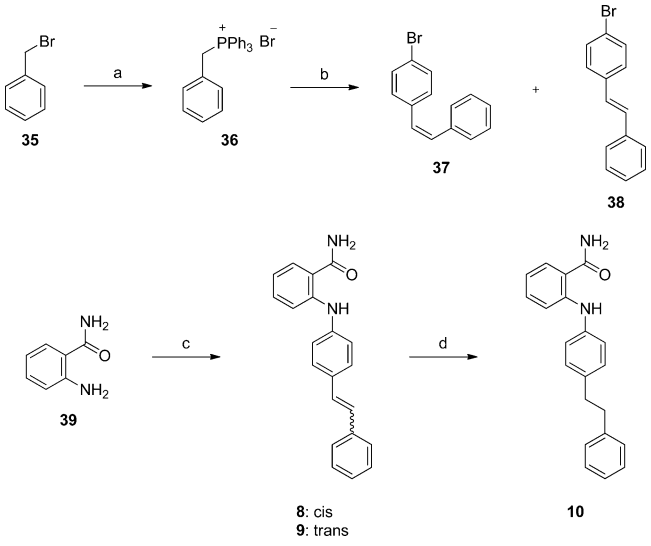
Chart 2. 2-Anilinobenzamide-Based SIRT1 Inhibitors



CHEMISTRY

The routes used for the synthesis of compounds **8**–**34**, which were prepared for this study, are shown in Schemes 1–6. Scheme 1 shows the preparation of 4-styryl compounds **8**, **9**, and phenethyl compound **10**. Treatment of benzyl bromide **35** with triphenylphosphine yielded benzyltriphenylphosphonium bromide **36**. Wittig reaction between 4-bromobenzaldehyde and the phosphonium ylide generated from **36** afforded a mixture of (*Z*)-4-bromostilbene **37** and (*E*)-4-bromostilbene **38**, which were separated by silica gel flash column chromatography. Buchwald–Hartwig reaction between 2-aminobenzamide **39** and (*Z*)- or (*E*)-4-bromostilbene using 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl as a ligand gave the desired styryl compounds **8** and **9**. The double bond of **9** was hydrogenated to yield phenethyl analogue **10**.

Scheme 1[a]



[a]Reagents and conditions: (a) PPh_3, CH_2Cl_2, reflux, 93%; (b) 4-bromobenzaldehyde, LiOH, *i*PrOH, reflux, 45% for **37**, 42% for **38**; (c) **37** or **38**, Pd_2dba_3, 2-dicyclohexylphosphino-2′,4′,6′-triisopropyl biphenyl, K_2CO_3, *tert*-BuOH, reflux, 58% for **8**, 13% for **9**; (d) H_2, Pd/C, MeOH, 60 °C, 99%.

The routes for the synthesis of 4-substituted amide compounds **11**–**14** are illustrated in Scheme 2. Condensation of amines **42**–**45** with an appropriate acid chloride **40** or **41** gave amides **46**–**49**. The nitro group of compounds **46**–**49** was hydrogenated to afford anilines **50**–**53**. Buchwald–Hartwig reaction of **50**–**53** with methyl 2-bromobenzoate yielded compounds **54**–**57**. Methyl esters **54**–**57** were hydrolyzed under alkaline conditions to give benzoic acids **58**–**61**. Benzamides **11**–**14** were obtained by the coupling of benzoic acids **58**–**61** and ammonia in the presence of EDCI and HOBt.

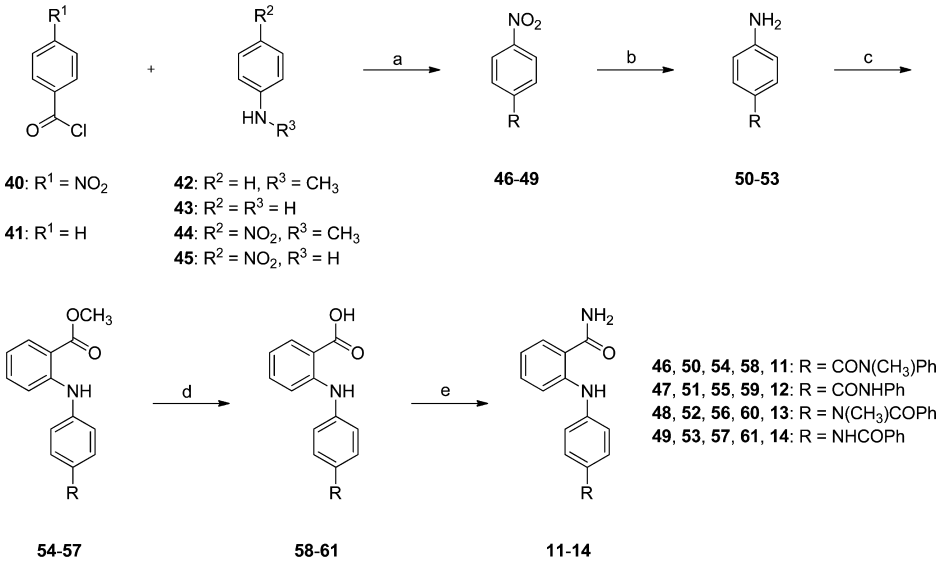
The preparation of 4-substituted anilide compounds **15**–**21** is shown in Scheme 3. Buchwald–Hartwig reaction of amine **39** with 4-bromonitrobenzene gave 2-(4-nitrophenylamino)-benzamide **62**. The nitro group of **62** was reduced to amine (**63**), and the product was acylated to afford anilides **15**–**17**, **64**, and **19**–**21**. Removal of the Boc group of compound **64** using TFA yielded the desired 4-piperidinyl compound **18**.

Scheme 4 shows the preparation of 3-substituted amide analogues **22**–**29**. Acid chlorides **65**–**67** were reacted with amines **68**–**71** to give corresponding amides **72**, **74**, **76**, **78**, and **79**. Treatment of amides **72**, **74**, and **76** with methyl iodide in the presence of sodium hydride yielded the *N*-methylated compounds **73**, **75**, and **77**. Bromides **72**–**79** were converted into compounds **22**–**29** by means of Buchwald–Hartwig reaction.

Scheme 5 illustrates the synthesis of 3-substituted ether compounds **30**–**32** and **33a**. Alkylation of phenols **80** and **81** gave compounds **82**–**85**, which were converted into compounds **30**–**32** and **33a** by means of Buchwald–Hartwig reaction.

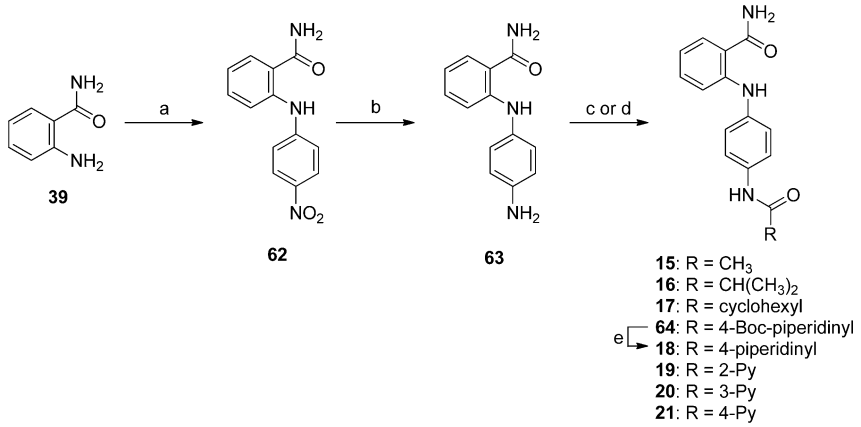
dx.doi.org/10.1021/jm3002108 | *J. Med. Chem.* 2012, 55, 5760–5773

Scheme 2[a]



[a]Reagents and conditions: (a) Et_3N, THF, 0 °C to room temp, 46−89%; (b) H_2, Pd/C, EtOH, room temp, 84−94%; (c) methyl 2-bromobenzoate, Pd_2dba_3, 2-dicyclohexylphosphino-2′,4′,6′-triisopropyl biphenyl, K_2CO_3, *tert*-BuOH, reflux, 82−85%; (d) LiOH, H_2O, MeOH, THF, room temp, 78−90%; (e) NH_4Cl, Et_3N, EDCI, HOBt, THF, room temp, 62−74%.

Scheme 3[a]



[a]Reagents and conditions: (a) 4-bromonitrobenzene, Pd_2dba_3, 2-dicyclohexylphosphino-2′,4′,6′-triisopropyl biphenyl, K_2CO_3, *tert*-BuOH, reflux, 82%; (b) Pd/C, MeOH, room temp, 95%; (c) Ac_2O, Et_3N, DMAP, THF, 38%; (d) RCOOH, PyBOP; Et_3N, CH_2Cl_2 or DMF, room temp, 35−99%; (e) TFA, room temp, 95%.

The synthesis of compounds **33b−p** and **34** is outlined in Scheme 6. Catalytic reduction of *O*-benzyl compound **32** gave 3-hydroxyl compound **86**. Compounds **33b−p** and **34** were obtained by the treatment of **86** with an appropriate alkyl bromide in the presence of K_2CO_3 in acetone.

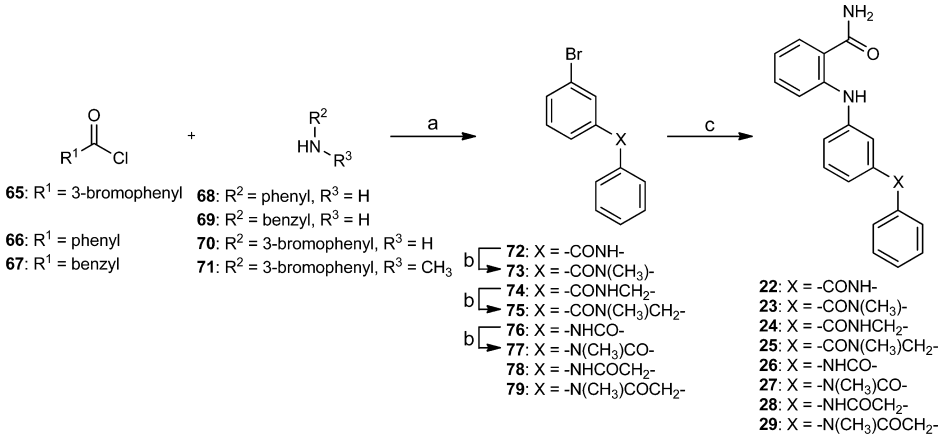
RESULTS AND DISCUSSION

We initially screened 2-anilinobenzamide derivatives[13] for SIRT2- and SIRT1-inhibitory activity with an in vitro assay using recombinant SIRT2 and SIRT1, aiming to find potent and selective SIRT2 inhibitors. The results are summarized in Table 1. Among meta-substituted 2-anilinobenzamides **7b−m**, phenyl analogue **7b**, phenoxy analogue **7e**, and methyl acrylate analogue **7h** showed relatively potent SIRT2 inhibition although they also inhibited SIRT1. On the other hand, several para-substituted 2-anilinobenzamides such as methyl **7o**, methoxy **7s**, and benzyloxy **7t** inhibited SIRT2 preferentially over SIRT1. In particular, benzyloxy compound **7t** displayed relatively strong SIRT2 inhibition (IC_{50} = 33 μM), whereas it was almost inactive against SIRT1 (13% inhibition at 100 μM). Compounds modified at the amide moiety (**7ab−ad**) or the amino moiety (**7ae−ag**) did not inhibit SIRT2 at concentrations up to 100 μM.

