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Journal

Journal of Medicinal Chemistry, 58(23)

ISSN

0022-2623

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Publication Date

2015-12-10

DOI

10.1021/acs.jmedchem.5b01188

Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Benzoxazolone Carboxamides as Potent Acid Ceramidase Inhibitors: Synthesis and Structure−Activity Relationship (SAR) Studies

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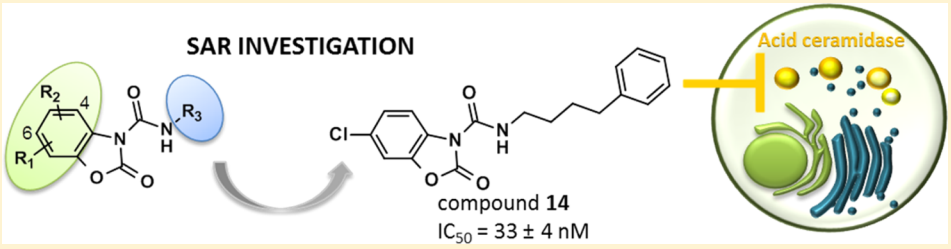
Supporting Information



ABSTRACT: Ceramides are lipid-derived intracellular messengers involved in the control of senescence, inflammation, and apoptosis. The cysteine amidase, acid ceramidase (AC), hydrolyzes these substances into sphingosine and fatty acid and, by doing so, regulates their signaling activity. AC inhibitors may be useful in the treatment of pathological conditions, such as cancer, in which ceramide levels are abnormally reduced. Here, we present a systematic SAR investigation of the benzoxazolone carboxamides, a recently described class of AC inhibitors that display high potency and systemic activity in mice. We examined a diverse series of substitutions on both benzoxazolone ring and carboxamide side chain. Several modifications enhanced potency and stability, and one key compound with a balanced activity−stability profile (**14**) was found to inhibit AC activity in mouse lungs and cerebral cortex after systemic administration. The results expand our arsenal of AC inhibitors, thereby facilitating the use of these compounds as pharmacological tools and their potential development as drug leads.

INTRODUCTION

Acid ceramidase (AC) is a cysteine amidase involved in the metabolism of ceramides, bioactive lipid molecules that belong to the class of sphingolipids.[1] Sphingolipids act as cellular messengers throughout the body and are involved in the survival, growth, and differentiation of cells,[2] as well as in pathophysiological process such as inflammation and neuropathic pain.[3] AC plays a pivotal role in balancing sphingolipid-mediated signaling.[4] The enzyme is mainly, albeit not uniquely, located in lysosomes,[5] where it cleaves the amide bond of ceramides producing the aliphatic amino alcohol sphingosine along with fatty acid (typically 14−26 carbon atoms in length) (Figure 1). Sphingosine is a metabolic precursor of sphingosine 1-phosphate, which enhances cell survival and proliferation,[6] while ceramides exert prosenescent and proapoptotic effects in both normal and tumor cells.[4,6c,7] Interestingly, ceramide concentrations increase in cells that are stressed with chemotherapeutic agents, DNA damage, and ionizing radiation,[7f] through stimulation of de novo ceramide biosynthesis, hydrolysis of sphingomyelin, or the salvage pathway;[1a] moreover, the cytotoxic effects of certain anticancer drugs partly depend on de novo ceramide biosynthesis.[8] AC is overexpressed in several cancer types,[9] and in prostate cancer this up-regulation renders cells more resistant to chemotherapy and radiotherapy, while genetic or pharmacological AC inhibition restores sensitivity to therapy in vivo.[10] Thus, modulating the ceramide/sphingosine 1-phosphate axis by inhibiting AC could provide a new strategy for future therapeutics against cancer and, possibly, other pathologies in which ceramide metabolism is dysregulated. To test this hypothesis, several AC inhibitors have been developed over the past decades.[11]

We have recently disclosed a small set of benzoxazolone carboxamides as the first example of systemically active inhibitors of intracellular AC activity.[12] We demonstrated that these molecules covalently inhibit AC through S-acylation of its catalytic nucleophile, Cys-143, with the benzoxazolone ring acting as leaving group. Preliminary structure−activity relationship (SAR) studies showed that a secondary carboxamide moiety is mandatory for activity, as in the initial hit compound **1** (h-AC IC_{50} = 64 nM) (Figure 2), and that introducing a bromine atom at position 6 on the heterocyclic scaffold leads to a 2-fold increase in inhibitory potency (**2**, IC_{50} = 31 nM), diminishing at the same time chemical and metabolic stability in aqueous buffer and mouse plasma. Also, we observed that replacing bromine with a *p*-fluorophenyl group, as in 6-(4-fluorophenyl)-2-oxo-*N*-(4-

Received: July 28, 2015
Published: November 11, 2015

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

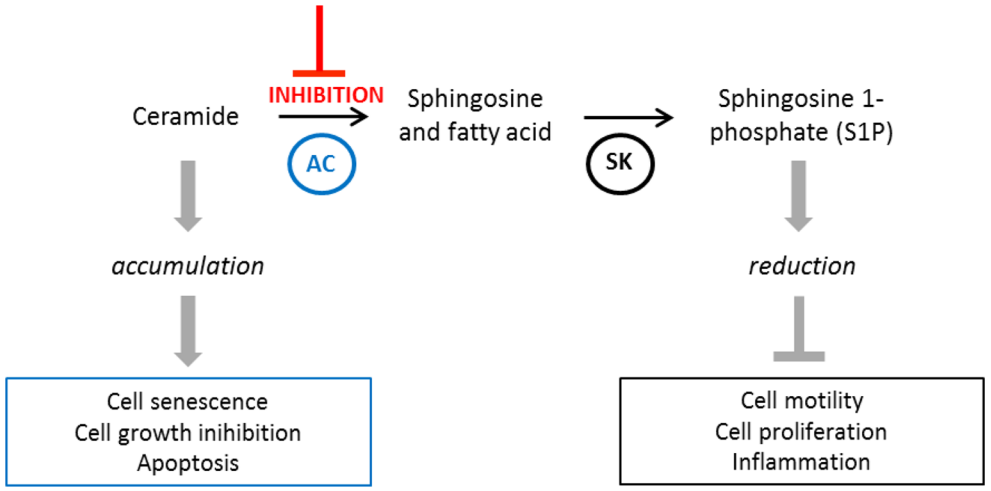


Figure 1. Biochemical pathway of ceramide to sphingosine 1-phosphate mediated by the enzymes acid ceramidase (AC) and sphingosine kinase (SK).

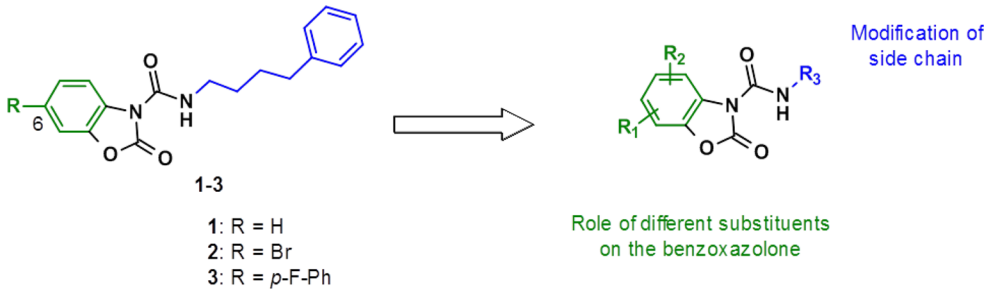
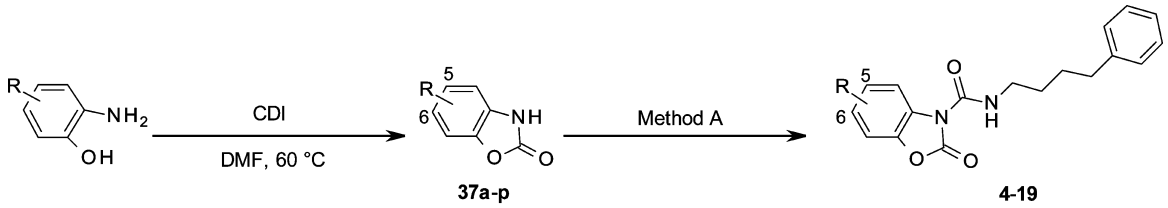


Figure 2. Benzoxazolone carboxamides **1**−**3** and illustration of the design strategy for structure−activity relationship (SAR) studies.

Scheme 1. Synthesis of Differently Substituted-Benzoxazolone Carboxamides 4−19[a]



37a: R = 4-Br (88%)
37b: R = 5-Br
37c: R = 7-Br (89%)
37d: R = 4-CH_3 (85%)
37e: R = 5-CH_3
37f: R = 6-CH_3
37g: R = 7-CH_3 (74%)
37h: R = 5-F
37i: R = 6-F
37j: R = 5-Cl
37k: R = 6-Cl
37l: R = 5-CF_3 (85%)
37m:R = 6-CF_3 (90%)
37n: R = 5-NO_2 (92%)
37o: R = 6-NO_2 (87%)
37p: R = 5,6-Cl (80%)

4: R = 4-Br (n.o.)[a]
5: R = 5-Br (48%)
6: R = 7-Br (56%)
7: R = 4-CH_3 (24%)
8: R = 5-CH_3 (91%)
9: R = 6-CH_3 (68%)
10: R = 7-CH_3 (71%)
11: R = 5-F (85%)
12: R = 6-F (79%)
13: R = 5-Cl (92%)
14: R = 6-Cl (67%)
15: R = 5-CF_3 (70%)
16: R = 6-CF_3 (60%)
17: R = 5-NO_2 (61%)
18: R = 6-NO_2 (60%)
19: R = 5,6-Cl (90%)

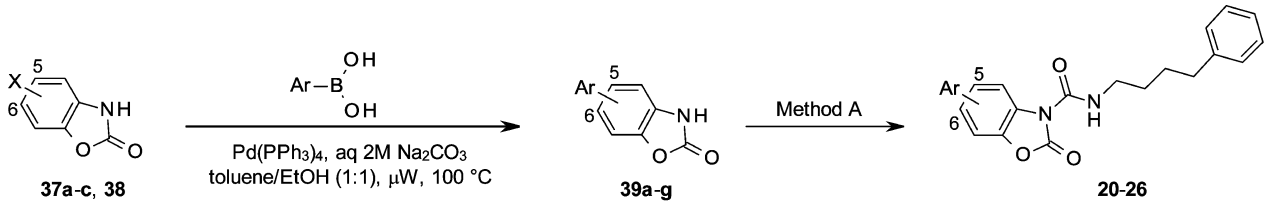
[a]Method A: 4-phenylbutyl isocyanate, DMAP, pryidine. n.o.: not obtained.

phenylbutyl)-1,3-benzoxazole-3-carboxamide (**3**, ARN14974)[12] allows for balancing potency (IC_{50} = 79 nM) with enhanced chemical and metabolic stability (Figure 2). We found that compound **3** inhibits AC in intact cells and in vivo, causing a substantial reduction in AC activity in multiple organs and resulting in expected changes of ceramide and sphingosine levels.