The preliminary structure−selectivity relationship study showed that compounds with a relatively bulky group at the meta position (**7b**, **7e**, and **7h**) tend to inhibit both SIRT1 and SIRT2, and compounds bearing a substituent at the para position (**7n−y**) tend not to inhibit SIRT1 (Table 1). To explore the origin of the SIRT2 selectivity of compound **7t**, we initially performed a binding mode study of SIRT1 inhibitor **7b** and SIRT2-selective inhibitor **7t** with homology models of SIRT1 and SIRT2, respectively. The binding mode studies were performed using the software packages Glide 3.5 and MacroModel 8.1. Inspection of the simulated SIRT1/compound **7b** complex shows that the NH_2 group of **7b**

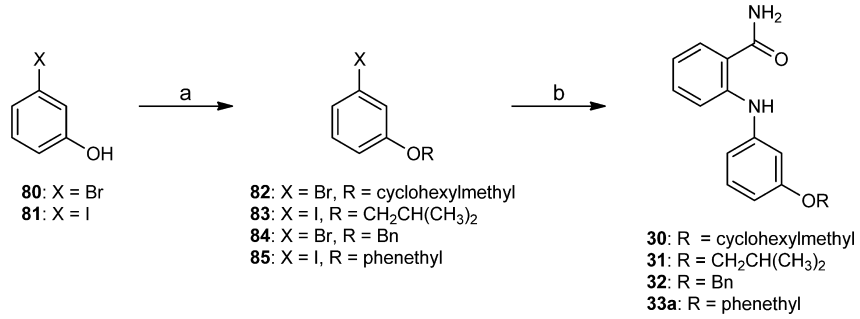
dx.doi.org/10.1021/jm3002108 | J. Med. Chem. 2012, 55, 5760−5773

Scheme 4[a]



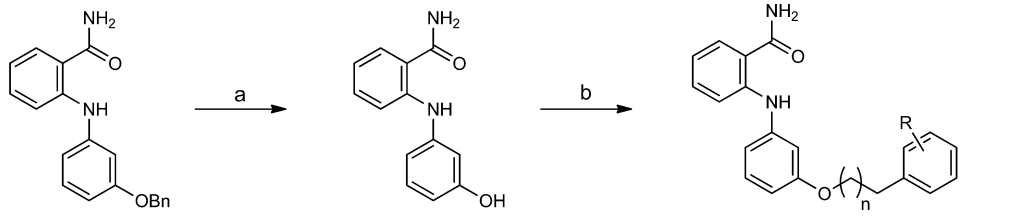
[a]Reagents and conditions: (a) Et_3N, CH_2Cl_2, 0 °C to room temp, 73−85%; (b) NaH, CH_3I, DMF, 0 °C to room temp, 82−86%; (c) **39**, Pd_2dba_3, 2-dicyclohexylphosphino-2′,4′,6′-triisopropyl biphenyl, K_2CO_3, *tert*-BuOH, reflux, 25−63%.

Scheme 5[a]



[a]Reagents and conditions: (a) R-Br, K_2CO_3, acetone or DMF, 60 °C, 30−63%; (b) **39**, Pd_2dba_3, 2-dicyclohexylphosphino-2′,4′,6′-triisopropyl biphenyl, K_2CO_3, *tert*-BuOH, reflux, 13−36%.

Scheme 6[a]

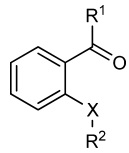


[a]Reagents and conditions: (a) H_2, Pd/C, EtOH, room temp, 73%; (b) R-Ph$CH_2(CH_2)_n$Br, K_2CO_3, acetone, reflux, 9−72%.

forms hydrogen bonds with the backbone carbonyls of Phe 413 and Val 412, while the CO group of **7b** forms a hydrogen bond with guanidine of Arg 446 in the active site of SIRT1 (Figure 1). An intramolecular hydrogen bond is observed between the CO and NH group of **7b**, which suggests the importance of the conformation of the inhibitor.[13b] The phenyl group of **7b** is located in the hydrophobic pocket formed by Ile 360, Pro 419, and Phe 422. In addition, Met 426 is positioned near the para position of the anilino group of **7b**. Steric hindrance between Met 426 and the substituent at the para position of **7o−z** may be the reason why these compounds do not inhibit SIRT1 (Table 1). In the simulated SIRT2/compound **7t** complex, the NH group and the CO group of **7t** form hydrogen bonds with Gln 267 (Figure 2). As is the case with SIRT1/**7b** complex, an intramolecular hydrogen bond is observed in the SIRT2/**7t** complex, suggesting the importance of the $CONH_2$ group and the NH group of the inhibitor. Val 233 is positioned around the para position of the anilino group of **7t**. A steric clash between Val 233 and the bulky group at the para position of **7q** and **7r** may be the reason why these compounds do not inhibit SIRT2

dx.doi.org/10.1021/jm3002108 | J. Med. Chem. 2012, 55, 5760−5773

Table 1. SIRT2- and SIRT1-Inhibitory Activities of Compounds 7a–7ag[a]



compd	R^1	R^2	X	IC_{50} (μM) SIRT2	IC_{50} (μM) SIRT1
7a	NH_2	Ph	NH	>100	56 ± 3.2
7b	NH_2	3-Ph-Ph	NH	33 ± 3.6	52 ± 2.1
7c	NH_2	3-Me-Ph	NH	72 ± 2.1	68 ± 6.4
7d	NH_2	3-CF_3-Ph	NH	100 ± 9.9	NE[b]
7e	NH_2	3-OPh-Ph	NH	25 ± 3.8	58 ± 3.7
7f	NH_2	3-COOMe-Ph	NH	>100	>100
7g	NH_2	3-COOH-Ph	NH	>100	>100
7h	NH_2	3-(*E*)-CH=CHCOOMe-Ph	NH	32 ± 1.6	65 ± 5.0
7i	NH_2	3-(*E*)-CH=CHCOOH-Ph	NH	>100	58 ± 5.4
7j	NH_2	3-CH_2CH_2COOMe-Ph	NH	93 ± 4.4	NE[b]
7k	NH_2	3-CH_2CH_2COOH-Ph	NH	>100	93 ± 7.4
7l	NH_2	3-CH_2OH-Ph	NH	>100	60 ± 3.2
7m	NH_2	3-CH_2CH_2OH-Ph	NH	>100	72 ± 6.0
7n	NH_2	4-Cl-Ph	NH	>100	56 ± 4.9
7o	NH_2	4-Me-Ph	NH	60 ± 5.4	>100
7p	NH_2	4-CF_3-Ph	NH	69 ± 9.6	NE[b]
7q	NH_2	4-*tert*-Bu-Ph	NH	>100	NE[b]
7r	NH_2	4-Ph-Ph	NH	>100	>100
7s	NH_2	4-OMe-Ph	NH	80 ± 5.0	>100
7t	NH_2	4-OBn-Ph	NH	33 ± 6.6	>100
7u	NH_2	4-CN-Ph	NH	93 ± 4.3	NE[b]
7v	NH_2	4-COOMe-Ph	NH	83 ± 7.8	>100
7w	NH_2	4-COOH-Ph	NH	>100	NE[b]
7x	NH_2	4-SMe-Ph	NH	>100	NE[b]
7y	NH_2	4-OPh-Ph	NH	>100	>100
7z	NH_2	2-Naph	NH	>100	>100
7aa	NH_2	Bn	NH	>100	NE[b]
7ab	OH	Ph	NH	>100	68 ± 5.4
7ac	NHMe	Ph	NH	>100	>100
7ad	NMe_2	Ph	NH	>100	>100
7ae	NH_2	Ph	O	>100	>100
7af	NH_2	Ph	S	>100	78 ± 3.8
7ag	NH_2	Ph	NMe	>100	75 ± 6.5

[a]Values are means of at least three experiments. [b]NE = Not evaluated.

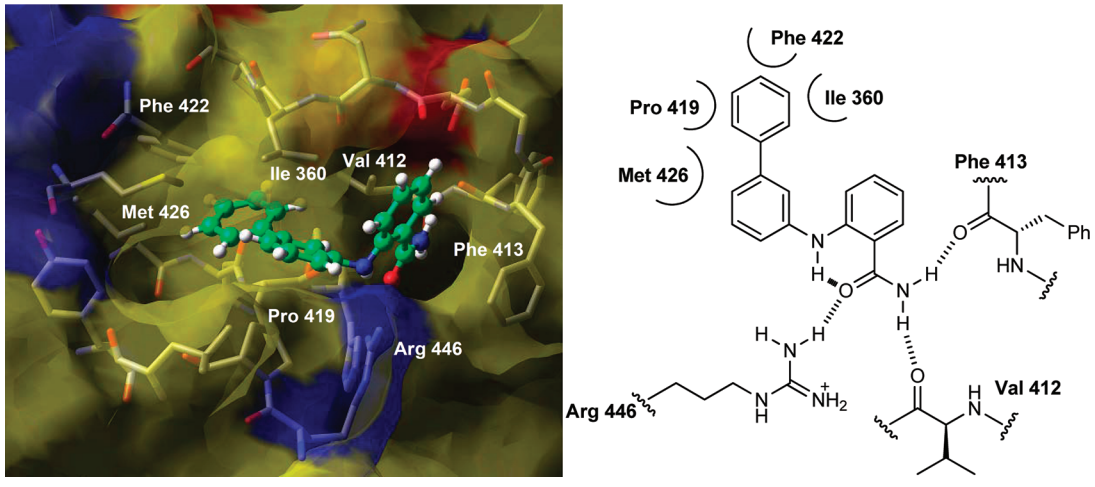


Figure 1. Calculated docking model of compound **7b** (ball-and-stick)/SIRT1.

dx.doi.org/10.1021/jm3002108 | *J. Med. Chem.* 2012, 55, 5760–5773

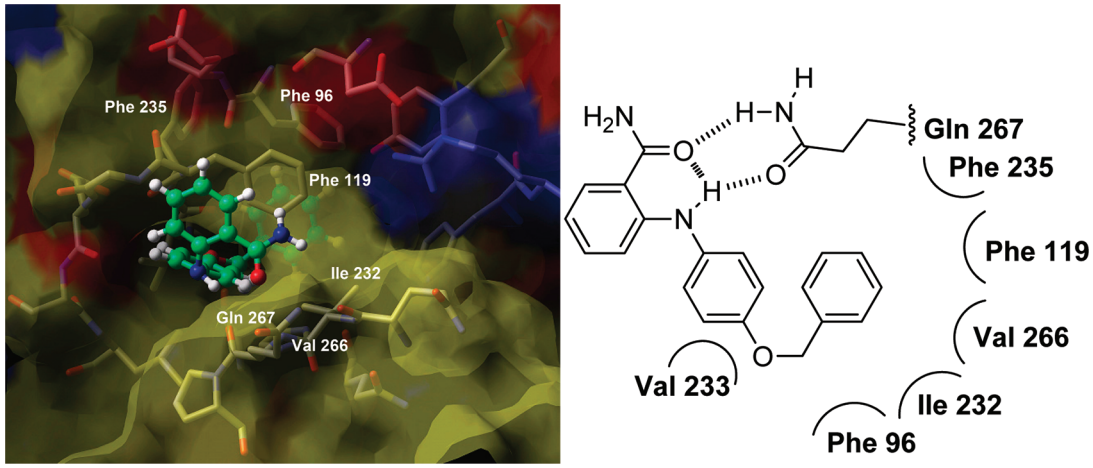


Figure 2. Calculated docking model of compound **7t** (ball-and-stick)/SIRT2.

(Table 1). In addition, the phenyl ring of the benzyloxy group of **7t** is positioned in the hydrophobic pocket delineated by Phe 96, Phe 119, Ile 232, Phe 235, and Val 266, which is larger and deeper than the corresponding pocket of SIRT1 (Figure 1).

On the basis of the SIRT inhibition data shown in Table 1, we selected compound **7t** as a lead compound. We initially focused on the *s*-cis conformation of the benzyloxyphenyl part of **7t** in the active site of SIRT2 (Figure 2). From the calculated docking model of **7t**/SIRT2, we hypothesized that the *s*-cis structure of **7t** is an active conformer in which the benzyl group can interact with hydrophobic amino acid residues (Figure 3).

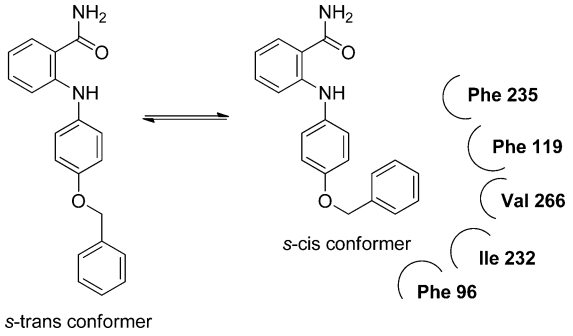
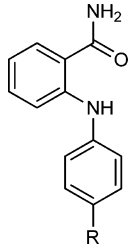


Figure 3. Predicted conformation of compound **7t** in the active site of SIRT2.

On the basis of this hypothesis, we designed and synthesized *cis*-olefin analogue **8** and *N*-methyl amide analogues **11** and **13** bearing an *N*-methyl benzanilide structure, which has been reported to display a "cis" conformation.[14] Compounds **9**, **10**, **11**, and **14** were prepared as reference compounds. The SIRT-inhibitory data for compounds **8**–**14** are shown in Table 2. In accordance with the data shown in Table 1, these para-substituted analogues did not inhibit SIRT1 at concentrations up to 100 μM. As expected, while *trans*-olefin analogue **9** did not inhibit SIRT2 (IC_{50} > 300 μM), *cis*-olefin analogue **8** inhibited SIRT2 more potently than **7t** (IC_{50} = 20 μM). The SIRT2-inhibitory activity of the reduced compound **10** was greater than that of *trans*-olefin **9** and less than that of *cis*-olefin **8**. These results support the hypothesis that *s*-cis structure of **7t** is an active conformer. However, unexpectedly, the *N*-methyl benzanilides **11** and **13** were less active than the reference compounds **12** and **14**, respectively. The reason for the weak activity of **11** and **13** in comparison with **12** and **14** is unclear

Table 2. SIRT2- and SIRT1-Inhibitory Activities of Compounds 8–21[a]



compd	R	IC_{50} (μM) SIRT2	IC_{50} (μM) SIRT1
7t	OCH_2Ph	33 ± 6.6	>100
8	(*Z*)-CH=CHPh	20 ± 2.4	>100
9	(*E*)-CH=CHPh	>300	>100
10	CH_2CH_2Ph	88 ± 2.2	>100
11	$CON(CH_3)Ph$	69 ± 8.7	>100
12	CONHPh	23 ± 1.5	>100
13	$N(CH_3)COPh$	>300	>100
14	NHCOPh	17 ± 1.5	>100
15	$NHCOCH_3$	77 ± 9.9	>100
16	$NHCOCH(CH_3)_2$	>100	>100
17	NHCOcyclohexyl	25 ± 3.5	>100
18	NHCO-4-piperidinyl	53 ± 8.2	>100
19	NHCO-2-Py	>100	>100
20	NHCO-3-Py	38 ± 5.6	>100
21	NHCO-4-Py	>100	>100

[a]Values are means of at least three experiments.

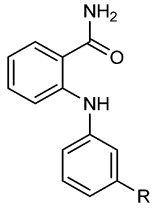
but might be related to steric repulsion between the methyl group of **11** or **13** and an amino acid residue such as Val 233 (Figure 2). The CONH amide of **12** and **14** displays cis conformation in the active site of SIRT2. Among compounds **8**–**14**, compound **14** showed the most potent SIRT2-inhibitory activity (IC_{50} = 17 μM). We therefore chose compound **14** for further study. We investigated the effect of replacement of the phenyl group of compound **14** with a methyl group (**15**), bulky alkyl groups (**16**–**18**), and pyridine rings (**19**–**21**), but the changes merely resulted in retention (compounds **17** and **20**) or decrease (compounds **15**, **16**, **18**, **19**, and **21**) of the activity.

We next turned our attention to the meta-substituted 2-anilinobenzamides. The results of the molecular modeling suggested that the hydrophobic pocket of SIRT2 is larger than

dx.doi.org/10.1021/jm3002108 | *J. Med. Chem.* 2012, 55, 5760–5773

that of SIRT1 (Figures 1 and 2). The calculation results also suggested that compounds with a lipophilic group more bulky than the phenyl group (**7b**) and phenoxy group (**7e**) at the meta position of 2-anilinobenzamide would not be able to bind to SIRT1, which has a smaller hydrophobic pocket, whereas they would be able to bind to SIRT2, which has a larger hydrophobic pocket, thereby resulting in selective inhibition of SIRT2. We thus prepared compounds bearing a substituent at the meta-position of 2-anilinobenzamide, focusing on benzene-containing amides and ethers, and evaluated their ability to inhibit SIRT2 and SIRT1 (Table 3). In the amide series, some

Table 3. SIRT2- and SIRT1-Inhibitory Activities of Compounds 22−34[a]



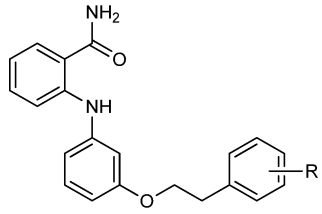
compd	R	IC50 (μM) SIRT2	IC50 (μM) SIRT1
22	CONHPh	83 ± 3.2	>100
23	$CON(CH_3)Ph$	>100	>100
24	$CONHCH_2Ph$	39 ± 5.4	>100
25	$CON(CH_3)CH_2Ph$	40 ± 2.2	>100
26	NHCOPh	57 ± 7.8	>100
27	$N(CH_3)COPh$	>100	>100
28	$NHCOCH_2Ph$	74 ± 5.7	>100
29	$N(CH_3)COCH_2Ph$	>100	>100
7e	OPh	25 ± 3.8	58 ± 3.7
30	OCH_2cyclohexyl	24 ± 5.0	210 ± 4.1
31	$OCH_2CH(CH_3)_2$	30 ± 4.1	>300
32	OCH_2Ph	54 ± 6.9	>300
33a	OCH_2CH_2Ph	1.0 ± 0.12	>300
34	$OCH_2CH_2CH_2Ph$	4.3 ± 0.59	>300

[a]Values are means of at least three experiments.

of the compounds (**22**, **24**, **25**, **26**, and **28**) showed SIRT2-selective inhibition, but the SIRT2-inhibitory activity was weaker than that of **7e**. In the ether series, compounds with an aliphatic substituent (**30** and **31**) displayed SIRT2-inhibitory activity similar to that of **7e**. However, compound **30** also inhibited SIRT1, although its activity was relatively weak. Compounds with a phenyl group (**32**−**34**) were found to be SIRT2-selective inhibitors. Especially, a pronounced inhibitory effect (IC_{50} = 1.0 μM) was observed with phenethyl ether **33a**, which was 25-fold more active than **7e**. SIRT2 inhibition was distinctly dependent on the methylene chain length, with n = 0 (**7e**), n = 1 (**32**), and n = 3 (**34**) resulting in less potent inhibitors.

We further synthesized several derivatives of **33a** and tested them in SIRT inhibition assays. As shown in Table 4, most of the compounds showed potent and selective inhibitory activity against SIRT2. While methyl or trifluoromethyl compounds **33b**−**g** showed slightly reduced SIRT2-inhibitory activity as compared with parent compound **33a**, two of the halogenated compounds (compounds **33i** and **33m**) inhibited SIRT2 with IC_{50} values in the submicromolar range.

Table 4. SIRT2- and SIRT1-Inhibitory Activities of Compounds 33a−33q[a]



compd	R	IC50 (μM) SIRT2	IC50 (μM) SIRT1
33a	H	1.0 ± 0.12	>100
33b	2-CH_3	1.8 ± 0.30	>100
33c	3-CH_3	1.8 ± 0.42	>100
33d	4-CH_3	1.6 ± 0.18	>100
33e	2-CF_3	3.1 ± 0.30	>100
33f	3-CF_3	2.1 ± 0.39	>100
33g	4-CF_3	1.4 ± 0.26	>100
33h	2-F	2.0 ± 0.64	>100
33i	3-F	0.57 ± 0.12	>100
33j	4-F	2.7 ± 0.082	>100
33k	2-Cl	1.5 ± 0.15	>100
33l	3-Cl	2.0 ± 0.057	>100
33m	4-Cl	0.83 ± 0.11	>100
33n	2-Br	1.1 ± 0.18	>100
33o	3-Br	3.4 ± 0.10	>100
33p	4-Br	1.2 ± 0.26	>100

[a]Values are means of at least three experiments.

We compared the SIRT2-inhibitory activity and selectivity of compounds **33a** and **33i** with those of AGK2 (**3**), a representative previously reported SIRT2-selective inhibitor.[5] As shown in Table 5, compounds **33a** and **33i** showed SIRT2-

Table 5. SIRT1- and SIRT2-Inhibitory Activities of AGK2 (3), Compounds 33a and 33i[a]

compd	IC50 (μM) SIRT2	IC50 (μM) SIRT1	IC50 (μM) SIRT3	selectivity SIRT1/SIRT2	selectivity SIRT3/SIRT2
3	3.5 ± 0.30	30 ± 0.40	91 ± 2.7	8.6	26
33a	1.0 ± 0.12	>300	>300	>300	>300
33i	0.57 ± 0.12	>300	>300	>530	>530

[a]Values are means of at least three experiments.

inhibitory activity 3.5- and 6.1-fold more potent than that of AGK2 (**3**), respectively. Furthermore, AGK2 (**3**) inhibited SIRT1 and SIRT3 with IC_{50} values of 30 and 91 μM, respectively, whereas compounds **33a** and **33i** did not inhibit either SIRT1 or SIRT3 at concentrations up to 300 μM, showing high selectivity for SIRT2 over both SIRT1 and SIRT3. Thus, compounds **33a** and **33i** are more potent and more selective SIRT2 inhibitors than AGK2 (**3**) in these enzyme assays.

Next, we evaluated the interaction between SIRT2 and compound **33a** using Biacore surface plasmon resonance (SPR) analysis. Recombinant SIRT2 was immobilized onto CM5 sensor chip, over which a compound solution was passed. Dose-dependent SPR responses were recorded for a series of concentrations of compound **33a** as shown in Supporting

dx.doi.org/10.1021/jm3002108 | J. Med. Chem. 2012, 55, 5760−5773

Information Figure S1, which clearly indicated its direct interactions with SIRT2.

To examine whether compounds **33** selectively inhibit SIRT2 in cells, we performed a cellular assay using Western blot analysis. Because SIRT2 and SIRT1 are known to catalyze the deacetylation of α-tubulin[3b] and p53,[15] respectively, the effect of the inhibitors on the acetylation levels of α-tubulin and p53 in HCT116 cells were analyzed. As can be seen in Figure 4,

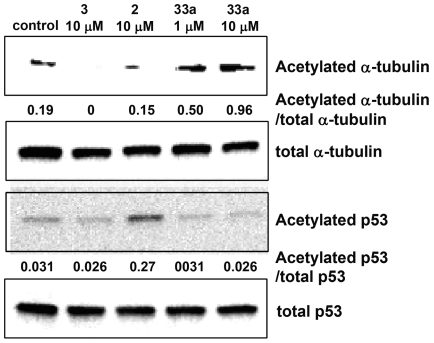


Figure 4. Western blot detection of acetylated α-tubulin and p53 levels in HCT116 cells after 8 h treatment with AGK2 (**3**), EX-527 (**2**), and compound **33a**. Each value means the ratio of acetylated α-tubulin amount to total α-tubulin or acetylated p53 amount to total p53.

compound **33a** induced a dose-dependent increase of α-tubulin acetylation, and its effect was greater than that of AGK (**3**). Furthermore, while EX-527 (**2**), a previously reported SIRT1-selective inhibitor,[9] caused selective acetylation of p53, the level of acetylated p53 was not elevated in the presence of **33a**. These results indicated that the compound **33a** selectively inhibits SIRT2 in preference to SIRT1 in cells.

Compound **33a** was also selective against zinc-dependent HDACs (IC_{50} > 30 μM). In addition, compound **33a** was weakly potent against CYP450s such as 3A4 or 2D6 (IC_{50} > 10 μM). Furthermore, compound **33a** displayed a certain level of stability in human liver microsomes (64% remaining after 15 min incubation of 0.5 mg **33a**).

CONCLUSION

We have designed and synthesized novel SIRT2-selective inhibitors based on homology models of SIRT1 and SIRT2. 3′-Phenethyloxy-2-anilinobenzamides such as compounds **33a** and **33i** exhibited potent and selective SIRT2 inhibition in enzyme assays, showing more than 3.5-fold greater SIRT2-inhibitory activity and more than 10-fold greater SIRT2-selectivity over SIRT1 and SIRT3, as compared with AGK2 (**3**), the most potent and selective SIRT2 inhibitor among previously reported SIRT inhibitors. Preliminary SAR study and molecular modeling suggested that the terminal phenethyl group of **33** is essential for the SIRT2 selectivity, and the methylene linker length is important for potent inhibition of SIRT2. In cellular assays, compound **33a** induced a selective increase of α-tubulin acetylation, suggesting that it is a cell-active SIRT2-selective inhibitor. Thus, compounds **33** are potent and selective SIRT2 inhibitors, and should be useful as tools for probing the biology of SIRT2.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined using a Yanagimoto micro melting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on JEOL JNM-LA500, JEOL JNM-A500, or BRUKER AVANCE600 spectrometers in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to the internal standard tetramethylsilane. Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within ±0.4% of the calculated values, indicating >95% purity. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-SX102A mass spectrometer. GC-MS analyses were performed on a Shimadzu GCMS-QP2010. Purity testing was done by means of analytical HPLC on a Shimadzu instrument equipped with an Inertsil ODS-3 column (4.6 mm × 150 mm, GL Science) eluted at 1 mL/min with Milli-Q water and CH_3CN. All tested compounds were ≥95% pure. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku and used without purification. Flash column chromatography was performed using silica gel 60 (particle size 0.046−0.063 mm) supplied by Merck.