In the present study we expanded our initial SAR study around compound **1** with the objective of demonstrating whether the benzoxazolone carboxamide scaffold is generally capable of providing potent AC inhibitors. We synthesized a series of benzoxazolone carboxamides to explore the roles of both position and stereoelectronic properties of substituents on the

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

Scheme 2. Synthesis of Phenyl-Substituted Benzoxazolone Carboxamides 20−26



37a-c, 38

37a: X = 4-Br
37b: X = 5-Br
37c: X = 7-Br
38: X = 6-Br

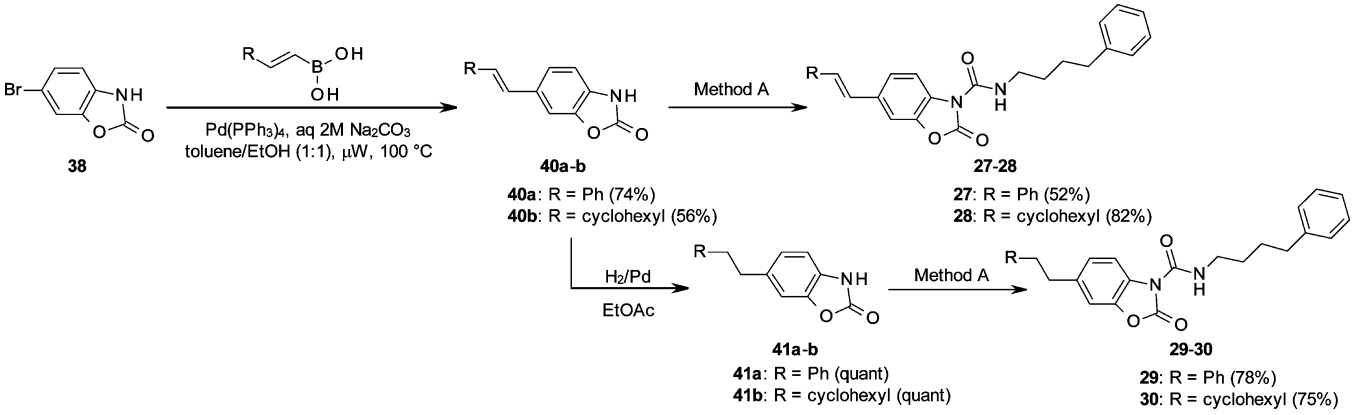
39a-g

39a: Ar = 4-Ph
39b: Ar = 5-Ph (19%)
39c: Ar = 6-Ph (37%)
39d: Ar = 7-Ph
39e: Ar = 5-(*p*-F-Ph) (30%)
39f: Ar = 5-(*p*-OCH_3-Ph) (45%)
39g: Ar = 6-(*p*-OCH_3-Ph) (40%)

20-26

20: Ar = 4-Ph (6% over 2 steps)
21: Ar = 5-Ph (63%)
22: Ar = 6-Ph (62%)
23: Ar = 7-Ph (18% over 2 steps)
24: Ar = 5-(*p*-F-Ph) (58%)
25: Ar = 5-(*p*-OCH_3-Ph) (47%)
26: Ar = 6-(*p*-OCH_3-Ph) (84%)

Scheme 3. Synthesis of 6-Substituted Benzoxazolone Carboxamides 27−30



38

40a-b

40a: R = Ph (74%)
40b: R = cyclohexyl (56%)

27-28

27: R = Ph (52%)
28: R = cyclohexyl (82%)

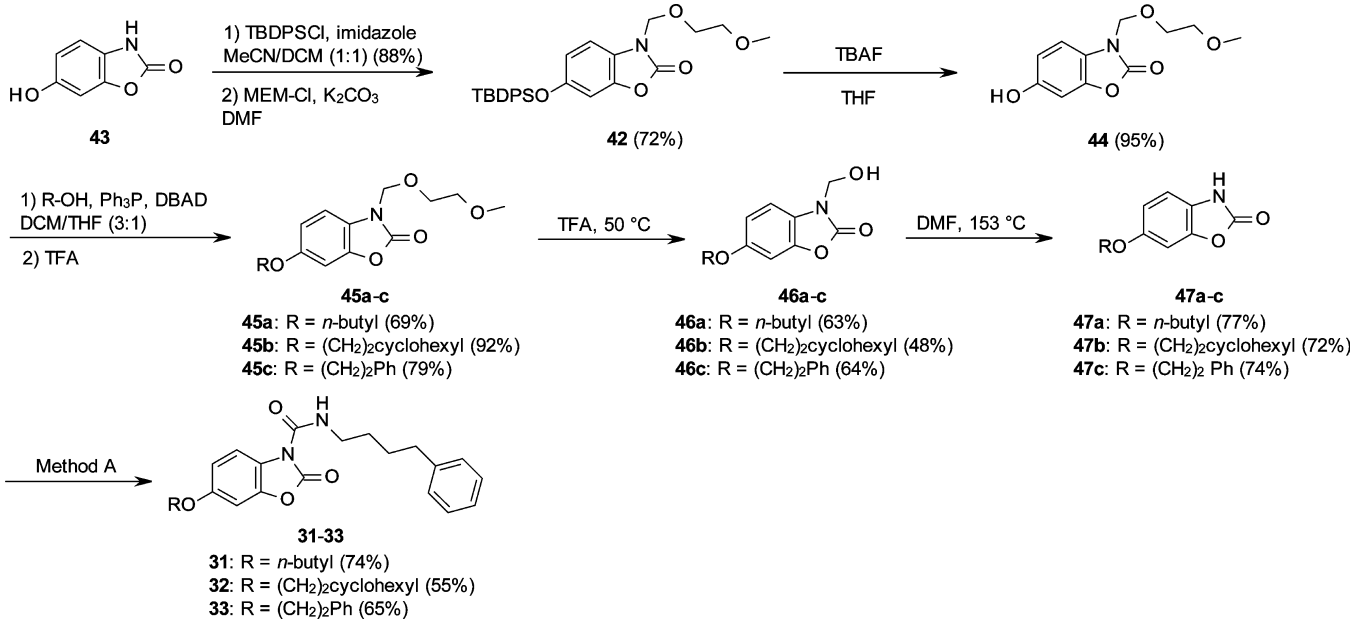
41a-b

41a: R = Ph (quant)
41b: R = cyclohexyl (quant)

29-30

29: R = Ph (78%)
30: R = cyclohexyl (75%)

Scheme 4. Synthesis of 6-Alkoxybenzoxazolone Carboxamides 31−33



43

42 (72%)

44 (95%)

45a-c

45a: R = *n*-butyl (69%)
45b: R = $(CH_2)_2$cyclohexyl (92%)
45c: R = $(CH_2)_2$Ph (79%)

46a-c

46a: R = *n*-butyl (63%)
46b: R = $(CH_2)_2$cyclohexyl (48%)
46c: R = $(CH_2)_2$Ph (64%)

47a-c

47a: R = *n*-butyl (77%)
47b: R = $(CH_2)_2$cyclohexyl (72%)
47c: R = $(CH_2)_2$ Ph (74%)

31-33

31: R = *n*-butyl (74%)
32: R = $(CH_2)_2$cyclohexyl (55%)
33: R = $(CH_2)_2$Ph (65%)

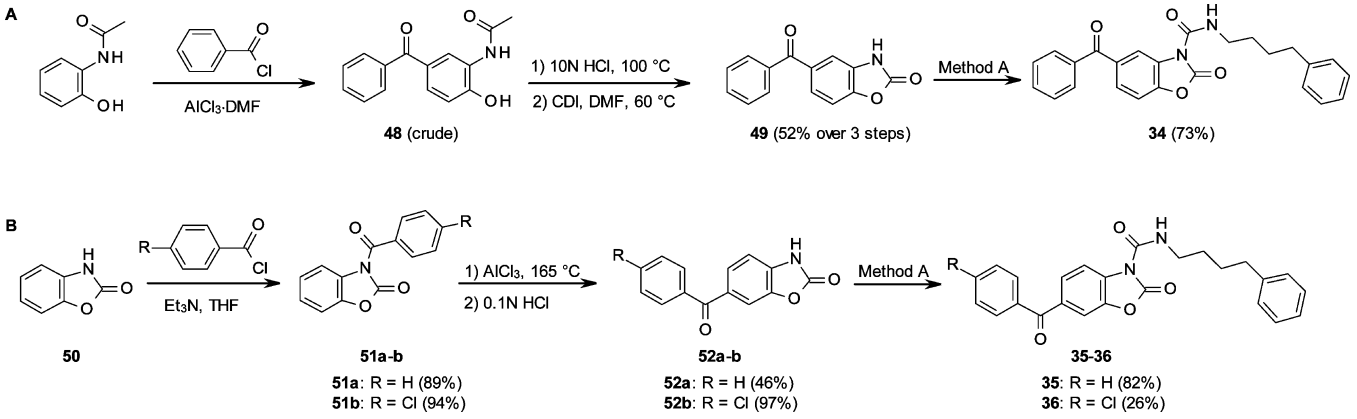
heterocyclic scaffold, as well as the effect of side chain modifications on AC inhibition (Figure 2). Finally, we tested selected derivatives for their stability in aqueous buffer and demonstrated in vivo AC inhibitory activity of the optimized compound, 6-chloro-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (**14**).

CHEMISTRY

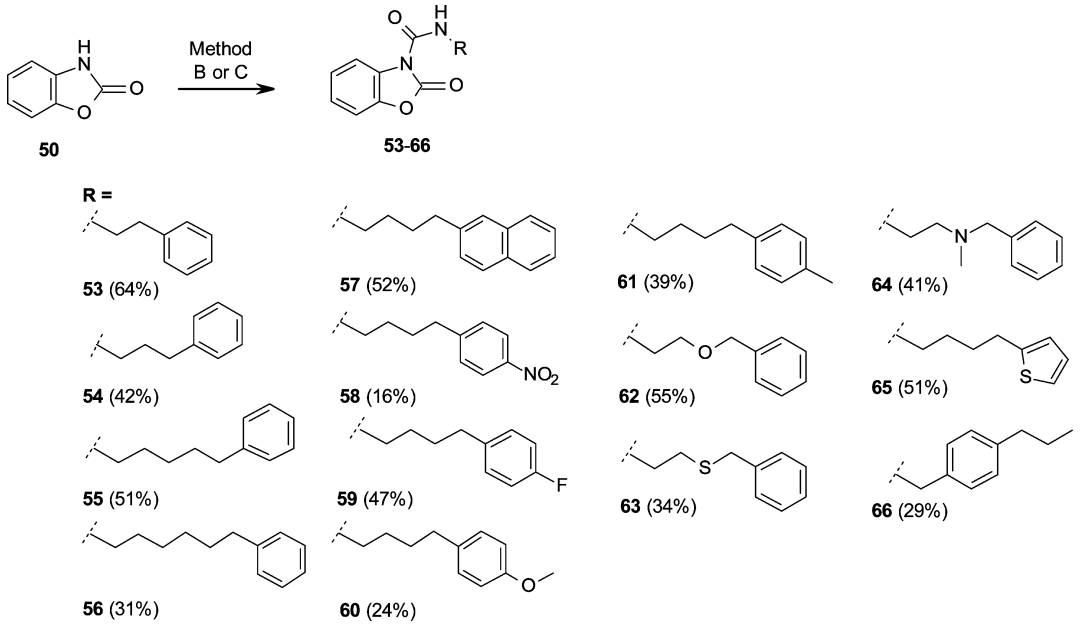
A series of benzoxazolone carboxamide derivatives (**4**−**36**) bearing different substituents on the bicyclic ring system were synthesized, as outlined in Schemes 1−5. In analogy to hit compound **1**, we initially coupled substituted benzoxazolones with the commercial 4-phenylbutyl isocyanate in the presence of

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

Scheme 5. Synthesis of Benzoylbenzoxazolone Carboxamides 34−36



Scheme 6. Synthesis of Benzoxazolone Carboxamide Derivatives 53−66[a]



[a]Method B: (1) triphosgene, pyridine; (2) R-NH_2 (**67e**, **67l−n**), pyridine. Method C: (1) triphosgene, Et_3N, DCM; (2) R-NH_2 (**67e−d**, **67f−k**), Et_3N, DCM.

4-dimethylaminopyridine (DMAP), under basic conditions (method A).[13] First, we prepared a set of derivatives (**4−19**) bearing groups with different stereoelectronic properties on the heterocyclic ring (Scheme 1). Coupling reactions proceeded smoothly to give the final carboxamides **4−19** in good yield, with the exception of compounds **4** and **7**, for which no or low conversion was observed, seemingly due to the substituent at position 4 leading to chemical instability. In most cases, the starting benzoxazolone scaffolds were commercially available (**37b**, **37e,f**, **37h,k**); alternatively the properly substituted heterocycles (**37a**, **37c,d**, **37g**, **37l−p**) were obtained from the corresponding 2-aminophenols by intramolecular cyclization reaction in the presence of 1,1′-carbonyldiimidazole (CDI) (Scheme 1).[14]

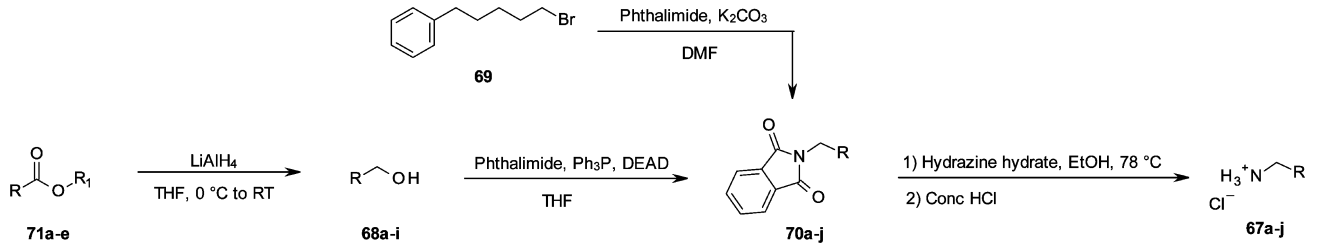
Second, we introduced aromatic substituents on the benzoxazolone scaffold by Suzuki−Miyaura coupling reaction between the appropriate bromo heterocycles (**37a−c**, **38**) and commercially available boronic acids (Scheme 2). Couplings at positions 5 and 6 of the benzoxazolone system were accomplished in satisfactory yields, while the reactions to get 4- and 7-phenylbenzoxazolones **39a** and **39d** led to a complex mixture, and the intermediates were used as crudes in the following step. N-acylation of **39a−g** with 4-phenylbutyl isocyanate yielded the targeted compounds **20−26**.