(*Z*)-2-(4-Styrylphenylamino)benzamide (8). *Steps 1 and 2: Preparation of (Z)-4-Bromostilbene (**37**) and (E)-4-Bromostilbene (**38**).* To a well-stirred solution of benzyl bromide **35** (0.40 mL, 3.34 mmol) in anhydrous CH_2Cl_2 (30 mL) was added triphenylphosphine (1.31 g, 5.01 mmol). The reaction mixture was refluxed for 12 h and then allowed to cool to room temperature. The solvent was removed in vacuo to afford the crude salt as a white powder. The powder was rinsed with Et_2O until no PPh_3 was detected by TLC in the ethereal filtrate, affording 1.35 g (93%) of benzyltriphenylphosphonium bromide **36** as a white powder.

To a stirred solution of **36** (760 mg, 1.76 mmol) in isopropyl alcohol (7 mL) was added lithium hydroxide (100 mg, 2.4 mmol). After 15 min, 4-bromobenzaldehyde (296 mg, 1.60 mmol) was further added. The reaction mixture was refluxed for 1 h, allowed to cool to room temperature, and then quenched with water and extracted with AcOEt. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo, and purification of the residue by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/20) gave 205 mg (45%) of **37** and 190 mg (42%) of **38**. **37**: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.34 (2H, d, J = 7.6 Hz), 7.25−7.16 (5H, m), 7.10 (2H, d, J = 7.6 Hz), 6.63 (1H, d, J = 12.5 Hz), 6.50 (1H, d, J = 12.5 Hz). **38**: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.53−7.45 (2H, m), 7.40−7.34 (2H, m), 7.30−7.24 (5H, m), 7.10 (1H, d, J = 16.4 Hz), 7.03 (1H, d, J = 16.4 Hz).

*Step 3: Preparation of (Z)-2-(4-Styrylphenylamino)benzamide (**8**).* A mixture of 2-aminobenzamide **39** (71 mg, 0.52 mmol), **37** (100 mg, 0.38 mmol) obtained above, Pd_2dba_3 (32 mg, 0.033 mmol), 2-dicyclohexylphosphino-2′-4′,6′-triisopropylbiphenyl (83 mg, 0.17 mmol), and K_2CO_3 (131 mg, 0.95 mmol) in *tert*-BuOH (3 mL) was refluxed for 15 h. The reaction mixture was diluted with AcOEt, washed with 1 N aqueous HCl and brine, and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo, and purification of the residue by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/3 to 1/2) gave 70 mg (59%) of **8** as a white solid: mp 137−139 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 10.0 (1H, broad s), 8.06 (1H, broad s), 7.70 (1H, dd, J = 8.0, 1.5 Hz), 7.49 (broad s, 1H), 7.35−7.26 (6H, m), 7.24−7.20 (1H, m), 7.16 (2H, d, J = 8.5 Hz) 7.02 (2H, d, J = 8.5 Hz), 6.81 (1H, t, J = 6.7 Hz), 6.56 (1H, d, J = 12.2 Hz), 6.52 (1H, d, J = 12.2 Hz). ^{13}C NMR (CD_3OD, 125 MHz, δ, ppm) 171.19, 144.22, 140.69, 137.21, 132.09, 129.82, 129.71, 129.33, 128.39, 128.37, 128.33, 127.67, 127.07, 126.17, 118.59, 118.15, 117.98. MS (EI) m/z 314 (M^+). HRMS calcd for $C_{21}H_{18}N_3O$, 314.142; found, 314.142; purity 96.2% (HPLC).

(*E*)-2-(4-Styrylphenylamino)benzamide (9). Compound **9** was prepared from **38** obtained above and **39** using the procedure described for **8** (step 3) in 13% yield as colorless crystals; mp 203−206 °C. ^{1}H NMR ($CDCl_3$, 600 MHz, δ, ppm) 9.61 (1H, s), 7.50 (2H, d, J = 7.8 Hz), 7.48 (1H, t, J = 7.8 Hz), 7.47 (2H, d, J = 8.4 Hz), 7.39 (1H, d, J = 8.4 Hz), 7.35 (2H, t, J = 7.8 Hz), 7.32 (1H, t, J = 7.2 Hz), 7.24 (1H, d, J = 7.8 Hz), 7.20 (2H, d, J = 8.4 Hz), 7.08 (1H, d, J = 16.2 Hz), 7.02 (1H, d, J = 16.2 Hz), 6.78 (1H, t, J = 7.2 Hz). MS (EI) m/z:

dx.doi.org/10.1021/jm3002108 | J. Med. Chem. 2012, 55, 5760−5773

314 (M^+). HRMS calcd for $C_{21}H_{18}N_2O$, 314.141; found, 314.142; purity 95.5% (HPLC).

2-(4-Phenethylphenylamino)benzamide (10). To a solution of **9** (42 mg, 0.130 mmol) obtained above in MeOH (14 mL) was added 10% Pd/C (8.4 mg). The reaction mixture was stirred for 4 h at 60 °C under a H_2 atmosphere and filtered through Celite. The filtrate was concentrated in vacuo, and the residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 3/2) to give 41 mg (99%) of **10** as a white solid: mp 112−114 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.49 (1H, s), 7.45 (1H, d, J = 8.0 Hz), 7.30−7.26 (7H, m), 7.25−7.16 (4H, m), 6.72 (1H, t, J = 8.0 Hz), 2.91 (4H, m). MS (EI) m/z: 316 (M^+). HRMS calcd for $C_{21}H_{20}N_2O$, 316.157; found, 316.160; purity 97.5% (HPLC).

4-(2-Carbamoylphenylamino)-*N*-methyl-*N*-phenylbenzamide (11). *Step 1: Preparation of N-Methyl-4-nitro-N-phenylbenzamide (**46**).* To a mixture of *N*-methyl-4-nitroaniline **42** (0.40 mL, 3.70 mmol) and triethylamine (2.00 mL, 14.8 mmol) in anhydrous THF (10 mL) was added 4-nitrobenzoyl chloride **40** (1 g, 5.5 mmol) in one portion at 0 °C. The resulting mixture was stirred for 30 min at 0 °C, allowed to warm to room temperature, and further stirred for 4 h. The solvent was removed in vacuo, and the residue was diluted with AcOEt, washed with saturated aqueous Na_2CO_3 and brine, and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo and purification of the residue by recrystallization from AcOEt/*n*-hexane gave 460 mg (49%) of **46** as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.02 (2H, d, J = 8.5 Hz), 7.44 (2H, d, J = 8.5 Hz), 7.29−7.23 (2H, m), 7.20 (1H, t, J = 7.3 Hz), 7.03 (2H, d, J = 7.3 Hz), 3.52 (3H, s, 3H). MS (GC) m/z: 256 (M^+).

*Step 2: Preparation of 4-Amino-N-methyl-N-phenylbenzamide (**50**).* A mixture of **46** (256 mg, 1.00 mmol) and 10% Pd/C (25 mg) in EtOH (5 mL) was stirred under H_2 for 2 h at room temperature. The reaction mixture was filtered through Celite. The filtrate was concentrated in vacuo, and the residue was recrystallized from AcOEt/*n*-hexane to afford 204 mg (90%) of **50** as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.24 (2H, d, J = 7.9 Hz), 7.19−7.10 (3H, m), 7.05 (2H, dd, J = 1.5, 7.9 Hz), 6.41 (2H, d, J = 8.5 Hz), 3.75 (2H, broad s), 3.47 (3H, s). MS (GC) m/z: 226 (M^+).

*Step 3: Preparation of Methyl 2-{4-(Methylphenylcarbamoyl)-phenylamino}benzoate (**54**).* Compound **54** was prepared from **50** obtained above and methyl 2-bromobenzoate using the procedure described for **8** (step 3) in 85% yield. ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 9.47 (1H, broad s), 7.94 (1H, dd, J = 1.5, 8.0 Hz), 7.32 (1H, dt, J = 1.8, 8.2 Hz), 7.28−7.23 (5H, m), 7.15 (1H, t, J = 7.3 Hz), 7.06 (2H, d, J = 8.8 Hz), 7.00 (2H, d, J = 8.8 Hz), 6.77 (1H, dt, J = 1.2, 8.2 Hz), 3.87 (3H, s), 3.52 (3H, s). MS (EI) m/z: 360 (M^+).

*Step 4: Preparation of 2-{4-(Methylphenylcarbamoyl)-phenylamino}benzoic Acid (**58**).* To a solution of **54** (150 mg, 0.41 mmol) obtained above in THF (6 mL) and MeOH (2 mL) was added an aqueous solution (2 mL) of lithium hydroxide monohydrate (175 mg, 4.10 mmol), and the resulting solution was stirred overnight at 50 °C. After removal of organic solvents in vacuo, the remaining aqueous phase was acidified with 2 N HCl and the precipitate was extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo, and purification of the residue by recrystallization from AcOEt/*n*-hexane gave 115 mg (80%) of **58** as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 9.33 (1H, broad s), 8.01 (1H, dd, J = 1.8, 8.2 Hz), 7.35 (1H, dt, J = 1.5, 7.3 Hz), 7.31−7.24 (4H, m), 7.18 (1H, t, J = 7.3 Hz), 7.08 (2H, d, J = 8.5 Hz), 7.02 (2H, d, J = 8.5 Hz), 6.79 (1H, t, J = 8.2 Hz), 3.51 (3H, s). MS (EI) m/z: 346 (M^+).

*Step 5: Preparation of 4-(2-Carbamoylphenylamino)-N-methyl-N-phenylbenzamide (**11**).* A mixture of **58** (100 mg, 0.29 mmol) obtained above, NH_4Cl (77 mg, 1.44 mmol), Et_3N (202 μL, 1.44 mmol), EDCI (164 mg, 0.85 mmol), and HOBt·H_2O (131 mg, 0.85 mmol) in THF (5 mL) was stirred at room temperature for 15 h. The reaction mixture was poured into water, and the whole was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo, and purification of the residue by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/1) gave 63 mg (63%) of **11** as a colorless solid; mp 202−204 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.53 (1H, s), 7.45 (1H, dd, J = 1.5, 7.8 Hz), 7.34 (1H, dt, J = 1.2, 8.2 Hz), 7.23−7.20 (5H, m), 7.16 (1H, t, J = 7.8 Hz), 7.06 (2H, d, J = 8.5 Hz), 6.96 (2H, d, J = 8.5 Hz), 6.80 (1H, dt, J = 1.2, 7.8 Hz), 3.49 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.38, 170.22, 145.44, 144.82, 142.92, 132.85, 130.58, 129.19, 128.90, 128.25, 126.84, 126.31, 118.68, 118.139, 117.19, 116.42, 38.59. MS (EI) m/z 345 (M^+). HRMS calcd for $C_{21}H_{19}N_3O_2$, 345.147; found, 345.147; purity 99.0% (HPLC).

Compounds **12**−**14** were prepared from an appropriate acid chloride (**40** or **41**) and aniline (**43**, **44**, or **45**) using the procedure described for **11**.

4-(2-Carbamoylphenylamino)-*N*-phenylbenzamide (12). Yield 18%; colorless crystals; mp 230−232 °C. ^{1}H NMR (CD_3OD, 500 MHz, δ, ppm) 7.79 (2H, d, J = 8.8 Hz), 7.62 (1H, dd, J = 1.5, 7.8 Hz), 7.54 (2H, d, J = 7.6 Hz), 7.41 (1H, d, J = 7.6 Hz), 7.32 (1H, dt, J = 1.2, 7.6 Hz), 7.25 (2H, t, J = 8.2 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.03 (1H, t, J = 7.6 Hz), 6.86 (1H, dt, J = 1.2, 7.3 Hz). ^{13}C NMR (CD_3OD, 125 MHz, δ, ppm) 174.02, 168.39, 147.37, 144.40, 140.05, 133.39, 130.41, 130.35, 129.75, 125.38, 122.34, 121.69, 121.24, 118.69, 118.21. MS (EI) m/z 331 (M^+). HRMS calcd for $C_{20}H_{17}N_3O_2$, 331.132; found, 331.1323. Anal. ($C_{20}H_{17}N_3O_2$·1/4H_2O) C, H, N.

***N*-(4-(2-Carbamoylphenylamino)phenyl)-*N*-methylbenzamide (13).** Yield 31%; colorless crystals; mp 245−247 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.53 (1H, broad s), 7.46 (1H, dd, J = 1.5, 7.9 Hz), 7.35−7.30 (2H, m), 7.28−7.22 (2H, m), 7.22−7.17 (3H, m), 7.07 (2H, d, J = 8.5 Hz), 6.96 (2H, d, J = 8.5 Hz), 6.77 (1H, dt, J = 1.5, 7.9 Hz), 3.49 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.51, 170.69, 145.91, 139.69, 139.37, 136.06, 132.97, 129.52, 128.68, 128.30, 127.81, 127.75, 121.39, 118.00, 116.10, 115.38, 38.47. MS (EI) m/z 345 (M^+). HRMS calcd for $C_{21}H_{19}N_3O_2$, 345.148; found, 345.148; purity 97.8% (HPLC).