Next, we synthesized a small set of benzoxazolone carboxamide derivatives (**27−30**) with alkyl/alkenyl-cyclohexyl/phenyl groups at position 6. The appropriate vinylboronic acid was reacted with 6-bromobenzoxazolone **38** under Suzuki−Miyaura reaction conditions to afford the corresponding alkenylic derivatives (**40a,b**) in good yields. Hydrogenation of the double bond yielded compounds **41a,b** quantitatively. Coupling of **40a,b** and **41a,b** to 4-phenylbutyl isocyanate gave the targeted derivatives **27**,**28** and **29**,**30**, respectively, in good yield (Scheme 3).

To further expand the series of 6-substituted derivatives, compounds **31−33** bearing electron-donating and bulky alkoxy groups were prepared as shown in Scheme 4. The benzoxazolone **42** was obtained by orthogonal protection of the commercial 6-hydroxy derivative **43** (Scheme 4).[15] *O*-Silyl group cleavage by treatment with tetrabutylammonium fluoride (TBAF) gave the

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

Scheme 7. Synthesis of Amines 67a−j[a]



71a: R = $(CH_2)_4$naphtyl, R_1 = CH_3
71b: R = $(CH_2)_4$*p*-F-Ph, R_1 = H
71c: R = $(CH_2)_4$*p*-CH_3-Ph, R_1 = H
71d: R = $(CH_2)_4$thiophenyl, R_1 = H
71e: R = $CH_2Ph(CH_2)_2CH_3$, R_1 = H

68a: R = $(CH_2)_4$naphtyl
68b: R = $(CH_2)_4$*p*-F-Ph
68c: R = $(CH_2)_4$*p*-CH_3-Ph
68d: R = $(CH_2)_4$thiophenyl
68e: R = $Bn(CH_2)_2CH_3$
68f: R = $(CH_2)_6Ph$
68g: R = $(CH_2)_4$*p*-NO_2Ph
68h: R = $(CH_2)_4$*p*-OCH_3Ph
68i: R = $(CH_2)_2NCH_3Bn$

70a: R = $(CH_2)_4$naphtyl (37% over two steps)
70b: R = $(CH_2)_4$*p*-F-Ph (70% over two steps)
70c: R = $(CH_2)_4$*p*-CH_3-Ph (52% over two steps)
70d: R = $(CH_2)_4$thiophenyl (76% over two steps)
70e: R = $Bn(CH_2)_2CH_3$ (63% over two steps)
70f: R = $(CH_2)_6Ph$ (78%)
70g: R = $(CH_2)_4$*p*-NO_2Ph (64%)
70h: R = $(CH_2)_4$*p*-OCH_3Ph (77%)
70i: R = $(CH_2)_2NCH_3Bn$ (63%)
70j: R = $(CH_2)_5Ph$

67a: R = $(CH_2)_4$naphtyl
67b: R = $(CH_2)_4$*p*-F-Ph
67c: R = $(CH_2)_4$*p*-CH_3-Ph
67d: R = $(CH_2)_4$thiophenyl
67e: R = $Bn(CH_2)_2CH_3$
67f: R = $(CH_2)_6Ph$
67g: R = $(CH_2)_4$*p*-NO_2Ph
67h: R = $(CH_2)_4$*p*-OCH_3Ph
67i: R = $(CH_2)_2NCH_3Bn$
67j: R = $(CH_2)_5Ph$

67k: R = $(CH_2)_2Ph$
67l: R = $(CH_2)_3Ph$
67m:R = $(CH_2)_2OBn$
67n: R = $(CH_2)_2SBn$

[a]Yields are not reported when compounds were used as crude in the following step. Alcohols **68f−i** and amines **67k−n** were commercially available.

N-protected 6-hydroxy derivative **44**, which was readily alkylated with commercial alcohols under Mitsunobu reaction conditions, using triphenylphosphine and di-*tert*-butyl azodicarboxylate (DBAD). DBAD was degraded under mild conditions in trifluoroactic acid (TFA),[16] affording N-protected 6-alkoxybenzoxazolones **45a−c** in good yield (Scheme 4). Cleavage of the methoxyethoxymethyl (MEM) group with refluxing TFA[15] led only to partial deprotection (**46a−c**); however, subsequent treatment in dimethylformamide (DMF) at 153 °C provided efficient and clean reaction conditions for obtaining benzoxazolones **47a−c**. Coupling with 4-phenylbutyl isocyanate delivered the desired final compounds **31−33** (Scheme 4).

Scheme 5 shows the syntheses of benzoxazolone carboxamides **34−36** bearing a benzoyl group at either position 5 or position 6. Acylation of the benzoxazolone ring could be efficiently achieved by means of Friedel−Crafts reaction. To get compound **34**, the commercial *N*-acetyl-2-aminophenol was treated with a mild electrophile, such as the complex $AlCl_3$·DMF in the presence of benzoyl chloride to regioselectively acylate position 4 (Scheme 5A). Subsequent *N*-acetyl group cleavage and ring closure with CDI afforded the targeted 5-benzoyl-3*H*-1,3-benzoxazol-2-one **49**, which was then coupled to 4-phenylbutyl isocyanate. To obtain compounds **35** and **36**, the unsubstituted benzoxazolone **50** was first N-acylated with benzoyl chloride or 4-chlorobenzoyl chloride to give **51a** and **51b**, respectively (Scheme 5B). Heating in the presence of $AlCl_3$ promoted the migration of acyl group from the nitrogen to the carbon atom at position 6 (**52a,b**), according to a "Fries-like" rearrangement mechanism.[17] Final N-acylation of nitrogen N3 with 4-phenylbutyl isocyanate led to the targeted compounds.

Finally, a series of unsubstituted benzoxazolone derivatives with different side chains at the carboxamide moiety was accessed as depicted in Scheme 6. Compounds **53−66** were obtained by activating benzoxazolone **50** with triphosgene, either in pyridine (method B) (**54**, **62**, **63**, **66**) or in DCM in the presence of Et_3N (method C) (**53**, **55−61**, **64**, **65**), and in situ quenching with the proper amine (**67a−n**) to incorporate the desired side chains (Scheme 6). Noncommercial amines (**67a−j**) were synthesized through a two-step sequence starting from alcohols **68a−i** and bromide **69** under Mitsunobu and Gabriel reaction conditions, respectively (Scheme 7). Hydrazine-mediated cleavage of phthalimide derivatives **70a−j** led to the desired amines as hydrochloride salts. Noncommercial alcohols **68a−e** were readily obtained by reduction of the corresponding ester (**71a**) or acids (**71b−e**).

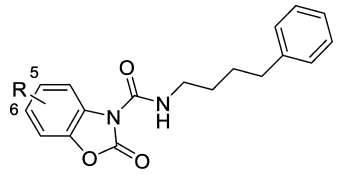
RESULTS AND DISCUSSION

We recently disclosed benzoxazolone carboxamides as the first class of potent and systemically active inhibitors of intracellular AC.[12] We demonstrated that these compounds covalently inhibit AC and identified derivative **3** as the first chemical probe that may be used to study the impact of AC inhibition in vivo (Figure 2). A preliminary SAR study around hit compound **1** revealed that a secondary carboxamide moiety is mandatory for activity, and substitution at position 6 of the benzoxazolone ring allows for modulation of the inhibitory potency against AC. These results prompted us to expand the class of benzoxazolone carboxamides by systematically investigating the effects of different substituents on the heterocyclic scaffold as well as of modifications of the side chain. The new compounds were tested for their ability to inhibit the hydrolysis of *N*-[(1*S*,2*R*)-2-hydroxy-1-(hydroxymethyl)-4-(2-oxochromen-7-yl)oxybutyl]-dodecanamide by recombinant human AC (h-AC) in a fluorescence-based assay.[18]

To probe for stereoelectronic effects on the benzoxazolone system, we initially monosubstituted **1** with bromine or methyl groups at position 4, 5, 6, or 7 (Table 1). As previously observed with the 6-bromo derivative **2** (IC_{50} = 31 nM), introduction of bromine at either position 5 or 7 led to a 2- to 4-fold increase in potency relative to **1** (**5**, IC_{50} = 22 nM, and **6**, IC_{50} = 18 nM). Bromine introduction at position 4 yielded the unstable derivative **4**, and the same result was obtained when a methyl group was introduced at the same position (**7**). As for the small set of methyl-substituted derivatives, with the exception of compound **10** bearing a methyl at position 7 and having an IC_{50} of 23 nM, the introduction of this group led to a decrease in potency when the methyl was placed in position 5 (**8**, IC_{50} = 117 nM) or to an equipotent compound, compared to **1**, when the

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

Table 1. Inhibitory Potencies (IC_{50}) of Compounds on Human AC Activity[a]



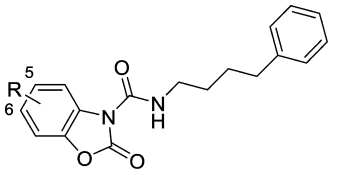
compd	R	IC_{50} ± SEM (nM)
1	H	64 ± 7[b]
2	6-Br	31 ± 9[b]
4	4-Br	nd
5	5-Br	22 ± 11
6	7-Br	18 ± 11
7	4-Me	nd
8	5-Me	117 ± 26
9	6-Me	66 ± 10
10	7-Me	23 ± 5
11	5-F	12 ± 4
12	6-F	31 ± 5
13	5-Cl	15 ± 7
14	6-Cl	33 ± 4
15	5-CF_3	18 ± 2
16	6-CF_3	20 ± 9
17	5-NO_2	3 ± 2
18	6-NO_2	3 ± 1
19	5,6-Cl	18 ± 8

[a]IC_{50} values are reported as mean values of two or more determinations. nd: not determined due to low chemical stability. [b]IC_{50} values from ref 12.

methyl was placed in position 6 (**9**, IC_{50} = 66 nM). Next, we expanded the set of substituted analogs at positions 5 and 6 investigating compounds **11**−**19**, which were 2- to 20-fold more potent than **1** (Table 1). The 5-fluoro derivative **11** (IC_{50} = 12 nM) turned out to be ∼3-fold more potent than the corresponding 6-substituted analog **12** (IC_{50} = 31 nM). The same trend in potency associated with halide introduction at either position 5 or 6 was observed for the chloro-substituted compounds **13** and **14** (and for the bromine-substituted **5** and **2**). Replacement of halides with strong electron-withdrawing groups such as trifluoromethyl and nitro groups diminished the difference in potency between 5- and 6-substituted derivatives (**15**−**18**). Of particular interest were the results obtained with 5-nitrobenzoxazolone carboxamide **17** and the corresponding 6-nitro analog **18**, which represent the first single-digit nanomolar compounds in this class, both inhibiting AC with an IC_{50} value of 3 nM. Finally, we explored whether incorporating simultaneously two favorable substitutions would provide additive effects. We observed that the disubstituted compound **19** was potent (IC_{50} = 18 nM) but not significantly more so than the related monosubstituted analogs **13** and **14** (Table 1).

As a continuation of the SAR study around the benzoxazolone system, we prepared a small series of derivatives bearing a phenyl ring (Table 2). First, we systematically explored the introduction of this moiety at positions 4, 5, 6, and 7. Interestingly, stability issues did not prevent isolation and testing of the 4-phenyl derivative **20**, which turned out to be 75-fold more potent than **1** (IC_{50} = 0.8 nM). Derivatives **21**−**23** bearing a phenyl ring at positions 5, 6, and 7, respectively, were approximately as potent as **1**. Next, we focused on positions 5 and 6 and prepared a small series of aryl-substituted derivatives (**24**−**36**). Overall, decorating the phenyl ring with fluorine or methoxy group in the para position (**3**, **24** and **25**, **26**) did not improve potency over the unsubstituted benzoxazolone derivative **1**. However, replacement of the phenyl with aryl ketones (**34**−**36**) led to 2- to 5-fold increases in potency relative to **1**, with compound **36** (IC_{50} = 14 nM) being the most potent within this small series. Finally, introduction of an alkylic or alkenylic spacer between the benzoxazolone system and the phenyl ring as well as the replacement of the phenyl with a cyclohexyl led to a loss in potency, with compounds **27**−**30** (Scheme 3) showing no inhibitory activity against AC at the concentrations tested (50 and 500 nM). Similarly, compounds **31**−**33** (Scheme 4) bearing various alkoxy groups at position 6 were inactive.

Table 2. Inhibitory Potencies (IC_{50}) of Compounds on Human AC Activity[a]



compd	R	IC_{50} ± SEM (nM)
3	6-(*p*-F-Ph)	79 ± 31[b]
20	4-Ph	0.8 ± 0.1
21	5-Ph	42 ± 21
22	6-Ph	84 ± 35
23	7-Ph	91 ± 10
24	5-(*p*-F-Ph)	56 ± 14
25	5-(*p*-OCH_3-Ph)	67 ± 58
26	6-(*p*-OCH_3-Ph)	67 ± 5
34	5-(COPh)	24 ± 6
35	6-(COPh)	29 ± 1
36	6-CO(*p*-Cl-Ph)	14 ± 4

[a]IC_{50} values are reported as mean values of two or more determinations. [b]IC_{50} values from ref 12.

We next examined the role of the side chain by preparing a series of unsubstituted benzoxazolone carboxamides. First, we evaluated the length of the carbon linker between the benzoxazolone and the phenyl ring of **1**. Compounds **53**−**56**, in which the spacer was progressively increased from two to six methylene units, were tested for their inhibitory activity (Table 3). The short linker with two methylene units led to a 2-fold decrease in potency (**53**, IC_{50} = 121 nM) compared to **1**, while compounds **54**−**56**, which contain a three-, five-, or six-carbon atom linker, respectively, turned out to be as potent as **1**. Replacement of one methylene unit of the four-carbon atom chain of **1** with a heteroatom (**62**−**64**) was detrimental for activity, with the exception of the thioether derivative **63**, which was equipotent to **1**. Next, to evaluate the role of the phenyl ring, we replaced it with a naphthyl (**57**) or thiophene (**65**) group, observing an increase in potency with the latter derivative (**65**, IC_{50} = 37 nM). Introduction of substituents with different electronic properties in the para position of the phenyl ring was generally well tolerated, as shown by compounds **59**−**61**, with the exception of derivative **58** (IC_{50} = 312 nM), which turned out to be 5-fold less potent than **1**. Interestingly, the most potent compound of this series showed the same side chain length as **1** but with the phenyl ring situated one carbon atom from the *N*3-carboxamide group (**66**, IC_{50} = 19 nM).

Previously, we reported that introducing bromine in position 6 (**2**) destabilizes compounds and leads to lower half-life in PBS

DOI: 10.1021/acs.jmedchem.5b01188
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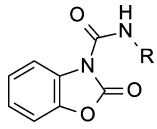
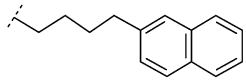
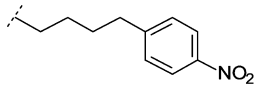
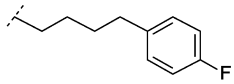
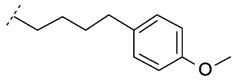
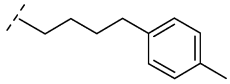
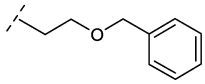
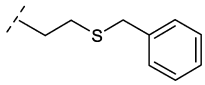
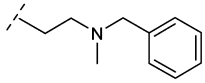
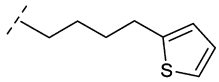
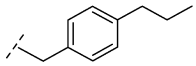
Table 3. Inhibitory Potencies (IC_{50}) of Compounds on Human AC Activity[a]



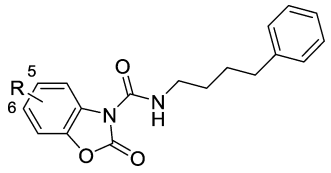
Compounds	R	IC_{50} (nM) ± S.E.M.
1	$(CH_2)_4Ph$	64 ± 7[b]
53	$(CH_2)_2Ph$	121 ± 19
54	$(CH_2)_3Ph$	55 ± 6
55	$(CH_2)_5Ph$	54 ± 12
56	$(CH_2)_6Ph$	51 ± 16
57		71 ± 13
58		312 ± 90
59		75 ± 1
60		63 ± 22
61		25 ± 14
62		649 ± 270
63		65 ± 26
64		No inhibition[c]
65		37 ± 21
66		19 ± 2

[a]IC_{50} values are reported as mean values of two or more determinations. [b]IC_{50} values from ref 12. [c]Tested at 5 μM.

and blood plasma compared to **1** but that having a *p*-fluorophenyl in position 6 (**3**) enhanced chemical and metabolic stability dramatically. Conversely, stability under standard AC assay conditions (pH 4.5) was not affected by these substitutions.[12] In order to gain a more comprehensive understanding of the structure−stability relationship of benzoxazolone carboxamides, compounds **14**, **19**−**26**, and **35**,**36** were tested for stability in aqueous buffer at physiological pH (Table 4). Representative compounds (**14**, **17**, **18**, **21**, **35**, **36**) were also evaluated for their stability at pH 4.5 (Table S1, Supporting Information). At acidic pH, all compounds were stable, as previously observed with derivatives **1**−**3**. The analysis at physiological pH showed that, in contrast to bromine, chlorine in position 6 (**14**) or positions 5 and 6 (**19**) did not induce instability relative to the unsubstituted compound **1**. Aromatic substitutions in positions 5−7 enhanced stability in all cases (**3**, **21**−**26**), with *p*-fluorophenyl in position 6 (**3**) being the most efficient substituent and *p*-methoxyphenyl in the same position (**26**) the least efficient one. Noticeably, incorporating a phenyl group in position 4 resulted in a very unstable compound (**20**), which tallies well with the chemical instability observed with other compounds (**4**, **7**) with substitutions in the same position. Finally, compound **35** with the benzoyl group in position 6 demonstrated enhanced stability, and interestingly its close analog **36** was even more stable with a half-life 6-fold higher than **1** (Table 4). Overall, these results demonstrate that stability properties are not simply explained by the electronegativity of the substituents on the benzoxazolone ring and that increased activity is not necessarily linked with

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

Table 4. Chemical Stability of Compounds 1−3, 14, 19−26, 35, and 36 in PBS (pH 7.4)[a]



compd	R	buffer stability, $t_{1/2}$ (min)
1	H	45[b]
2	6-Br	24[b]
3	6-(*p*-F-Ph)	>300[b]
14	6-Cl	58
19	5,6-Cl	55
20	4-Ph	7
21	5-Ph	296
22	6-Ph	>300
23	7-Ph	>300
24	5-(*p*-F-Ph)	>300
25	5-(*p*-OCH_3-Ph)	176
26	6-(*p*-OCH_3-Ph)	132
35	6-(COPh)	98
36	6-CO(*p*-Cl-Ph)	274

[a]Determined at room temperature by LC−MS analysis. [b]$t_{1/2}$ values from ref 12.

decreased stability. Indeed, we see that it is possible to increase potency without decreasing stability, as exemplified by compounds **14** and **19**, and that it is even possible to enhance both potency and stability relative to **1** as seen for compounds **35** and **36**. However, the most potent compound in this series, compound **20** (IC_{50} = 0.8 nM, Table 2) also showed the lowest half-life, providing a case in which activity and stability are seemingly related.

To test whether the explored structural modifications of the benzoxazolone carboxamide scaffold could also provide novel systemically active compounds, we selected derivative **14** for further pharmacological studies in vivo, based on its balanced profile in terms of potency, stability, solubility, and druglikeness. Indeed, compound **14** shows an improved potency compared to **3** and an adequate solubility in the vehicle used for systemic administration. In this regard, other slightly more potent and stable derivatives of this series, such as compounds **35** and **36**, were found to be poorly soluble and thus difficult to handle in in vivo studies. As additional criteria of selection, we considered the druglikeness of **14** in comparison with other considerably more potent compounds, such as the single-digit nanomolar inhibitors **17** and **18**. The nitro-substituted benzoxazolone ring makes these derivatives important for SAR purposes but not promising as candidates for further development, due to the potential in vivo toxicity of aromatic nitro groups.

Compound **14** was injected in mice at the dose of 10 mg kg^{-1} (ip), and AC activity was evaluated at different time points in lysosomal fractions prepared from lung tissue and cerebral cortex. We selected the lungs because of the high basal AC activity in this tissue, and the cerebral cortex to evaluate the ability of **14** to reach the brain. As shown in Figure 3, the compound significantly inhibited AC activity in both compartments. The effect was long lasting, as AC inhibition was still observed 24 h after administration, although a slight recovery in activity was seen at this time point (Figure 3A and 3C). In the lungs, AC inhibition was accompanied by an increase in the levels of ceramide and dihydroceramide (namely, d18:1/16:0 and d18:0/16:0) for up to 6 h; sphingosine and sphingosine 1-phosphate were also decreased at 3 h (Figure 3B). No statistically significant changes in sphingolipid levels were observed in the cerebral cortex (Figure 3D), presumably owing to the relative low level of AC inhibition achieved in that brain region. The results indicate that **14** engages its intended target in two relevant tissues, with results that are comparable to those previously obtained with compound **3** under the same experimental conditions.[12]

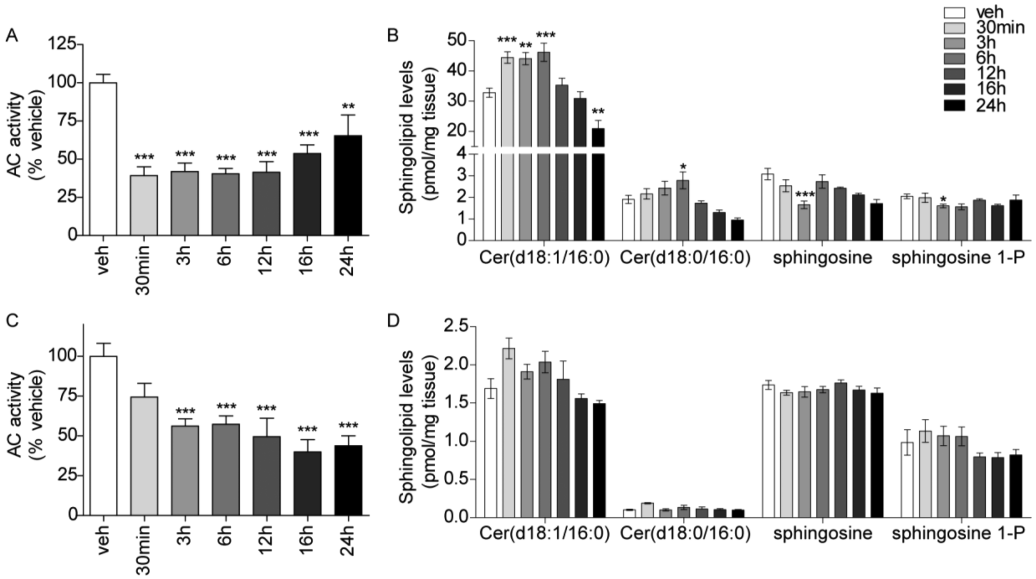


Figure 3. Effects of compound **14** (10 mg kg^{-1}, ip) on AC activity and sphingolipid levels in mice lungs (A, B) and cerebral cortex (C, D) after 30 min, 3 h, 6 h, 12 h, 16 h, and 24 h. Activity is expressed as percentage of vehicle (basal AC activity in lungs, 98 ± 7.1 pmol min^{-1} mg^{-1}; cerebral cortex, 74 ± 8.6 pmol min^{-1} mg^{-1}). Values are reported as the mean ± SEM (n = 6). Repeated experiments gave similar results: (***) $p < 0.001$ vs vehicle, one-way ANOVA followed by Dunnett's test.