***N*-(4-(2-Carbamoylphenylamino)phenyl)benzamide (14).** Yield 44%; colorless crystals; mp 209−211 °C. ^{1}H NMR (CD_3OD, 500 MHz, δ, ppm) 7.93 (2H, d, J = 8.8 Hz), 7.67−7.61 (3H, m), 7.57 (1H, t, J = 7.6 Hz), 7.51 (2H, t, J = 7.6 Hz), 7.32−7.27 (2H, m), 7.18 (2H, d, J = 8.8 Hz), 6.78 (1H, dt, J = 1.5, 7.6 Hz). ^{13}C NMR (CD_3OD, 125 MHz, δ, ppm) 174.47, 168.75, 147.26, 139.77, 136.34, 134.61, 133.58, 132.80, 130.29, 129.62, 128.57, 123.68, 122.22, 118.95, 118.49, 116.10. MS (EI) m/z 331 (M^+). HRMS calcd for $C_{20}H_{17}N_3O_2$, 331.132; found, 331.133. Anal. ($C_{20}H_{17}N_3O_2$·1/3H_2O) C, H, N.

2-(4-Acetamidophenylamino)benzamide (15). *Step 1: Preparation of 2-(4-Nitrophenylamino)benzamide (**62**).* Compound **62** was prepared from **39** and 4-bromonitrobenzene using the procedure described for **8** (step 3) in 82% yield. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.97 (1H, s), 8.16 (2H, dd, J = 2.0, 7.0 Hz), 7.57−7.55 (2H, m), 7.46 (1H, t, J = 7.0 Hz), 7.20 (2H, dd, J = 2.0, 7.5 Hz), 7.02 (1H, t, J = 7.0 Hz).

*Step 2: 2-(4-Aminophenylamino)benzamide (**63**).* Compound **63** was prepared from **62** obtained above using the procedure described for **11** (step 2) in 95% yield. ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm); 9.32 (1H, s), 7.21 (1H, t, J = 7.0 Hz), 7.04 (2H, dd, J = 2.0, 6.5 Hz), 6.98 (1H, dd, J = 1.0, 8.5 Hz), 6.69 (2H, dd, J = 2.0, 6.5 Hz), 6.63 (1H, t, J = 7.0 Hz).

*Step 3: 2-((4-Acetamidophenyl)amino)benzamide (**15**).* To a solution of **63** (50.0 mg, 0.220 mmol) obtained above, DMAP (13.4 mg, 0.110 mmol) and Et_3N (33.4 mg, 0.330 mmol) in THF (3 mL) was added acetic anhydride (34 mg, 0.330 mmol). The mixture was stirred at room temperature for 3 h and then poured into 2 N aqueous HCl, and the whole was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo, and purification of the residue by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/1) gave 23 mg (38%) of **15** as a gray powder. The powder was recrystallized from AcOEt/*n*-hexane to give 18 mg of **15** as a gray powder; mp 181−183 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.50 (1H, s), 7.45 (1H, d, J = 7.5 Hz), 7.44 (2H, d, J = 7.0 Hz), 7.27 (1H, t, J = 7.0 Hz), 7.23 (1H, d, J = 7.0 Hz), 7.17 (2H, d, J = 8.5 Hz), 7.11 (1H, s), 6.73 (1H, t, J = 7.0 Hz), 2.18 (3H, s). ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm) 171.29, 167.76, 145.87, 136.15, 134.22, 132.12,

dx.doi.org/10.1021/jm3002108 | J. Med. Chem. 2012, 55, 5760−5773

129.19, 121.16, 120.10, 116.83, 116.22, 113.71, 23.77. MS (EI) *m/z*: 269 (M^+). HRMS calcd for $C_{15}H_{15}N_3O_2$, 269.116; found, 269.117; purity 99.6% (HPLC).

2-(4-Isobutyramidophenylamino)benzamide (16). To a solution of **63** (100 mg, 0.44 mmol) obtained above, isobutyric acid (32 mg, 0.360 mmol), PyBop (281 mg, 0.540 mmol), and Et_3N (89 mg, 0.880 mmol) in dry CH_2Cl_2 (5 mL) was stirred at room temperature for 23 h. The reaction mixture was poured into aqueous saturated $NaHCO_3$, and the whole was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo, and purification of the residue by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/2) gave 93 mg (87%) of **16** as a white powder. The powder was recrystallized from AcOEt/*n*-hexane to give 45 mg of **16** as a white powder; mp 227−230 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 10.54 (1H, s), 10.05 (1H, s), 8.38 (2H, d, J = 9.0 Hz), 8.19 (2H, d, J = 9.0 Hz), 7.71 (1H, dd, J = 1.5, 8.0 Hz), 7.32 (1H, t, J = 7.0 Hz), 7.23 (1H, d, J = 8.5 Hz), 7.19 (2H, d, J = 9.0 Hz), 6.77 (1H, t, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm) 171.25, 163.38, 148.97, 145.36, 140.61, 137.42, 133.15, 132.13, 129.02, 123.44, 121.64, 120.48, 117.26, 116.74, 114.11. MS (EI) *m/z*: 376 (M^+). HRMS calcd for $C_{20}H_{16}N_4O_4$, 376.117; found, 376.117; purity 95.6% (HPLC).

2-(4-Cyclohexanecarboxamidophenylamino)benzamide (17). Compound **17** was prepared from **63** obtained above and cyclohexanecarboxylic acid using the procedure described for **16** in 38% yield; mp 207−209 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 9.95 (1H, s), 9.75 (1H, s), 7.69 (1H, dd, J = 1.5, 8.0 Hz), 7.56 (2H, d, J = 9.0 Hz), 7.27 (1H, t, J = 7.5 Hz), 7.12 (1H, d, J = 8.5 Hz), 7.05 (2H, d, J = 9.0 Hz), 6.72 (1H, d, J = 7.5 Hz), 1.80−1.17 (11H, m). ^{13}C NMR (DMSO, 125 MHz, δ, ppm) 173.83, 171.31, 145.94, 136.02, 134.42, 132.13, 129.20, 121.22, 120.15, 116.79, 116.16, 113.66, 29.07, 25.31, 25.15. MS (EI) *m/z*: 337 (M^+). Anal. ($C_{20}H_{23}N_3O_2$) C, H, N.

***N*-(4−2-Carbamoylphenylaminophenyl)piperidine-4-carboxamide Trifluoroacetic Acid Salt (18·TFA).** *Step 1: Preparation of tert-Butyl 4-((4−2-Carbamoylphenylaminophenyl)carbamoyl)-piperidine-1-carboxylate (64).* Compound **64** was prepared from **63** and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid using the procedure described for **16** in 35% yield. ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 9.96 (1H, s), 9.86 (1H, s), 7.68 (1H, dd, J = 1.5, 8.0 Hz), 7.54 (2H, d, J = 9.0 Hz), 7.27 (1H, t, J = 7.5 Hz), 7.12 (1H, d, J = 8.0 Hz), 6.89 (2H, d, J = 8.5 Hz), 6.72 (1H, t, J = 7.0 Hz), 1.40 (9H, s).

Step 2: N-(4-2-Carbamoylphenylaminophenyl)piperidine-4-carboxamide Trifluoroacetic Acid Salt (18·TFA). A solution of **64** (26 mg, 0.06 mmol) obtained above in TFA (2 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, and the residue was recrystallized from $CHCl_3$/MeOH to give 19 mg (95%) of **18·TFA** as a gray powder; mp 207−209 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 9.99 (1H, s), 9.97 (1H, s), 7.69 (1H, d, J = 7.5 Hz), 7.55 (2H, d, J = 8.5 Hz), 7.28 (1H, t, J = 7.5 Hz), 7.14−7.11 (3H, m), 6.74 (1H, t, J = 6.3 Hz), 2.93−1.82 (9H, m). MS (EI) *m/z*: 338 (M^+). HRMS calcd for $C_{19}H_{21}N_4O_2$, 338.174; found, 338.174; purity 98.7% (HPLC).

Compounds **19**−**21** were prepared from **63** and an appropriate carboxylic acid using the procedure described for **16**.

***N*-(4-2-Carbamoylphenylaminophenyl)picolinamide (19).** Yield 19%; mp 231−233 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 10.60 (1H, s), 10.00 (1H, s), 8.75−8.74 (1H, m), 8.07 (1H, t, J = 8.0 Hz), 7.87 (2H, dd, J = 2.5, 9.0 Hz), 7.71−7.67 (2H, m), 7.31 (1H, t, J = 7.5 Hz), 7.21 (1H, d, J = 8.3 Hz), 7.17 (2H, dd, J = 2.0, 9.0 Hz), 6.76 (1H, t, J = 7.0 Hz). MS (EI) *m/z*: 332 (M^+). Anal. ($C_{19}H_{16}N_4O_2$) C, H, N.

***N*-(4-2-Carbamoylphenylaminophenyl)nicotinamide (20).** Yield 97%; mp 177−179 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 10.41 (1H, s), 10.04 (1H, s), 9.11 (1H, d, J = 1.5 Hz), 8.76 (1H, dd, J = 1.5, 5.0 Hz), 8.29 (1H, t, J = 8.0 Hz), 7.73 (2H, d, J = 8.0 Hz), 7.71 (1H, d, J = 8.0 Hz), 7.57 (1H, dd, J = 1.5, 5.0 Hz), 7.31 (1H, t, J = 8.5 Hz), 7.21 (1H, d, J = 8.5 Hz), 6.76 (1H, t, J = 8.0 Hz). MS (EI) *m/z*: 332 (M^+). Anal. ($C_{19}H_{16}N_4O_2$) C, H, N.

***N*-(4-2-Carbamoylphenylaminophenyl)isonicotinamide (21).** Yield 75%; mp 249−252 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 10.46 (1H, s), 10.52 (1H, s), 8.79 (2H, dd, J = 2.0, 4.5 Hz), 7.86 (2H, dd, J = 1.5, 4.5 Hz), 7.72 (3H, m), 7.22 (2H, d, J = 8.0 Hz), 7.71 (1H, d, J = 8.0 Hz), 7.57 (1H, t, J = 5.0 Hz), 8.29 (1H, t, J = 8.0 Hz), 7.73 (2H, d, J = 8.0 Hz), 7.71 (1H, d, J = 8.0 Hz), 7.57 (1H, t, J = 5.0 Hz), 7.31 (1H, t, J = 8.5 Hz), 7.21 (1H, dd, J = 1.5, 8.5 Hz), 6.77 (1H, t, J = 7.0 Hz). MS (EI) *m/z*: 332 (M^+). HRMS calcd for $C_{19}H_{16}N_4O_2$, 332.127; found, 332.128; purity 96.3% (HPLC).

Compounds **22**, **24**, **26**, **28**, and **29** were prepared from an appropriate acid chloride (**65**−**67**) and amine (**68**−**71**) using the procedure described for **11** (step 1) and **8** (step 3).

2-(3-*N*-Phenylbenzamidoamino)benzamide (22). Yield 45%; mp 182−184 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.66 (1H, s), 7.79 (1H, broad s), 7.73 (1H, t, J = 2.1 Hz), 7.64 (2H, d, J = 7.9 Hz), 7.50 (1H, dd, J = 1.2, 7.8 Hz), 7.47 (1H, td, J = 1.5, 7.5 Hz), 7.41 (1H, t, J = 7.6 Hz), 7.30−7.38 (5H, m), 7.15 (1H, t, J = 7.6 Hz), 6.83 (1H, dt, J = 1.5, 8.2 Hz);. ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.52, 165.53, 145.47, 142.22, 137.92, 136.36, 133.14, 129.80, 129.12, 128.36, 124.57, 123.98, 120.59, 120.12, 119.04, 118.58, 116.80, 115.81. MS (EI) *m/z*: 331 (M^+). HRMS (EI) *m/z*: calcd for $C_{20}H_{17}N_3O_2$, 331.132; found, 331.132. Anal. ($C_{20}H_{17}N_3O_2$) C, H, N.

2-(3-*N*-Benzylbenzamidoamino)benzamide (24). Yield 37%; mp 194−196 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.62 (1H, s), 7.66 (1H, s), 7.46 (1H, d, J = 7.9 Hz), 7.40−7.33 (m, 8H), 7.32−7.28 (2H, m), 6.81 (1H, t, J = 7.5 Hz), 6.35 (1H, broad s), 4.65 (2H, d, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.52, 167.17, 148.48, 145.67, 141.99, 138.10, 135.75, 133.12, 129.58, 128.83, 128.31, 127.95, 127.67, 123.86, 120.65, 119.34, 118.36, 115.1, 44.20. MS (EI) *m/z*: 345 (M^+). HRMS (EI) *m/z*: calcd for $C_{21}H_{19}N_3O_2$, 345.148; found, 345.147. Anal. ($C_{21}H_{19}N_3O_2 \cdot 1/10H_2O$) C, H, N.