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

CONCLUSIONS

The present study describes a systematic SAR investigation of a recently disclosed class of AC inhibitors that features the benzoxazolone carboxamide scaffold.[12] We show that this scaffold is able to provide stable systemically active AC inhibitors and may thus represent a good starting point for further discovery. We present various synthetic approaches to obtain diversified analogs in high yields and with great feasibility. We show that electron-withdrawing groups in positions 5−7 of the benzoxazolone scaffold (**2**, **5**, **6**, **10**−**19**) lead to enhanced inhibitory activity toward AC. This is consistent with the mechanism of action of these agents, as electron-withdrawing groups are likely to make the carboxamide carbonyl more prone to nucleophilic attack by AC's active site cysteine. Introducing a phenyl group at position 4 gave a highly potent but very unstable compound (**20**), which correlates with the chemical instability seen with other molecules substituted in the same position (**4** and **7**). Additionally, we investigated a range of aromatic substitutions on positions 5−7 of the benzoxazolone scaffold (**21**−**26**), which all provide stable compounds but do not improve activity toward AC. Also, we demonstrated that long and bulky aliphatic groups are not favorable for inhibitory activity, as seen with compounds **27**−**30** and **31**−**33**. Instead, we found that substitutions with benzoyl (**34**, **35**) or 4-chlorobenzoyl (**36**) groups combine good potency and high stability: the most favorable of these derivatives, **36**, shows a 5- to 6-fold improvement in AC inhibition relative to **1** and **3** and a 6-fold improvement in stability relative to **1**. Finally, we addressed the role of the carboxamide side chain and demonstrated that the inhibitory potency of **1** could be improved 2- to 3-fold by incorporating the phenyl group within the linker (**66**) or replacing it with thiophene (**65**). On the basis of potency, stability, and adequate solubility, we chose to investigate compound **14** for in vivo activity. We show that following systemic administration in mice, this compound inhibits AC in peripheral tissues as well as in the brain, leading to the expected variations in the sphingolipid profile. This provides essential evidence that AC inhibition can be successfully obtained in live animals using potent and reasonably stable benzoxazolone carboxamides. Compound **14** might be used as a tool to unravel the biological role of AC and define the potential value of this enzyme as a therapeutic target.

EXPERIMENTAL SECTION

Chemistry. General. All commercial available reagents and solvents were used as purchased from vendors without further purification. Dry solvents (pyridine, DCM) were purchased from Sigma-Aldrich. Automated column chromatography purifications were done using a Teledyne ISCO apparatus (CombiFlash Rf) with prepacked silica gel columns of different sizes (from 4 g up to 40 g). Mixtures of increasing polarity of cyclohexane and ethyl acetate (EtOAc) were used as eluents (unless otherwise stated). Microwave heating was performed using Explorer-48 positions instrument (CEM). Hydrogenation reactions were performed using H-Cube continuous hydrogenation equipment (SS-reaction line version), employing disposable catalyst cartridges (CatCart) preloaded with the required heterogeneous catalyst. NMR experiments were run on a Bruker Avance III 400 system (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C), equipped with a BBI probe and Z-gradients. Spectra were acquired at 300 K, using deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as solvents. Chemical shifts for ^{1}H and ^{13}C spectra were recorded in parts per million using the residual nondeuterated solvent as the internal standard (for $CDCl_3$, 7.26 ppm, ^{1}H, and 77.16 ppm, ^{13}C; for DMSO-d_6, 2.50 ppm, ^{1}H, and 39.52 ppm, ^{13}C). UPLC−MS analyses were run on a Waters ACQUITY UPLC−MS system consisting of a SQD (single quadrupole detector) mass spectrometer equipped with an electrospray ionization interface and a photodiode array detector. PDA range was 210−400 nm. Analyses were performed on an ACQUITY UPLC HSS T3 C_{18} column (50 mm × 2.1 mm i.d., particle size 1.8 μm) with a VanGuard HSS T3 C_{18} precolumn (5 mm × 2.1 mm i.d., particle size 1.8 μm). Mobile phase was either 10 mM NH_4OAc in H_2O at pH 5 adjusted with AcOH (A) or 10 mM NH_4OAc in MeCN−H_2O (95:5) at pH 5 (B). Electrospray ionization in positive and negative mode was applied. All final compounds showed ≥95% purity by NMR (^{1}H, ^{13}C, ^{1}H−^{1}H COSY, ^{1}H−^{13}C HSQC) and UPLC−MS (UV). DMSO stock solutions of final compounds (10 mM) used for biological tests were evaluated prior to tests (NMR, LC−MS), and concentration was assessed by quantitative ^{1}H NMR.

General Procedure for the Synthesis of Final Benzoxazolone *N*-Carboxamides 4−36 via Method A (Schemes 1−5 and Tables 1 and 2). The properly substituted 3*H*-1,3-benzoxazol-2-one (1.0 equiv) was dissolved in dry pyridine (6 mL per mmol benzoxazol-2-one). DMAP (1.1 equiv) was added and the reaction mixture stirred under nitrogen atmosphere at rt for 30 min. 4-Phenylbutyl isocyanate (1.1 equiv) was added and the resulting mixture was stirred for 15 h. The solvent was removed under reduced pressure, and the compound was purified by silica gel column chromatography using the Teledyne ISCO apparatus (cyclohexane/EtOAc).

5-Bromo-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (5). The reaction was carried out following method A, using **37b** (100 mg, 0.467 mmol) and 4-phenylbutyl isocyanate (90 mg, 0.088 mL, 0.514 mmol). White solid (88 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.77 (m, 4H), 2.67 (t, J = 7.2 Hz, 2H), 3.44 (td, J = 6.8, 5.6 Hz, 2H), 7.11 (d, J = 8.6 Hz, 1H), 7.16−7.21 (m, 3H), 7.26−7.31 (m, 2H), 7.37 (dd, J = 8.6, 2.1 Hz, 1H), 7.97 (t, J = 4.6 Hz, 1H), 8.27 (d, J = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.1, 35.6, 40.4, 111.3, 117.9, 119.0, 126.0, 127.6, 128.5 (4C), 129.2, 140.8, 142.0, 149.5, 152.9. MS (ESI) m/z: 212 and 214 [M − $CONH(CH_2)_4Ph$]$^-$.

7-Bromo-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (6). The reaction was carried out following method A, using **37c** (140 mg, 0.654 mmol) and 4-phenylbutyl isocyanate (126 mg, 0.123 mL, 0.720 mmol). White solid (143 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61−1.78 (m, 4H), 2.67 (t, J = 7.1 Hz, 2H), 3.44 (q, J = 6.4 Hz, 2H), 7.13−7.21 (m, 4H), 7.26−7.31 (m, 2H), 7.38 (dd, J = 8.3, 1.1 Hz, 1H), 7.99 (t, J = 6.0 Hz, 1H), 8.02 (dd, J = 8.2, 1.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.1, 35.6, 40.4, 102.4, 114.7, 126.0, 126.2, 128.0, 128.5 (4C), 129.0, 140.0, 142.0, 149.6, 152.4. MS (ESI) m/z: nonionizable compound under routine conditions used.

4-Methyl-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (7). The reaction was carried out following method A, using **37d** (94 mg, 0.630 mmol) and 4-phenylbutyl isocyanate (121 mg, 0.119 mL, 0.693 mmol). Clear colorless oil (49 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.79 (m, 4H), 2.53 (s, 3H), 2.68 (t, J = 7.2 Hz, 2H), 3.45 (q, J = 6.6 Hz, 2H), 7.04−7.09 (m, 2H), 7.11−7.21 (m, 4H), 7.26−7.31 (m, 2H), 7.60 (t, J = 4.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.5, 28.6, 29.1, 35.6, 40.9, 107.6, 124.8, 126.0, 126.4, 126.6, 128.4, 128.5 (2C), 128.5 (2C), 142.1, 143.0, 149.3, 154.0. MS (ESI) m/z: 325 [M + H]$^+$, 342 [M + NH_4]$^+$.

5-Methyl-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (8). The reaction was carried out following method A, using **37e** (50 mg, 0.334 mmol) and 4-phenylbutyl isocyanate (64 mg, 0.063 mL, 0.367 mmol). White solid (99 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.77 (m, 4H), 2.41 (s, 3H), 2.67 (t, J = 7.2 Hz, 2H), 3.44 (td, J = 6.7, 5.5 Hz, 2H), 7.00−7.04 (m, 1H), 7.08−7.11 (m, 1H), 7.16−7.21 (m, 3H), 7.26−7.30 (m, 2H), 7.90 (br s, 1H), 8.08 (t, J = 4.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.7, 28.7, 29.2, 35.6, 40.2, 109.5, 116.1, 125.0, 126.0, 128.0, 128.5 (2C), 128.5 (2C), 135.2, 139.9, 142.0, 150.1, 153.6. MS (ESI) m/z: 325 [M + H]$^+$, 342 [M + NH_4]$^+$.

6-Methyl-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (9). The reaction was carried out following method A, using **37f** (50 mg, 0.334 mmol) and 4-phenylbutyl isocyanate (64 mg, 0.063 mL, 0.367 mmol). White solid (73 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.78 (m, 4H), 2.41 (s, 3H), 2.67 (t, J = 7.2 Hz, 2H), 3.44 (td, J = 6.7, 5.5 Hz, 2H), 7.03−7.08 (m, 2H), 7.15−7.21 (m, 3H), 7.26−

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

7.30 (m, 2H), 7.89−7.92 (m, 1H), 8.04 (t, J = 5.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.6, 28.7, 29.2, 35.6, 40.2, 110.5, 115.2, 125.6, 125.7, 126.0, 128.5 (2C), 128.5 (2C), 135.0, 142.0, 142.1, 150.0, 153.5. MS (ESI) m/z: 325 $[M + H]^+$, 342 $[M + NH_4]^+$.

7-Methyl-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (10). The reaction was carried out following method A, using **37g** (83 mg, 0.556 mmol) and 4-phenylbutyl isocyanate (107 mg, 0.105 mL, 0.612 mmol). White solid (128 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.78 (m, 4H), 2.39 (s, 3H), 2.67 (t, J = 7.1 Hz, 2H), 3.44 (q, J = 6.6 Hz, 2H), 7.04 (d, J = 7.8 Hz, 1H), 7.12−7.20 (m, 4H), 7.26−7.31 (m, 2H), 7.88 (dd, J = 8.0, 1.2 Hz, 1H), 8.09 (t, J = 5.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.5, 28.7, 29.2, 35.6, 40.2, 113.11, 120.5, 124.9, 126.0, 126.1, 127.8, 128.5 (2C), 128.5 (2C), 140.5, 142.0, 150.1, 153.5. MS (ESI) m/z: 325 $[M + H]^+$, 342 $[M + NH_4]^+$.

5-Fluoro-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (11). The reaction was carried out following method A, using **37h** (61 mg, 0.398 mmol) and 4-phenylbutyl isocyanate (76.7 mg, 0.075 mL, 0.438 mmol). White solid (111 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.78 (m, 4H), 2.67 (t, J = 7.1 Hz, 2H), 3.44 (q, J = 6.6 Hz, 2H), 6.94 (td, J = 9.0, 2.7 Hz, 1H), 7.15−7.21 (m, 4H), 7.26−7.31 (m, 2H), 7.85 (dd, J = 8.5, 2.7 Hz, 1H), 8.01 (t, J = 6.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.6, 29.1, 35.5, 40.3, 104.3 (d, J = 31.3 Hz), 110.5 (d, J = 9.5 Hz), 111.2 (d, J = 25.1 Hz), 126.0, 128.5 (4C), 128.7 (d, J = 14.0 Hz), 137.8 (d, J = 2.2 Hz), 142.0, 149.5, 153.4, 159.8 (d, J = 242.4 Hz). MS (ESI) m/z: 152 $[M - CONH(CH_2)_4Ph]^-$.

6-Fluoro-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (12). The reaction was carried out following method A, using **37i** (51 mg, 0.334 mmol) and 4-phenylbutyl isocyanate (64 mg, 0.063 mL, 0.367 mmol). White solid (87 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.78 (m, 4H), 2.67 (t, J = 7.2 Hz, 2H), 3.44 (td, J = 6.7, 5.6 Hz, 2H), 6.96−7.04 (m, 2H), 7.16−7.21 (m, 3H), 7.25−7.31 (m, 2H), 7.96 (t, J = 5.7 Hz, 1H), 8.00−8.05 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.1, 35.6, 40.3, 99.1 (d, J = 28.8 Hz), 111.8 (d, J = 23.4 Hz), 116.3 (d, J = 9.0 Hz), 124.3 (d, J = 2.6 Hz), 126.0, 128.5 (4C), 141.8, 142.0 (d, J = 2.3 Hz), 149.6, 153.2, 159.7 (d, J = 245.2 Hz). MS (ESI) m/z: 329 $[M + H]^+$, 346 $[M + NH_4]^+$.