2-(3-Benzamidophenylamino)benzamide (26). Yield 18%; mp 158−160 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.54 (1H, s), 7.86 (2H, d, J = 8.2 Hz), 7.75 (1H, broad s), 7.60 (1H, t, J = 2.3 Hz), 7.58−7.53 (1H, m), 7.52−7.46 (3H, m), 7.43 (1H, d, J = 8.2 Hz), 7.33 (2H, dt, J = 1.2, 7.2 Hz), 7.29 (2H, d, J = 7.9 Hz), 6.99 (1H, td, J = 2.1, 7.2 Hz), 6.79 (1H, dt, J = 1.2, 8.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.54, 165.65, 145.89, 142.26, 138.86, 135.00, 133.04, 131.88, 129.89, 128.83, 128.27, 126.99, 118.05, 117.28, 116.47, 115.95, 114.29, 112.47. MS (EI) *m/z*: 331 (M^+). HRMS (EI) *m/z*: calcd for $C_{20}H_{17}N_3O_2$, 331.132; found, 331.132. Anal. ($C_{20}H_{17}N_3O_2 \cdot 1/5H_2O$) C, H, N.

2-(3-2-Phenylacetamidophenylamino)benzamide (28). Yield 18%; mp 159−161 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.46 (1H, s), 7.45 (1H, dd, J = 1.2, 7.5 Hz), 7.40 (2H, t, J = 7.5 Hz), 7.36−7.28 (6H, m), 7.20 (1H, t, J = 7.9 Hz), 7.20 (1H, d, J = 7.9 Hz), 6.99 (1H, broad s), 6.94 (1H, d, J = 7.5 Hz), 6.78−6.73 (1H, m), 3.74 (2H, s). ^{13}C NMR ($CDCl_3$, 150 MHz, δ, ppm) 171.53, 168.95, 145.94, 142.05, 138.57, 134.34, 133.01, 129.68, 129.55, 129.32, 128.23, 127.77, 117.95, 117.16, 116.32, 115.80, 113.99, 112.39, 44.96. MS (EI) *m/z*: 345 (M^+). HRMS (EI) *m/z*: calcd for $C_{21}H_{19}N_3O_2$, 345.148; found, 345.148; purity 99.0% (HPLC).

2-(3-*N*-Methyl-2-phenylacetamidophenylamino)benzamide (29). Yield 45%; mp 173−175 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.63 (1H, s), 7.49 (1H, dd, J = 1.2, 7.5 Hz), 7.34−7.27 (2H, m), 7.25−7.16 (5H, m), 7.10 (2H, d, J = 7.3 Hz), 6.97−6.93 (1H, m), 6.82 (1H, t, J = 7.5 Hz), 6.77 (1H, d, J = 7.3 Hz), 3.53 (2H, s), 3.26 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.46, 170.92, 145.29, 144.96, 142.89, 135.50, 133.02, 130.40, 129.06, 128.36, 128.34, 126.57, 121.18, 119.92, 118.98, 118.55, 116.75, 115.77, 40.99, 37.52. MS (EI) *m/z*: 359 (M^+). HRMS (EI) *m/z*: calcd for $C_{22}H_{21}N_3O_2$, 359.163; found, 359.163; purity 97.1% (HPLC).

2-(3-*N*-Methyl-*N*-phenylbenzamidoamino)benzamide (23). *Step 1: Preparation of 3-Bromo-N-methyl-N-phenylbenzamide (73).* To a solution of **72** (300 mg, 1.08 mmol) in DMF (4 mL) was added NaH (87 mg, 60% in oil, 2.16 mmol), and the mixture was stirred at room temperature for 30 min. MeI (101 μL, 1.62 mmol) was then added dropwise at 0 °C, and stirring was continued at 0 °C for 1 h and at room temperature for 2 h. The reaction was quenched with saturated aqueous NH_4Cl, and the whole was extracted with AcOEt.

dx.doi.org/10.1021/jm3002108 | *J. Med. Chem.* 2012, 55, 5760−5773

The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo, and purification of the residue by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/3) gave 270 mg (86%) of **73** as a colorless solid. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.50 (1H, t, J = 1.8 Hz), 7.35 (1H, d, J = 7.9 Hz), 7.27−7.24 (2H, m), 7.20−7.11 (2H, m), 7.03 (2H, d, J = 7.6 Hz), 7.00 (1H, t, J = 7.9 Hz), 3.50 (3H, s).

*Step 2: Preparation of 2-(3-N-Methyl-N-phenylbenzamidoamino)benzamide (**23**).* Compound **23** was prepared from **73** and **39** using the procedure described for **8** (step 3) in 53% yield; mp 157−159 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.45 (1H, s), 7.43 (1H, dd, J = 1.5, 7.8 Hz), 7.28 (2H, t, J = 7.8 Hz), 7.21−7.12 (4H, m), 7.06 (2H, d, J = 8.5 Hz), 7.01 (2H, dd, J = 1.5, 7.5 Hz), 6.76−6.70 (2H, m), 3.49 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.50, 170.56, 145.79, 144.91, 140.70, 137.06, 133.02, 129.24, 129.07, 128.19, 126.77, 126.35, 123.03, 122.79, 120.70, 117.82, 116.08, 115.16, 38.37. MS (EI) m/z: 345 (M^+). HRMS (EI) m/z: calcd for $C_{21}H_{19}N_3O_2$, 345.148; found, 345.148. Anal. ($C_{21}H_{19}N_3O_2 \cdot 1/3H_2O$) C, H, N.

Compounds **25** and **27** were prepared from **74** and **76**, respectively, using the procedure described for **23**.

2-(3-*N*-Methyl-*N*-benzylbenzamidoamino)benzamide (25). Yield 45%; mp 60−62 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.60 (1H, s), 9.56 (1H, s), 7.45 (2H, m), 7.37−7.28 (14H, m), 7.20−7.16 (6H, m), 7.10−7.05 (2H, m), 6.80−6.76 (2H, m), 4.75 (2H, s), 4.55 (2H, s), 3.01 (3H, s), 2.88 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.53, 133.04, 129.61, 129.49, 128.87, 128.73, 128.29, 128.17, 127.61, 126.82, 122.05, 121.03, 120.53, 119.24, 118.49, 118.22, 115.72, 55.21, 5.78, 37.00, 33.10. MS (EI) m/z: 359 (M^+). HRMS (EI) m/z: calcd for $C_{22}H_{21}N_3O_2$, 359.163; found, 359.163; purity 98.2% (HPLC).

2-(3-*N*-Methylbenzamidophenylamino)benzamide (27). Yield 53%; mp 178−180 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.49 (1H, s), 7.41 (1H, dd, J = 1.8, 7.8 Hz), 7.38 (2H, dd, J = 1.5, 7.8 Hz), 7.32 (1H, tt, J = 1.2, 7.2 Hz), 7.28 (2H, td, J = 1.5, 8.2 Hz), 7.21 (1H, t, J = 8.0 Hz), 7.07 (1H, dt, J = 1.5, 8.5 Hz), 6.90−6.74 (1H, m), 6.84−6.80 (2H, m), 6.73 (1H, dt, J = 0.9, 8.2 Hz), 6.40 (1H, d, J = 8.5 Hz), 3.49 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.47, 170.46, 145.82, 145.44, 141.99, 135.95, 133.04, 130.18, 129.70, 128.92, 128.19, 127.90, 120.08, 119.49, 119.41, 118.13, 116.31, 115.07, 38.27. MS (EI) m/z: 345 (M^+). HRMS (EI) m/z: calcd for $C_{21}H_{19}N_3O_2$, 345.148; found, 345.148. Anal. ($C_{21}H_{19}N_3O_2 \cdot 1/10H_2O$) C, H, N.

2-(3-Isobutoxyphenylamino)benzamide (31). *Step 1: Preparation of 1-Iodo-3-isobutoxybenzene (**83**).* A mixture of 3-iodophenol **81** (5.00 g, 22.7 mmol) and 1-bromo-2-methylpropane (3.10 g, 22.7 mmol) in acetone (10 mL) was refluxed for 18 h. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration, concentration of the filtrate in vacuo, and purification of the residue by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/10) gave 2.90 g (46%) of **83** as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.26 (2H, m), 6.98 (1H, t, J = 8.0 Hz), 6.85 (1H, m), 3.68 (2H, d, J = 7.0 Hz), 2.06 (1H, m), 1.01 (6H, m, J = 7.0 Hz).

*Step 2: Preparation of 2-(3-Isobutoxyphenylamino)benzamide (**31**).* Compound **31** was prepared from **83** and **39** using the procedure described for **8** (step 3) in 13% yield; mp 77−79 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 9.48 (1H, s), 7.48 (1H, dd, J = 1.5, 8.0 Hz), 7.38 (1H, dd, J = 1.0, 8.5 Hz), 7.31 (1H, t, J = 7.5 Hz), 7.20 (1H, t, J = 8.0 Hz), 6.80 (1H, d, J = 8.0 Hz), 6.80−6.75 (2H, m), 6.58 (1H, dd, J = 2.0, 8.0 Hz), 3.70 (2H, d, J = 6.5 Hz), 2.07 (1H, m), 1.01 (6H, d, J = 6.5 Hz). MS (EI) m/z: 284 (M^+). HRMS calcd for $C_{17}H_{20}N_2O_2$, 284.153; found, 284.153; purity 96.1% (HPLC).

Compounds **30**, **32**, and **33a** were prepared from 3-bromophenol **80** or 3-iodophenol **81** using the procedure described for **31**.

2-(3-Cyclohexylmethoxyphenylamino)benzamide (30). Yield 13%; mp 123−125 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.48 (1H, s), 7.46 (1H, dd, J = 1.5, 8.0 Hz), 7.38 (1H, d, J = 7.5 Hz), 7.19 (1H, t, J = 8.0 Hz), 6.79 (1H, dd, J = 1.5, 8.0 Hz), 6.76 (1H, m), 6.75 (1H, s), 6.58 (1H, dd, J = 2.0, 8.0 Hz), 3.73 (2H, d, J = 6.5 Hz), 1.87−1.84 (2H, m), 1.78−1.74 (4H, m), 1.31−1.28 (3H, m). MS (EI) m/z: 324 (M^+). HRMS calcd for $C_{20}H_{25}N_2O_2$, 324.184; found, 324.184; purity 95.9% (HPLC).

2-(3-Benzyloxyphenylamino)benzamide (32). Yield 31%; mp 105−107 °C. ^{1}H NMR ($CDCl_3$, 600 MHz, δ, ppm) 9.51 (1H, s), 7.46 (1H, dd, J = 1.5, 8.0 Hz), 7.43 (2H, d, J = 9.0 Hz), 7.39 (2H, t, J = 7.5 Hz), 7.33 (1H, t, J = 2.5 Hz), 7.28 (1H, dd, J = 1.5, 7.0 Hz), 7.26 (1H, s), 7.21 (1H, t, J = 7.5 Hz), 6.85 (1H, t, J = 2.5 Hz), 6.80 (1H, dd, J = 1.5, 8.0 Hz), 6.76 (1H, t, J = 7.0 Hz), 6.66 (1H, dd, J = 1.5, 7.5 Hz), 5.03 (2H, s). MS (EI) m/z: 318 (M^+). HRMS calcd for $C_{20}H_{18}N_2O_2$, 318.137; found, 318.137; purity 97.4% (HPLC).

2-((3-Phenethoxyphenyl)amino)benzamide (33a). Yield 6%; mp 129−131 °C. ^{1}H NMR ($CDCl_3$, 600 MHz, δ; ppm) 9.49 (1H, s), 7.47 (1H, d, J = 8.4 Hz), 7.36 (1H, t, J = 8.0 Hz), 7.34−7.22 (3H, m), 7.19 (1H, d, J = 8.5 Hz), 6.82−6.74 (4H, m), 6.58 (1H, d, J = 8.5 Hz), 4.16 (2H, t, J = 7.0 Hz), 3.09 (2H, t, J = 7.0 Hz). MS (EI) m/z: 332 (M^+). HRMS calcd for $C_{21}H_{20}N_2O_2$, 332.152; found, 332.153; purity 98.7% (HPLC).