5-Chloro-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (13). The reaction was carried out following method A, using **37j** (68 mg, 0.401 mmol) and 4-phenylbutyl isocyanate (76.7 mg, 0.075 mL, 0.438 mmol). Clear colorless oil (127 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.79 (m, 4H), 2.67 (t, J = 7.1 Hz, 2H), 3.44 (td, J = 6.7, 5.5 Hz, 2H), 7.13−7.24 (m, 5H), 7.26−7.31 (m, 2H), 7.98 (t, J = 5.2 Hz, 1H), 8.12 (d, J = 2.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.1, 35.6, 40.4, 110.8, 116.3, 124.7, 126.0, 128.5 (4C), 128.9, 130.8, 140.3, 142.0, 149.5, 153.0. MS (ESI) m/z: 345 and 347 $[M + H]^+$.

6-Chloro-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (14). The reaction was carried out following method A, using **37k** (56 mg, 0.334 mmol) and 4-phenylbutyl isocyanate (64 mg, 0.063 mL, 0.367 mmol). White solid (77 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.78 (m, 4H), 2.67 (t, J = 7.2 Hz, 2H), 3.44 (td, J = 6.7, 5.6 Hz, 2H), 7.16−7.20 (m, 3H), 7.24−7.31 (m, 4H), 7.93−8.02 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.1, 35.6, 40.3, 110.9, 116.4, 125.4, 126.1, 126.8, 128.5 (4C), 130.2, 142.0, 142.1, 149.6, 152.9. MS (ESI) m/z: 168 and 170 $[M - CONH(CH_2)_4Ph]^-$.

2-Oxo-*N*-(4-phenylbutyl)-5-(trifluoromethyl)-1,3-benzoxazole-3-carboxamide (15). The reaction was carried out following method A, using **37l** (128 mg, 0.630 mmol) and 4-phenylbutyl isocyanate (121 mg, 0.119 mL, 0.693 mmol). White solid (166 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.46 (q, J = 6.5 Hz, 2H), 7.16−7.21 (m, 3H), 7.26−7.31 (m, 2H), 7.34 (d, J = 8.5 Hz, 1H), 7.53−7.57 (m, 1H), 7.96 (t, J = 5.7 Hz, 1H), 8.40 (d, J = 1.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.1, 35.5, 40.4, 110.2, 113.5 (q, J = 4.0 Hz), 122.2 (q, J = 3.9 Hz), 123.8 (q, J = 272.0 Hz), 126.0, 127.8 (q, J = 33.3 Hz), 128.5, 128.5 (4C), 142.0, 143.8, 149.4, 152.8. MS (ESI) m/z: 379 $[M + H]^+$, 396 $[M + NH_4]^+$.

2-Oxo-*N*-(4-phenylbutyl)-6-(trifluoromethyl)-1,3-benzoxazole-3-carboxamide (16). The reaction was carried out following method A, using **37m** (81 mg, 0.399 mmol) and 4-phenylbutyl isocyanate (76.7 mg, 0.075 mL, 0.438 mmol). White solid (91 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.79 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.46 (q, J = 6.6 Hz, 2H), 7.15−7.21 (m, 3H), 7.26−7.31 (m, 2H), 7.51 (d, J = 1.7 Hz, 1H), 7.55−7.59 (m, 1H), 7.98 (t, J = 5.9 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.6, 29.1, 35.6, 40.4, 107.6 (q, J = 4.0 Hz), 115.9, 122.6 (q, J = 3.9 Hz), 123.7 (q, J = 272.1 Hz), 126.0, 127.2 (q, J = 33.3 Hz), 128.5 (4C), 130.9, 141.6, 141.9, 149.3, 152.8. MS (ESI) m/z: 202 $[M - CONH(CH_2)_4Ph]^-$.

5-Nitro-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (17). The reaction was carried out following method A, using **37n** (124 mg, 0.688 mmol) and 4-phenylbutyl isocyanate (133 mg, 0.130 mL, 0.757 mmol). White solid (150 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.79 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.47 (td, J = 6.7, 5.6 Hz, 2H), 7.16−7.21 (m, 3H), 7.26−7.31 (m, 2H), 7.37 (d, J = 8.9 Hz, 1H), 7.88 (t, J = 5.3 Hz, 1H), 8.24 (dd, J = 8.9, 2.4 Hz, 1H), 8.98 (d, J = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.6, 29.1, 35.5, 40.5, 110.2, 112.0, 121.1, 126.1, 128.5 (2C), 128.5 (2C), 128.6, 141.9, 145.3, 145.6, 148.9, 152.6. MS (ESI) m/z: 354 $[M + H]^+$.

6-Nitro-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (18). The reaction was carried out following method A, using **37o** (72 mg, 0.400 mmol) and 4-phenylbutyl isocyanate (76.7 mg, 0.075 mL, 0.438 mmol). White solid (86 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.79 (m, 4H), 2.68 (t, J = 7.0 Hz, 2H), 3.47 (q, J = 6.4 Hz, 2H), 7.15−7.21 (m, 3H), 7.26−7.31 (m, 2H), 7.94 (t, J = 5.8 Hz, 1H), 8.15 (t, J = 1.2 Hz, 1H), 8.23−8.28 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.6, 29.0, 35.5, 40.5, 106.3, 115.6, 121.5, 126.1, 128.5 (2C), 128.6 (2C), 133.3, 141.3, 141.9, 144.7, 149.0, 152.7. MS (ESI) m/z: nonionizable compound under routine conditions used.

5,6-Dichloro-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (19). The reaction was carried out following method A, using **37p** (121 mg, 0.593 mmol) and 4-phenylbutyl isocyanate (114 mg, 0.112 mL, 0.652 mmol). White solid (203 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.78 (m, 4H), 2.67 (t, J = 7.1 Hz, 2H), 3.44 (td, J = 6.7, 5.5 Hz, 2H), 7.16−7.21 (m, 3H), 7.26−7.31 (m, 2H), 7.37 (s, 1H), 7.84 (t, J = 5.8 Hz, 1H), 8.22 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.6, 29.1, 35.6, 40.4, 112.0, 117.3, 126.1, 127.5, 128.5 (4C), 128.6, 129.3, 140.4, 141.9, 149.2, 152.6. MS (ESI) m/z: nonionizable compound under routine conditions used.

2-Oxo-4-phenyl-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (20). The reaction was carried out following method A, using crude **39a** (140 mg) and 4-phenylbutyl isocyanate (127 mg, 0.124 mL, 0.724 mmol). In total, four portions of 4-phenylbutyl isocyanate were added with about 3 h in between. Waxy white solid (18 mg, 6% over steps from **37a**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47−1.58 (m, 2H), 1.59−1.68 (m, 2H), 2.61 (t, J = 7.5 Hz, 2H), 3.20 (td, J = 6.8, 5.7 Hz, 2H), 7.09 (t, J = 5.1 Hz, 1H), 7.15−7.25 (m, 4H), 7.26−7.38 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.5, 29.1, 35.6, 40.8, 108.8, 124.9, 125.0, 126.0, 126.4, 127.3, 127.3 (2C), 127.6, 128.4 (2C), 128.5 (2C), 128.5 (3C), 139.4, 142.1, 143.6, 147.9. MS (ESI) m/z: 387 $[M + H]^+$, 404 $[M + NH_4]^+$.

2-Oxo-5-phenyl-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (21). The reaction was carried out following method A, using **39b** (14.9 mg, 0.0705 mmol) and 4-phenylbutyl isocyanate (33 mg, 0.032 mL, 0.187 mmol). Clear colorless sticky oil (17 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.80 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.46 (q, J = 6.4 Hz, 2H), 7.15−7.21 (m, 3H), 7.25−7.31 (m, 3H), 7.33−7.38 (m, 1H), 7.41−7.47 (m, 3H), 7.57−7.61 (m, 2H), 8.08 (t, J = 5.3 Hz, 1H), 8.34 (d, J = 1.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 40.3, 110.1, 114.2, 123.6, 126.0, 127.5 (2C), 127.7, 128.5 (2C), 128.5 (2C), 128.6, 129.0 (2C), 139.0, 140.4, 141.3, 142.0, 150.0, 153.5. MS (ESI) m/z: 210 $[M - CONH(CH_2)_4Ph]^-$.

2-Oxo-6-phenyl-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22). The reaction was carried out following method A, using **39c** (29 mg, 0.138 mmol) and 4-phenylbutyl isocyanate (29 mg, 0.028 mL, 0.165 mmol). White solid (33 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.79 (m, 4H), 2.68 (t, J = 7.2 Hz, 2H), 3.47 (td, J = 6.7, 5.6 Hz, 2H), 7.16−7.21 (m, 3H), 7.25−7.31 (m, 2H), 7.34−7.40 (m, 1H), 7.43−7.50 (m, 4H), 7.55−7.59 (m, 2H), 8.06 (t, J = 5.3 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 40.3, 108.7, 115.8, 124.1, 126.0, 127.3 (2C), 127.3, 127.9, 128.5

DOI: 10.1021/acs.jmedchem.5b01188
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(2C), 128.6 (2C), 129.1 (2C), 138.6, 140.05, 142.0, 142.4, 149.9, 153.4. MS (ESI) m/z: 387 $[M + H]^+$, 404 $[M + NH_4]^+$.

2-Oxo-7-phenyl-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23). The reaction was carried out following method A, using crude **39d** (399 mg) and 4-phenylbutyl isocyanate (145 mg, 0.141 mL, 0.825 mmol). The reaction was stirred for 68 h. Yellow solid (52 mg, 18% over two steps from **37c**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.80 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.47 (q, J = 6.6 Hz, 2H), 7.16−7.21 (m, 3H), 7.25−7.32 (m, 2H), 7.33−7.36 (m, 1H), 7.38−7.44 (m, 2H), 7.46−7.52 (m, 2H), 7.69−7.73 (m, 2H), 8.07 (dd, J = 7.9, 1.3 Hz, 1H), 8.11 (t, J = 5.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 40.3, 114.6, 124.4, 124.5, 125.4, 126.0, 128.5 (4C), 128.5 (3C), 128.7, 129.0 (2C), 134.4, 139.0, 142.0, 150.0, 153.3. MS (ESI) m/z: 387 $[M + H]^+$, 404 $[M + NH_4]^+$.

5-(4-Fluorophenyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (24). The reaction was carried out following method A, using **39e** (26 mg, 0.113 mmol) and 4-phenylbutyl isocyanate (33 mg, 0.032 mL, 0.187 mmol). White solid (26 mg, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.78 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.46 (q, J = 6.5 Hz, 2H), 7.09−7.16 (m, 2H), 7.17−7.21 (m, 3H), 7.26−7.31 (m, 3H), 7.39 (dd, J = 8.4, 1.9 Hz, 1H), 7.51−7.57 (m, 2H), 8.07 ((t, J = 5.5 Hz, 1H), 8.28 (d, J = 1.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 40.3, 110.2, 114.4, 115.9 (d, J = 21.5 Hz, 2C), 123.5, 126.0, 128.5 (2C), 128.5 (2C), 128.7, 129.1 (d, J = 7.7 Hz, 2C), 136.5, 136.6, 141.3, 142.0, 150.0, 153.4, 162.8 (d, J = 246.9 Hz). MS (ESI) m/z: 228 $[M - CONH(CH_2)_4Ph]^-$.

5-(4-Methoxyphenyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (25). The reaction was carried out following method A, using **39f** (41 mg, 0.170 mmol) and 4-phenylbutyl isocyanate (33 mg, 0.032 mL, 0.187 mmol). White solid (33 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.78 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.46 (q, J = 6.6 Hz, 2H), 3.86 (s, 3H), 6.94−6.99 (m, 2H), 7.15−7.21 (m, 3H), 7.24−7.30 (m, 3H), 7.40 (dd, J = 8.4, 1.9 Hz, 1H), 7.50−7.54 (m, 2H), 8.09 (t, J = 5.3 Hz, 1H), 8.29 (d, J = 1.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 40.3, 55.5, 110.0, 114.1, 114.4 (2C), 123.1, 126.0, 128.5 (2C), 128.5 (2C), 128.6 (2C), 128.6, 132.9, 138.6, 140.9, 142.0, 150.1, 153.5, 159.5. MS (ESI) m/z: 417 $[M + H]^+$, 416 $[M + NH_4]^+$.

6-(4-Methoxyphenyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (26). The reaction was carried out following method A, using **39g** (36 mg, 0.149 mmol) and 4-phenylbutyl isocyanate (29 mg, 0.028 mL, 0.165 mmol). White solid (52 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.78 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.46 (td, J = 6.8, 5.6 Hz, 2H), 3.86 (s, 3H), 6.96−7.01 (m, 2H), 7.15−7.21 (m, 3H), 7.26−7.31 (m, 2H), 7.41 (d, J = 1.5 Hz, 1H), 7.44 (dd, J = 8.4, 1.7 Hz, 1H), 7.48−7.52 (m, 2H), 8.03−8.09 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 40.3, 55.5, 108.2, 114.6 (2C), 115.7, 123.6, 126.0, 126.8, 128.3 (2C), 128.5 (2C), 128.6 (2C), 132.6, 138.2, 142.0, 142.5, 149.9, 153.4, 159.7. MS (ESI) m/z: nonionizable compound under routine conditions used.

2-Oxo-*N*-(4-phenylbutyl)-6-[(*E*)-styryl]-1,3-benzoxazole-3-carboxamide (27). The reaction was carried out following method A, using **40a** (80 mg, 0.337 mmol) and 4-phenylbutyl isocyanate (65 mg, 0.064 mL, 0.371 mmol). White solid (72 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.79 (m, 4H), 2.68 (t, J = 7.1 Hz, 2H), 3.45 (q, J = 6.6 Hz, 2H), 7.09 (br s, 2H), 7.16−7.21 (m, 3H), 7.24−7.31 (m, 3H), 7.35−7.42 (m, 4H), 7.49−7.53 (m, 2H), 8.02 (d, J = 8.2 Hz, 1H), 8.02−8.06 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 40.3, 107.2, 115.7, 124.0, 126.0, 126.7 (2C), 127.3, 127.5, 128.1, 128.5 (2C), 128.6 (2C), 128.9 (2C), 129.6, 134.8, 136.9, 142.0, 142.5, 149.8, 153.4. MS (ESI) m/z: nonionizable compound under routine conditions used.

6-[(*E*)-2-Cyclohexylvinyl]-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (28). The reaction was carried out following method A, using **40b** (58 mg, 0.238 mmol) and 4-phenylbutyl isocyanate (46 mg, 0.045 mL, 0.262 mmol). White solid (81 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13−1.39 (m, 5H), 1.63−1.86 (m, 9H), 2.08−2.18 (m, 1H), 2.67 (t, J = 7.1 Hz, 2H), 3.44 (q, J = 6.4 Hz, 2H), 6.16 (dd, J = 15.9, 6.8 Hz, 1H), 6.33 (dd, J = 15.9, 1.2 Hz, 1H), 7.15−7.27 (m, 5H), 7.26−7.30 (m, 2H), 7.93 (d, J = 8.3 Hz, 1H), 8.04 (t, J = 5.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 26.1 (2C), 26.3, 28.7, 29.2, 33.0 (2C), 35.6, 40.26, 41.3, 106.8, 115.4, 123.3, 126.0, 126.3, 126.6, 128.5 (2C), 128.5 (2C), 135.6, 137.9, 142.0, 142.3, 149.9, 153.5. MS (ESI) m/z: 419 $[M + H]^+$, 436 $[M + NH_4]^+$.

2-Oxo-6-phenethyl-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (29). The reaction was carried out following method A, using **41a** (48 mg, 0.201 mmol) and 4-phenylbutyl isocyanate (46 mg, 0.045 mL, 0.262 mmol). White solid (65 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.80 (m, 4H), 2.70 (t, J = 7.2 Hz, 2H), 2.91−3.03 (m, 4H), 3.46 (q, J = 6.4 Hz, 2H), 7.03 (d, J = 1.6 Hz, 1H), 7.07 (dd, J = 8.2, 1.6 Hz, 1H), 7.14−7.17 (m, 2H), 7.18−7.24 (m, 4H), 7.27−7.33 (m, 4H), 7.95 (d, J = 8.2 Hz, 1H), 8.07 (t, J = 5.7 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 37.9, 38.0, 40.2, 110.0, 115.4, 125.2, 126.0, 126.1, 126.3, 128.5 (2C), 128.6 (4C), 128.6 (2C), 138.1, 141.1, 141.9, 142.1, 150.0, 153.5. MS (ESI) m/z: nonionizable compound under routine conditions used.

6-(2-Cyclohexylethyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (30). The reaction was carried out following method A, using **41b** (42 mg, 0.173 mmol) and 4-phenylbutyl isocyanate (40 mg, 0.033 mL, 0.194 mmol). White solid (55 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87−0.98 (m, 2H), 1.10−1.29 (m, 4H), 1.45−1.56 (m, 2H), 1.62−1.78 (m, 9H), 2.62−2.69 (m, 4H), 3.44 (q, J = 6.6 Hz, 2H), 7.05 (s, 1H), 7.07 (d, J = 1.2 Hz, 1H), 7.15−7.20 (m, 3H), 7.26−7.31 (m, 2H), 7.92 (d, J = 8.2 Hz, 1H), 8.05 (t, J = 5.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 26.4 (2C), 26.8, 28.7, 29.2, 33.3, 33.4 (2C), 35.6, 37.3, 39.5, 40.2, 109.8, 115.3, 125.0, 125.8, 126.0, 128.5 (2C), 128.5 (2C), 140.3, 142.0, 142.1, 150.1, 153.5. MS (ESI) m/z: nonionizable compound under routine conditions used.

6-Butoxy-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (31). The reaction was carried out following method A, using **47a** (49 mg, 0.238 mmol) and 4-phenylbutyl isocyanate (46 mg, 0.045 mL, 0.262 mmol). White solid (67 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (t, J = 7.4 Hz, 3H), 1.44−1.55 (m, 2H), 1.63−1.81 (m, 6H), 2.67 (t, J = 7.2 Hz, 2H), 3.44 (q, J = 6.4 Hz, 2H), 3.95 (t, J = 6.5 Hz, 2H), 6.78 (d, J = 2.3 Hz, 1H), 6.79−6.82 (m, 1H), 7.14−7.21 (m, 3H), 7.26−7.31 (m, 2H), 7.91 (d, J = 8.7 Hz, 1H), 8.00 (t, J = 5.2 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 13.14.0, 19.3, 28.7, 29.2, 31.3, 35.6, 40.2, 68.7, 97.6, 111.1, 116.0, 121.4, 126.0, 128.5 (2C), 128.5 (2C), 142.0, 142.6, 150.0, 153.5, 156.9. MS (ESI) m/z: 383 $[M + H]^+$, 400 $[M + NH_4]^+$.

6-(2-Cyclohexylethoxy)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (32). The reaction was carried out following method A, using **47b** (50 mg, 0.190 mmol) and 4-phenylbutyl isocyanate (37 mg, 0.036 mL, 0.209 mmol). White solid (45 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91−1.04 (m, 2H), 1.10−1.33 (m, 3H), 1.43−1.54 (m, 1H), 1.62−1.80 (m, 11H), 2.67 (t, J = 7.2 Hz, 2H), 3.43 (q, J = 6.4 Hz, 2H), 3.98 (t, J = 6.7 Hz, 2H), 6.77 (d, J = 2.3 Hz, 1H), 6.79−6.82 (m, 1H), 7.15−7.20 (m, 3H), 7.26−7.30 (m, 2H), 7.91 (d, J = 8.7 Hz, 1H), 8.00 (t, J = 5.7 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 26.4 (2C), 26.7, 28.7, 29.2, 33.4 (2C), 34.7, 35.6, 36.6, 40.2, 67.0, 97.6, 111.1, 116.0, 121.4, 126.0, 128.5 (2C), 128.5 (2C), 142.1, 142.6, 150.01, 153.5, 156.9. MS (ESI) m/z: 260 $[M - CONH(CH_2)_4Ph]^-$.

2-Oxo-6-phenethyloxy-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (33). The reaction was carried out following method A, using **47c** (65 mg, 0.254 mmol) and 4-phenylbutyl isocyanate (49 mg, 0.048 mL, 0.279 mmol). White solid (71 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61−1.77 (m, 4H), 2.67 (t, J = 7.2 Hz, 2H), 3.10 (t, J = 7.0 Hz, 2H), 3.43 (q, J = 6.4 Hz, 2H), 4.17 (t, J = 7.0 Hz, 2H), 6.78 (d, J = 2.2 Hz, 1H), 6.79−6.82 (m, 1H), 7.15−7.21 (m, 4H), 7.22−7.36 (m, 6H), 7.91 (d, J = 8.5 Hz, 1H), 7.99 (t, J = 5.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.2, 35.6, 35.8, 40.2, 69.7, 97.8, 111.2, 116.0, 121.7, 126.0, 126.8, 128.5 (2C), 128.5 (2C), 128.7 (2C), 129.1 (2C), 130.4, 138.0, 142.1, 142.6, 150.0, 156.6. MS (ESI) m/z: 431 $[M + H]^+$.

5-Benzoyl-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (34). The reaction was carried out following method A, using **49** (100 mg, 0.418 mmol) and 4-phenylbutyl isocyanate (81 mg, 0.079 mL, 0.460 mmol). White solid (127 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62−1.79 (m, 4H), 2.67 (t, J = 7.2 Hz, 2H), 3.44 (td, J = 6.7 Hz, 2H), 7.14−7.23 (m, 3H), 7.24−7.32 (m, 2H), 7.35 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 7.8 Hz, 2H), 7.56−7.65 (m, 1H), 7.79 (dd, J = 1.5, 8.3 Hz, 3H), 7.98 (t, J = 5.4 Hz, 1H), 8.51 (d, J = 1.7 Hz, 1H). ^{13}C NMR

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

(101 MHz, $CDCl_3$) δ 28.7, 29.4, 35.6, 40.4, 110.0, 117.7, 126.0, 127.4, 128.0, 128.5 (4C), 128.6 (2C), 130.1 (2C), 132.8, 135.1, 137.4 142.0, 144.5, 149.5, 153.1, 195.2. MS (ESI) *m/z*: 415 $[M + H]^+$, 432 $[M + NH_4]^+$.

6-Benzoyl-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (35). The reaction was carried out following method A, using **52a** (100 mg, 0.418 mmol) and 4-phenylbutyl isocyanate (81 mg, 0.079 mL, 0.461 mmol). White solid (142 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61−1.79 (m, 4H), 2.68 (t, J = 6.6 Hz, 2H), 3.47 (q, J = 6.3 Hz, 2H), 7.15−7.21 (m, 3H), 7.26−7.32 (m, 2H), 7.46−7.54 (m, 2H), 7.58−7.65 (m, 1H), 7.69−7.80 (m, 4H), 8.03 (t, J = 5.3 Hz, 1H), 8.16 (d, J = 8.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.1, 35.6, 40.4, 111.6, 115.2, 126.1, 128.1, 128.5 (4C), 128.6 (2C), 130.0 (2C), 131.6, 132.8, 134.4, 137.4, 141.8, 142.0, 149.5, 153.2, 194.9. MS (ESI) *m/z*: 238 $[M - CONH(CH_2)_4Ph]^-$.

6-(4-Chlorobenzoyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (36). The reaction was carried out following method A, using **52b** (169 mg, 0.616 mmol) and 4-phenylbutyl isocyanate (119 mg, 0.116 mL, 0.678 mmol). White solid (66 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.79 (m, 4H), 2.68 (t, J = 7.0 Hz, 2H), 3.47 (q, J = 6.3 Hz, 2H), 7.16−7.22 (m, 3H), 7.26−7.31 (m, 2H), 7.47−7.51 (m, 2H), 7.69−7.75 (m, 4H), 8.02 (t, J = 5.4 Hz, 1H), 8.17 (d, J = 8.2 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.7, 29.1, 35.6, 40.4, 111.5, 115.2, 126.1, 128.0, 128.5 (4C), 129.0 (2C), 131.4 (2C), 131.7, 133.9, 135.6, 139.4, 141.8, 141.9, 149.4, 153.1 193.7. MS (ESI) *m/z*: 272 and 274 $[M - CONH(CH_2)_4Ph]^-$.

General Procedure for the Synthesis of Benzoxazolone *N*-Carboxamides 54, 62, 63, and 66 via Method B (Scheme 6, Table 3). Triphosgene (1.0 equiv) was placed in an oven-dried flask, cooled to 0 °C, and dissolved in dry pyridine (5 mL per mmol triphosgene). 3*H*-1,3-Benzoxazol-2-one (**50**, 1.0 equiv) dissolved in dry pyridine (1.9 mL per mmol of **50**) was added, and the reaction mixture was stirred under nitrogen for 30 min at rt. The solution was cooled to 0 °C, and the proper amine (1.5 equiv), dissolved in dry pyridine (3.5 mL per mmol amine), was added. The reaction was allowed to warm to rt and stirred for 3 h. The solvent was removed under reduced pressure, and the compound was purified by column chromatography using the Teledyne ISCO apparatus (cyclohexane/EtOAc).