2-{3-(3-Fluorophenethyloxy)phenylamino}benzamide (33i). *Step 1: Preparation of 2-(3-Hydroxyphenylamino)benzamide (**86**).* A solution of **32** (150 mg, 0.47 mmol) in EtOH (4 mL) and 10% Pd/C (30 mg) was vigorously stirred under hydrogen gas at room temperature for 6 h. After complete disappearance of the starting material, the reaction mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was purified by silica gel flash column chromatography (AcOEt/*n*-hexane = 2/1) to give **86** (78 mg, 73%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.45 (1H, s), 7.46 (1H, dd, J = 1.5, 7.8 Hz), 7.38 (1H, d, J = 8.5 Hz), 7.30 (1H, dt, J = 1.5, 8.2 Hz), 7.15 (1H, t, J = 8.2 Hz), 6.82−6.75 (2H, m), 6.72 (1H, t, J = 2.1 Hz), 6.51 (1H, dd, J = 1.5, 8.2 Hz), 5.55 (broad s, 1H).

*Step 2: 2-{3-(3-Fluorophenethyloxy)phenylamino}benzamide (**33i**).* To a stirred solution of **86** (100 mg, 0.430 mmol) obtained above and potassium carbonate (191 mg, 1.31 mmol) in acetone (10 mL) was added portionwise 3-fluorophenethyl bromide (185 μL, 1.31 mmol). The mixture was refluxed for 12 h, allowed to cool to room temperature, and then filtered. The filtrate was concentrated, and the residue was purified by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/3 to 1/2) to give **33i** as a colorless solid, which was recrystallized from $CHCl_3$/*n*-hexane to give a colorless solid (76 mg, 50%); mp 114−116 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.49 (1H, s), 7.47 (1H, dd, J = 1.5, 7.8 Hz), 7.37 (1H, dd, J = 1.2, 8.5 Hz), 7.32−7.27 (2H, m), 7.20 (1H, t, J = 8.2 Hz), 7.05 (1H, d, J = 7.6 Hz), 7.00 (1H, td, J = 1.8, 8.2 Hz), 6.93 (1H, ddt, J = 0.9, 2.4, 8.5 Hz), 6.81 (1H, dd, J = 1.5, 7.5 Hz), 6.79−6.75 (2H, m), 6.57 (1H, ddd, J = 0.9, 1.5, 8.5 Hz), 4.15 (2H, t, J = 7.0 Hz), 3.08 (2H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz, δ, ppm) 171.58, 162.80 (d, $^1J_{C-F}$ = 244.2 Hz),159.67, 146.14, 142.62, 140.88 (d, $^3J_{C-F}$ = 7.5 Hz), 132.95, 130.00, 129.86 (d, $^3J_{C-F}$ = 7.9 Hz), 128.26, 124.64, 124.62, 117.76, 116.18, 115.91 (d, $^2J_{C-F}$ = 19.3 Hz), 113.81, 113.37 (d, $^2J_{C-F}$ = 21.0 Hz), 108.94, 107.477, 68.19, 35.48. MS (EI) m/z: 350 (M^+). HRMS calcd for $C_{21}H_{19}FN_2O_2$, 350.143; found, 350.142. Anal. ($C_{21}H_{19}FN_2O_2$) C, H, N.

Compounds **33b−h**, **33j−p**, and **34** were prepared from **86** and an appropriate alkyl bromide using the procedure described for **33i** (step 2).

2-{3-(2-Methylphenethyloxy)phenylamino}benzamide (33b). Yield 30%; mp 120−122 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.48 (1H, s), 7.45 (1H, dd, J = 1.5, 7.9 Hz), 7.36 (1H, dd, J = 0.9, 8.5 Hz), 7.30 (1H, dd, J = 1.5, 7.0 Hz), 7.23−7.18 (2H, m), 7.17−7.13 (3H, m), 6.80 (1H, dd, J = 1.5, 7.9 Hz), 6.78−6.73 (2H, m), 6.58 (1H, dd, J = 2.4, 8.0 Hz), 4.13 (2H, t, J = 7.3 Hz), 3.10 (2H, t, J = 7.3 Hz), 2.37 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.58, 159.81, 146.19, 142.59, 136.49, 136.23, 132.95, 130.32, 129.98, 129.52, 128.24, 126.66, 126.10, 117.70, 116.14, 115.85, 113.72, 108.90, 107.49, 67.73, 33.04, 19.49. MS (EI) m/z: 346 (M^+). HRMS calcd for $C_{22}H_{22}N_2O_2$, 346.168; found, 346.168; Anal. ($C_{22}H_{22}N_2O_2$) C, H, N.

2-{3-(3-Methylphenethoxy)phenylamino}benzamide (33c). Yield 43%; mp 109−111 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm)

dx.doi.org/10.1021/jm3002108 | J. Med. Chem. 2012, 55, 5760−5773

9.48 (1H, s), 7.46 (1H, dd, J = 1.5, 7.5 Hz), 7.37 (1H, dd, J = 1.2, 8.5 Hz), 7.29 (1H, dt, J = 1.2, 7.2 Hz), 7.23−7.16 (2H, m), 7.10 (2H, s), 7.06 (1H, t, J = 7.6 Hz), 6.80 (1H, d, J = 6.4 Hz), 6.79−6.74 (2H, m), 6.58 (1H, ddd, J = 0.9, 2.4, 7.2 Hz), 4.14 (2H, t, J = 7.3 Hz), 3.05 (2H, t, J = 7.3 Hz), 2.34 (3H, s). MS (EI) m/z: 346 (M^+). HRMS calcd for $C_{22}H_{22}N_2O_2$, 346.168; found, 346.168. Anal. ($C_{22}H_{22}N_2O_2 \cdot 1/5H_2O$) C, H, N.

2-{3-(4-Methylphenethyloxy)phenylamino}benzamide (33d). Yield 69%; mp 189−191 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.47 (1H, s), 7.45 (1H, dd, J = 1.5, 7.8 Hz), 7.36 (1H, dd, J = 1.2, 8.5 Hz), 7.29 (1H, dt, J = 1.2, 7.0 Hz), 7.21−7.15 (3H, m), 7.12 (2H, d, J = 8.0 Hz), 6.79 (1H, dd, J = 1.5, 7.8 Hz), 6.78−6.73 (2H, m), 6.57 (1H, ddd, J = 0.9, 1.5, 7.5 Hz), 4.13 (3H, t, J = 7.3 Hz), 3.05 (3H, t, J = 7.3 Hz), 2.33 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171. 59, 159.83, 146.21, 142.56, 136.01, 135.09, 132.94, 129.95, 129.17, 128.88, 128.24, 117.68, 116.13, 115.85, 113.72, 108.99, 107.56, 68.82, 35.33, 21.04. MS (EI) m/z: 346 (M^+). HRMS calcd for $C_{22}H_{22}N_2O_2$, 346.168; found, 346.167. Anal. ($C_{22}H_{22}N_2O_2$) C, H, N.

2-{3-(2-Trifluoromethylphenethyloxy)phenylamino}-benzamide (33e). Yield 18%; mp 89−91 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.48 (1H, d, J = 7.6 Hz), 7.65 (1H, d, J = 7.8 Hz), 7.51−7.45 (3H, m), 7.36 (1H, dd, J = 0.9, 8.5 Hz), 7.34−7.28 (2H, m), 7.20 (1H, t, J = 8.2 Hz), 6.81 (1H, dd, J = 2.1, 7.8 Hz), 6.78−6.74 (2H, m), 6.57 (1H, dd, J = 2.1, 8.5 Hz), 4.17 (2H, t, J = 6.8 Hz), 3.28 (2H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.58, 159.67, 146.19, 142.64, 136.79, 132.95, 132.01, 131.74, 130.01, 128.25, 126.64, 126.07, 126.03, 117.75, 116.18, 115.86, 113.83, 108.89, 107.59, 67.96, 32.50. MS (EI) m/z: 400 (M^+). HRMS calcd for $C_{22}H_{19}F_3N_2O_2$, 400.140; found, 400.140. Anal. ($C_{22}H_{19}F_3N_2O_2 \cdot 1/3H_2O$) C, H, N.

2-{3-(3-Trifluoromethylphenethyloxy)phenylamino}-benzamide (33f). Yield 26%; mp 90−92 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.49 (1H, s), 7.54 (1H, broad s), 7.50 (1H, d, J = 8.2 Hz), 7.46 (2H, dd, J = 1.2, 7.6 Hz), 7.42 (1H, t, J = 7.6 Hz), 7.36 (1H, dd, J = 1.2, 8.2 Hz), 7.30 (1H, dt, J = 1.5, 7.0 Hz), 7.19 (1H, t, J = 8.0 Hz), 6.81 (1H, dd, J = 1.5, 7.5 Hz), 6.79−6.70 (2H, m), 6.57 (1H, dd, J = 2.4, 8.0 Hz), 4.17 (2H, t, J = 6.7 Hz), 3.14 (2H, t, J = 6.7 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.60, 159.60, 146.11, 142.64, 139.35, 132.94, 132.43, 130.02, 128.87, 128.26, 125.80, 123.40, 123.37, 123.0, 117.79, 116.20, 115.86, 113.84, 108.89, 107.43, 68.08, 35.56. MS (EI) m/z: 400 (M^+). HRMS calcd for $C_{22}H_{19}F_3N_2O_2$, 400.140; found, 400.141. Anal. ($C_{22}H_{19}F_3N_2O_2$) C, H, N.

2-{3-(4-Trifluoromethylphenethyloxy)phenylamino}-benzamide (33g). Yield 11%; mp 179−181 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.49 (1H, s), 7.56 (2H, d, J = 8.2 Hz), 7.46 (1H, dd, J = 1.5, 7.9 Hz), 7.40 (2H, d, J = 7.9 Hz), 7.36 (1H, dd, J = 0.9, 8.2 Hz), 7.30 (1H, dt, J = 1.5, 7.2 Hz), 7.19 (1H, t, J = 8.2 Hz), 6.81 (1H, dd, J = 1.5, 7.9 Hz), 6.79−6.70 (2H, m), 6.56 (1H, dd, J = 2.7, 8.2 Hz), 4.18 (2H, t, J = 6.5 Hz), 3.14 (2H, t, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.56, 159.61, 146.13, 142.65, 142.56, 132.94, 130.03, 129.34, 128.27, 125.39, 125.335, 125.31, 117.80, 116.20, 115.84, 113.85, 108.90, 107.46, 67.99, 35.57. MS (EI) m/z: 400 (M^+). HRMS calcd for $C_{22}H_{19}F_3N_2O_2$, 400.140; found, 400.140; purity 99.2% (HPLC).

2-{3-(2-Fluorophenethyloxy)phenylamino}benzamide (33h). Yield 21%; mp 113−115 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.48 (1H, s), 7.45 (1H, dd, J = 1.5, 7.6 Hz), 7.36 (1H, dd, J = 0.9, 7.6 Hz), 7.32−7.27 (2H, m), 7.24−7.16 (2H, m), 7.08 (1H, dt, J = 1.2, 6.3 Hz), 7.05 (1H, dt, J = 1.2, 8.5 Hz), 6.80 (1H, d, J = 1.5, 6.3 Hz), 6.78−6.73 (2H, m), 6.57 (1H, dd, J = 1.8, 8.0 Hz), 4.16 (2H, t, J = 7.0 Hz), 3.13 (2H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.57, 161.25 (d, $^1J_{C-F}$ = 243.7 Hz), 159.71, 146.18, 142.58, 132.94, 131.41, 129.97, 128.27 (d, $^3J_{C-F}$ = 10.2 Hz), 125.00 (d, $^3J_{C-F}$ = 16.4 Hz), 124.05 (d, $^4J_{C-F}$ = 4.1 Hz), 124.03, 117.70, 116.00 (d, $^2J_{C-F}$ = 37.0 Hz), 115.28 (d, $^2J_{C-F}$ = 22.6 Hz), 115.12, 113.76, 108.93, 107.55, 67.17, 29.16. MS (EI) m/z: 350 (M^+). HRMS calcd for $C_{21}H_{19}FN_2O_2$, 350.143; found, 350.143. Anal. ($C_{21}H_{19}FN_2O_2$) C, H, N.

2-{3-(4-Fluorophenethyloxy)phenylamino}benzamide (33j). Yield 72%; mp 163−165 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.49 (1H, s), 7.46 (1H, dd, J = 1.5, 7.2 Hz), 7.36 (1H, d, J = 8.5 Hz), 7.31 (1H, dd, J = 1.5, 7.0 Hz), 7.25−7.21 (2H, m), 7.19 (1H, t, J = 8.2 Hz), 7.00 (2H, t, J = 8.5 Hz), 6.81 (1H, dd, J = 2.1, 7.8 Hz), 6.77 (1H, dd, J = 1.5, 7.0 Hz), 6.76−6.73 (1H, m), 6.57 (1H, dd, J = 2.4, 7.2 Hz), 4.13 (2H, t, J = 6.8 Hz), 3.05 (2H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ; ppm) 171.57, 161.75 (d, $^1J_{C-F}$ = 238.7 Hz),159.74, 146.19, 142.61, 134.00, 132.94, 130.42 (d, $^3J_{C-F}$ = 8.3 Hz), 129.99, 128.26, 117.74, 116.16, 115.84, 115.24 (d, $^3J_{C-F}$ = 22.2 Hz), 113.78, 108.97, 107.53, 68.59, 34.97. MS (EI) m/z: 350 (M^+). HRMS calcd for $C_{21}H_{19}FN_2O_2$, 350.143; found, 350.143. Anal. ($C_{21}H_{19}FN_2O_2$) C, H, N.

2-{3-(2-Chlorophenethyloxy)phenylamino}benzamide (33k). Yield 28%; mp 118−120 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.48 (1H, s), 7.46 (1H, dd, J = 1.8, 8.0 Hz), 7.38−7.34 (2H, m), 7.32 (1H, dd, J = 2.1, 7.0 Hz), 7.30 (1H, dt, J = 1.5, 7.0 Hz), 7.23−7.16 (3H, m), 6.80 (1H, dd, J = 1.8, 7.0 Hz), 6.78−6.73 (2H, m), 6.59 (1H, dd, J = 2.4, 8.0 Hz), 4.18 (2H, t, J = 7.0 Hz), 3.23 (2H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.57, 159.70, 146.18, 142.59, 135.79, 134.21, 132.95, 131.32, 129.98, 129.52, 128.23, 128.04, 126.84, 117.71, 116.14, 115.85, 113.75, 108.95, 107.54, 66.73, 33.53. MS (EI) m/z: 366, 368 (M^+). HRMS calcd for $C_{21}H_{19}{}^{35}ClN_2O_2$, 366.114; found, 366.112; calcd for $C_{21}H_{19}{}^{37}ClN_2O_2$, 368.111; found, 368.109. Anal. ($C_{21}H_{19}ClN_2O_2$) C, H, N.

2-{3-(3-Chlorophenethyloxy)phenylamino}benzamide (33l). Yield 65%; mp 129−131 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.49 (1H, s), 7.46 (1H, dd, J = 1.2, 7.8 Hz), 7.36 (1H, dd, J = 1.2, 8.5 Hz), 7.31 (1H, dd, J = 1.5, 7.0 Hz), 7.29−7.27 (1H, m), 7.24−7.19 (2H, m), 7.18−7.15 (2H, m), 6.81 (1H, dd, J = 1.8, 7.0 Hz), 6.79−6.74 (2H, m), 6.57 (1H, dd, J = 1.5, 7.0 Hz), 4.15 (2H, t, J = 6.7 Hz), 3.06 (2H, t, J = 6.7 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.55, 159.65, 146.15, 142.62, 140.38, 134.20, 132.95, 135.01, 129.70, 129.14, 128.25, 127.21, 126.69, 117.76, 116.17, 115.85, 113.83, 108.93, 107.48, 68.16, 35.42. MS (EI) m/z: 366, 368 (M^+). HRMS calcd for $C_{21}H_{19}{}^{35}ClN_2O_2$, 366.114; found, 366.113; calcd for $C_{21}H_{19}{}^{37}ClN_2O_2$, 368.111; found, 368.109. Anal. ($C_{21}H_{19}ClN_2O_2 \cdot 1/10H_2O$) C, H, N.

2-{3-(4-Chlorophenethoxy)phenylamino}benzamide (33m). Yield 41%; mp 192−194 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.45 (1H, s), 7.46 (1H, dd, J = 1.5, 7.8 Hz), 7.36 (1H, dd, J = 0.9, 8.2 Hz), 7.32−7.26 (3H, m), 7.23−7.16 (3H, m), 6.81 (1H, dd, J = 1.5, 7.8 Hz), 6.79−6.72 (2H, m), 6.56 (1H, dd, J = 2.4, 8.2 Hz), 4.13 (2H, t, J = 7.0 Hz), 3.05 (2H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.56, 159.68, 146.15, 142.61, 136.83, 132.94, 132.29, 130.35, 129.99, 128.57, 128.25, 117.76, 116.17, 115.83, 113.80, 108.93, 107.48, 68.31, 35.11. MS (EI) m/z: 366, 368 (M^+). HRMS calcd for $C_{21}H_{19}{}^{35}ClN_2O_2$, 366.114; found, 366.112; calcd for $C_{21}H_{19}{}^{37}ClN_2O_2$, 368.111; found, 368.112. Anal. ($C_{21}H_{19}ClN_2O_2 \cdot 1/10H_2O$) C, H, N.

2-{3-(2-Bromophenethyloxy)phenylamino}benzamide (33n). Yield 24%; mp 118−120 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.48 (1H, s), 7.55 (1H, dd, J = 1.2, 7.9 Hz), 7.46 (1H, dd, J = 1.5, 7.5 Hz), 7.37 (1H, dd, J = 0.9, 7.3 Hz), 7.32 (1H, dt, J = 1.8, 7.9 Hz), 7.30−7.27 (2H, m), 7.19 (1H, t, J = 8.2 Hz), 7.10 (1H, dt, J = 1.5, 7.5 Hz), 6.80 (1H, dd, J = 1.8, 7.5 Hz), 6.78−6.74 (2H, m), 6.60 (1H, dd, J = 2.4, 8.2 Hz), 4.18 (2H, t, J = 7.0 Hz), 3.23 (2H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.58, 159.70, 146.17, 132.95, 132.85, 131.33, 131.25, 129.98, 128.29, 128.24, 128.20, 127.49, 127.40, 117.72, 115.90, 115.86, 113.74, 108.97, 107.53, 66.81, 36.01. MS (EI) m/z: 410, 412 (M^+). HRMS calcd for $C_{21}H_{19}{}^{79}BrN_2O_2$, 410.063; found, 410.062; calcd for $C_{21}H_{19}{}^{81}BrN_2O_2$, 412.061; found, 412.062. Anal. ($C_{21}H_{19}BrN_2O_2 \cdot 1/10H_2O$) C, H, N.

2-{3-(3-Bromophenethoxy)phenylamino}benzamide (33o). Yield 31%; mp 133−135 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.49 (1H, s), 7.47 (1H, dd, J = 1.5, 7.8 Hz), 7.44 (1H, t, J = 2.1 Hz), 7.36 (2H, d, J = 7.8 Hz), 7.30 (1H, dt, J = 1.2, 7.0 Hz), 7.23−7.15 (3H, m), 6.82−6.76 (1H, m), 6.78 (1H, t, J = 0.9 Hz), 6.75 (1H, t, J = 2.1 Hz), 6.57 (1H, ddd, J = 0.9, 1.8, 8.2 Hz), 4.14 (2H, t, J = 7.0 Hz), 3.05 (2H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.55, 159.64, 146.14, 142.62, 140.70, 132.96, 132.05, 130.00, 129.62, 128.24, 127.68, 122.47, 117.76, 116.18, 115.85, 113.84, 108.93, 107.48, 100.57, 68.16, 35.38. MS (EI) m/z: 410, 412 (M^+). HRMS calcd for $C_{21}H_{19}{}^{79}Br\,N_2O_2$, 410.063; found, 410.062; calcd for $C_{21}H_{19}N_2O_2{}^{81}Br$, 412.061; found, 412.061. Anal. ($C_{21}H_{19}N_2O_2B \cdot 1/4H_2O$) C, H, N.

dx.doi.org/10.1021/jm3002108 | J. Med. Chem. 2012, 55, 5760−5773

2-{3-(4-Bromophenethoxy)phenylamino}benzamide (33p). Yield 16%; mp 154−156 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.47 (1H, dd, J = 1.5, 8.0 Hz), 7.43 (2H, d, J = 8.0 Hz), 7.37−7.29 (2H, m), 7.19 (1H, t, J = 8.3 Hz), 7.16 (2H, d, J = 8.5 Hz), 6.81 (1H, d, J = 7.5 Hz), 6.77 (1H, t, J = 8.5 Hz), 6.74 (1H, m), 6.62−6.60 (1H, m), 6.56 (1H, dd, J = 2.0, 8.0 Hz), 4.13 (2H, t, J = 6.8 Hz), 3.04 (2H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm) 171.76, 159.67, 146.14, 142.62, 137.37, 132.94, 131.63, 130.08, 129.04, 128.25, 117.76, 115.84, 114.02, 113.80, 109.22, 108.92, 107.48, 68.22, 35.17. MS (EI) m/z: 410, 412 (M^+). HRMS calcd for $C_{21}H_{19}{}^{79}BrN_2O_2$, 410.063; found, 410.063; calcd for $C_{21}H_{19}N_2O_2{}^{81}Br$, 412.061; found, 412.061; purity 97.0% (HPLC).

Biology. *SIRT Enzyme Assays.* SIRT activity assay was performed using SIRT fluorimetric drug discovery kits (AK-555, AK-556, and AK-557, BIOMOL Research Laboratories), according to the supplier's protocol. SIRT (human, recombinant) (15 μL/well), NAD^+ (1 mM), and various concentrations of samples were incubated at 37 °C for 60 min, and Fluor de Lys-SIRT substrate (25 μM) was added to the mixture. Reactions were stopped after 60 min by adding Fluor de Lys Developer II with nicotinamide, which stops further deacetylation. Then, 45 min after addition of this developer, the fluorescence of the wells was measured on a fluorometric reader with excitation set at 360 nm and emission detection set at 460 nm. The value of % inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells. The compound concentration resulting in 50% inhibition was determined by plotting log[Inh] versus the logit function of % inhibition. IC_{50} values were determined by means of regression analysis of the concentration/inhibition data.

Western Blot Analysis. HCT116 human colon cancer cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, U.S.A.) and cultured in McCoy's 5A culture medium containing penicillin and streptomycin, which was supplemented with fetal bovine serum as described in the ATCC instructions. HCT-116 cells (5×10^5) were treated for 8 h with 20 μM etoposide and 10 μM AGK2 (**3**), 10 μM EX-527 (**2**), and 1 μM and 10 μM **33a** in 10% FBS-supplemented McCoy's 5A medium and then collected and extracted with SDS buffer. Protein concentrations of lysates were determined using a Bradford protein assay kit (Bio-Rad Laboratories), with which equivalent amounts of protein from each lysate were resolved in 15% SDS-polyacrylamide gels and then transferred onto nitrocellulose membranes (Bio-Rad Laboratories). After having been blocked for 30 min with Tris-buffered saline (TBS) containing 3% skim milk, the transblotted membrane was incubated overnight at 4 °C with acetylated p53 antibody (Cell Signaling) (1:500 dilution), p53 antibody (Calbiochem) (1:500 dilution), acetylated α-tubulin antibody (SIGMA) (1:2000 dilution), or α-tubulin antibody (SIGMA) (1:2500 dilution) in TBS containing 3% skim milk. The membrane was probed with the primary antibody and then washed twice with water, incubated with goat antirabbit (for acetylated p53) or antimouse (for p53, α-tubulin, and acetylated α-tubulin) IgG-horseradish peroxidase conjugates (1:2500 dilution) for 2 h at room temperature, and again washed twice with water. The immunoblots were visualized by enhanced chemiluminescence.

Molecular Modeling. Homology models for SIRT1 and SIRT2 based on the crystal structure of Hst2 (PDB code 1Q17) were built using the homology modeling procedure of the Full Automatic Modeling System (FAMS). Although the X-ray crystal structure of SIRT2 has been reported,[16] we used the crystal structure of Hst2 complexed with an NAD^+ analogue[17] for homology modeling, because the reported SIRT2 structure is an apo-structure, not a substrate-bound structure, which is not suitable for our drug design of inhibitors competitive with the acetylated lysine substrate.[13b] The homology models of SIRT1 and SIRT2 were used as the target structures for docking. Protein preparation, receptor grid generation, and ligand docking were performed using the software Glide 3.5. Compounds **7b** and **7t** were docked into the active site of the protein. The standard precision mode of Glide was used to determine favorable binding poses, which allowed the ligand conformation to be flexibly explored while holding the protein as a rigid structure during docking. The predicted complex structure was then fully energy-minimized, with both the protein and the ligand allowed to move, using Macromodel 8.1 software. The conformation of compounds **7b** and **7t** in the active site was minimized by MM calculation based upon the OPLS-AA force field with parameters set as follows; Solvent, water; method, LBFGS; max no. of iterations, 10000; converge on, gradient; convergence threshold, 0.05.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Results of elemental analysis of compounds **12**, **14**, **17**, **19**, **20**, **22**−**24**, **26**, **27**, **33b**−**f**, and **33h**−**o**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported in part by the JST A-STEP program (T.S.) and by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (M.N., A.K.).

■ ABBREVIATIONS USED

SIRT, sirtuin; HDAC, histone deacetylase; SPR, surface plasmon resonance

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