2-Oxo-*N*-(3-phenylpropyl)-1,3-benzoxazole-3-carboxamide (54). The reaction was carried out following method B, using **50** (70 mg, 0.520 mmol) and 3-phenylpropan-1-amine **67l** (106 mg, 0.111 mL, 0.784 mmol). White solid (65 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (p, J = 7.4 Hz, 2H), 2.73 (t, J = 7.6 Hz, 2H), 3.46 (q, J = 6.6 Hz, 2H), 7.16−7.32 (m, 8H), 8.07 (d, J = 7.5 Hz, 1H), 8.07−8.14 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 31.0, 33.2, 39.9, 110.0, 115.7, 124.7, 125.1, 126.2, 128.1, 128.5 (2C), 128.6 (2C), 141.1, 141.9, 150.0, 153.3. MS (ESI) *m/z*: 297 $[M + H]^+$, 314 $[M + NH_4]^+$.

2-Oxo-*N*-[2-(benzyloxy)ethyl]-1,3-benzoxazole-3-carboxamide (62). The reaction was carried out following method B, using **50** (70 mg, 0.520 mmol) and 2-(benzyloxy)ethan-1-amine hydrochloride **67m** (584 mg, 3.11 mmol, 6.0 equiv). The reaction was stirred for 22 h. White solid (89 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62−3.69 (m, 4H), 4.58 (s, 2H), 7.22−7.29 (m, 4H), 7.31−7.39 (m, 4H), 8.03−8.08 (m, 1H), 8.36 (br s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 40.4, 68.3, 73.4, 110.0, 115.7, 124.6, 125.1, 127.9 (3C), 128.1, 128.6 (2C), 137.9, 141.9, 150.1, 153.1. MS (ESI) *m/z*: 313 $[M + H]^+$, 330 $[M + NH_4]^+$.

2-Oxo-*N*-[2-(benzylthio)ethyl]-1,3-benzoxazole-3-carboxamide (63). The reaction was carried out following method B, using **50** (70 mg, 0.520 mmol) and 2-(benzylthio)ethan-1-amine hydrochloride **67n** (159 mg, 0.780 mmol). White powder (58 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.69 (t, J = 6.6 Hz, 2H), 3.55 (q, J = 6.5 Hz, 2H), 3.77 (s, 2H), 7.17−7.37 (m, 8H), 8.03−8.07 (m, 1H), 8.29 (t, J = 6.2 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 31.0, 36.3, 39.4, 110.1, 115.7, 124.7, 125.1, 127.3, 128.1, 128.7 (2C), 129.0 (2C), 138.1, 141.9, 149.9, 153.1. MS (ESI) *m/z*: 329 $[M + H]^+$, 346 $[M + NH_4]^+$.

2-Oxo-*N*-[(4-propylphenyl)methyl]-1,3-benzoxazole-3-carboxamide (66). The reaction was carried out following method B, using **50** (60 mg, 0.444 mmol) and amine **67e** (125 mg, 0.673 mmol). White powder (40 mg, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (t, J = 7.3 Hz, 3H), 1.58−1.71 (m, 2H), 2.58 (dd, J = 8.4, 6.8 Hz, 2H), 4.58 (d, J = 5.7 Hz, 2H), 7.17 (d, J = 8.1 Hz, 2H), 7.21−7.32 (m, 5H), 8.07−8.14 (m, 1H), 8.35−8.46 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 13.9, 24.7, 37.8, 44.1, 110.0, 115.8, 124.7, 125.1, 128.1, 129.0, 134.6, 141.9, 142.5, 150.0, 153.2. MS (ESI) *m/z*: 311 $[M + H]^+$, 328 $[M + NH_4]^+$.

General Procedure for the Synthesis of Benzoxazolone *N*-Carboxamides 53, 55−61, 64, 65 via Method C (Scheme 6, Table 3). In an oven-dried flask, triphosgene (1.0 equiv) was dissolved in dry DCM (5 mL per mmol triphosgene). A solution of 3*H*-1,3-benzoxazol-2-one (**50**, 1.0 equiv) and Et_3N (4.0 equiv) in dry DCM (5.6 mL per mmol of **50**) was added at 0 °C, and the reaction was stirred for 1 h at rt under nitrogen. Then a solution of the appropriate amine (1.5 equiv) and Et_3N (1.5 equiv) in dry DCM (6.5 mL per mmol of amine) was added at 0 °C, and the reaction was stirred at rt for 3 h. The reaction mixture was diluted with DCM (20 mL per mmol of **50**) and quenched with sat aq NH_4Cl (30 mL per mmol of **50**). The two phases were separated and the aq layer was extracted with DCM (3 × 20 mL per mmol of **50**). The combined organic phases were dried over Na_2SO_4, evaporated on Celite or silica, and the compound was purified by silica gel column chromatography using the Teledyne ISCO apparatus (cyclohexane/EtOAc).

2-Oxo-*N*-(phenethyl)-1,3-benzoxazole-3-carboxamide (53). The reaction was carried out following method C, using **50** (60 mg, 0.444 mmol) and 2-phenylethan-1-amine **67k** (80 mg, 0.084 mL, 0.660 mmol). White solid (79 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96 (t, J = 7.2 Hz, 2H), 3.69 (td, J = 5.8, 7.2 Hz, 2H), 7.19−7.29 (m, 6H), 7.34 (dd, J = 5.8, 8.9 Hz, 2H), 8.05−8.09 (m, 1H), 8.09−8.17 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 35.9, 41.8, 110.0, 115.7, 124.7, 125.1, 126.9, 128.1, 128.9, 128.9, 138.4, 141.9, 149.9, 153.2. MS (ESI) *m/z*: 283 $[M + H]^+$, 300 $[M + NH_4]^+$. MS (ESI) *m/z*: 281 $[M - H]^-$.

2-Oxo-*N*-(5-phenylpentyl)-1,3-benzoxazole-3-carboxamide (55). The reaction was carried out following method C, using **50** (68 mg, 0.503 mmol) and amine **67j** (100 mg, 0.501 mmol). White solid (83 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42−1.51 (m, 2H), 1.65−1.76 (m, 4H), 2.66 (t, J = 7.6 Hz, 2H), 3.45 (td, J = 7.1, 5.9 Hz, 2H), 7.16−7.22 (m, 3H), 7.24−7.32 (m, 5H), 8.06−8.13 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 26.4, 29.3, 31.0, 35.7, 40.2, 109.9, 115.6, 124.5, 125.0, 125.7, 128.0, 128.3, 128.4, 141.7, 142.3, 149.8, 153.2. MS (ESI) *m/z*: 325 $[M + H]^+$.

2-Oxo-*N*-(6-phenylhexyl)-1,3-benzoxazole-3-carboxamide (56). The reaction was carried out following method C, using **50** (40 mg, 0.300 mmol) and amine **67f** (95 mg, 0.440 mmol). White solid (32 mg, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32−1.52 (m, 4H), 1.59−1.71 (m, 4H), 2.51−2.74 (m, 2H), 3.31−3.51 (m, 2H), 7.13−7.20 (m, 3H), 7.21−7.31 (m, 5H), 8.02−8.11 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 26.8, 29.0, 29.5, 31.4, 36.0, 40.4, 110.0, 115.7, 124.6, 125.1, 125.8, 128.2, 128.4, 128.5, 141.9, 142.7, 149.9, 153.3. MS (ESI) *m/z*: 339 $[M + H]^+$, 356 $[M + NH_4]^+$.

***N*-[4-(2-Naphthyl)butyl]-2-oxo-1,3-benzoxazole-3-carboxamide (57).** The reaction was carried out following method C, using **50** (26 mg, 0.190 mmol) and amine **67a** (68 mg, 0.300 mmol). White solid (36 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66−1.79 (m, 2H), 1.78−1.91 (m, 2H), 2.84 (t, J = 7.4 Hz, 2H), 3.47 (td, J = 7.0, 5.7 Hz, 2H), 7.21−7.30 (m, 3H), 7.34 (dd, J = 8.4, 1.7 Hz, 1H), 7.38−7.49 (m, 2H), 7.60−7.66 (m, 1H), 7.74−7.84 (m, 3H), 8.01−8.12 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.5, 29.2, 35.7, 40.2, 110.1, 115.7, 124.6, 125.1, 125.3, 126.0, 126.6, 127.3, 127.5, 127.7, 128.1, 128.1, 132.2, 133.7, 139.5, 141.9, 150.0, 153.3. MS (ESI) *m/z*: nonionizable compound under routine conditions used.

***N*-[4-(4-Nitrophenyl)butyl]-2-oxo-1,3-benzoxazole-3-carboxamide (58).** The reaction was carried out following method C, using **50** (70 mg, 0.518 mmol) and amine **67g** (180 mg, 0.780 mmol). White solid (29 mg, 16%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.82 (m, 4H), 2.78 (t, J = 7.4 Hz, 2H), 3.47 (q, J = 6.7 Hz, 2H), 7.21−7.32 (m, 3H), 7.32−7.36 (m, 2H), 8.03−8.07 (m, 1H), 8.10 (t, J = 5.0 Hz, 1H), 8.15−8.17 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.2, 29.2, 35.5, 40.0, 110.1, 115.7, 123.9 (2C), 124.8, 125.2, 128.1, 129.3 (2C), 141.9, 146.6, 149.8, 150.0, 153.3. MS (ESI) *m/z*: nonionizable compound under routine conditions used.

***N*-[4-(4-Fluorophenyl)butyl]-2-oxo-1,3-benzoxazole-3-carboxamide (59).** The reaction was carried out following method C,

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

using **50** (70 mg, 0.518 mmol) and amine **67b** (160 mg, 0.786 mmol). White solid (80 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.80 (m, 4H), 2.67 (t, J = 7.0 Hz, 2H), 3.39−3.57 (m, 2H), 6.92−7.05 (m, 2H), 7.11−7.21 (m, 2H), 7.23−7.27 (m, 2H), 7.27−7.32 (m, 1H), 7.83−8.38 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.8, 29.1, 34.8, 40.2, 110.0, 115.20 (d, J = 21.4 Hz, 2C), 115.7, 124.7, 125.1, 128.1, 129.8 (d, J = 7.9 Hz, 2C), 137.6, 141.9, 150.0, 153.3, 161.4 (d, J = 242.8 Hz, 1C). MS (ESI) m/z: 329 $[M + H]^+$, 346 $[M + NH_4]^+$.

***N*-[4-(4-Methoxyphenyl)butyl]-2-oxo-1,3-benzoxazole-3-carboxamide (60).** The reaction was carried out following method C, using **50** (70 mg, 0.52 mmol) and amine **67h** (168 mg, 0.78 mmol). White solid (42 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61−1.75 (m, 4H), 2.61 (t, J = 6.9 Hz, 2H), 3.44 (q, J = 6.5 Hz, 2H), 3.78 (s, 3H), 6.79−6.85 (m, 2H), 7.07−7.12 (m, 2H), 7.21−7.33 (m, 3H), 8.03−8.09 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.9, 29.1, 34.7, 40.3, 55.4, 110.0, 113.9 (2C), 115.7, 124.6, 125.1, 128.2, 129.4 (2C), 134.1, 141.9, 150.0, 153.3, 158.0. MS (ESI) m/z: 341 $[M + H]^+$, 358 $[M + NH_4]^+$.

2-Oxo-*N*-[4-(*p*-tolyl)butyl]-1,3-benzoxazole-3-carboxamide (61). The reaction was carried out following method C, using **50** (50 mg, 0.370 mmol) and amine **67c** (110 mg, 0.551 mmol). White solid (47 mg, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.83 (m, 4H), 2.31 (s, 3H), 2.63 (t, J = 7.1 Hz, 2H), 3.44 (td, J = 6.7, 5.5 Hz, 2H), 7.04−7.15 (m, 4H), 7.22−7.29 (m, 3H), 8.01−8.13 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.1, 28.8, 29.2, 35.1, 40.3, 110.0, 115.8, 124.6, 125.1, 128.2, 128.4, 129.2, 135.5, 138.9, 141.9, 150.0, 153.3. MS (ESI) m/z: nonionizable compound under routine conditions used.

2-Oxo-*N*-[2-(benzyl(methyl)amino)ethyl]-1,3-benzoxazole-3-carboxamide (64). The reaction was carried out following method C, using **50** (70 mg, 0.518 mmol) and amine **67i** (216 mg, 0.777 mmol). Clear colorless oil (70 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 3H), 2.64 (t, J = 6.0 Hz, 2H), 3.54 (q, J = 5.7 Hz, 2H), 3.58 (s, 2H), 7.18−7.27 (m, 4H), 7.27−7.33 (m, 2H), 7.36−7.40 (m, 2H), 8.03−8.07 (m, 1H), 8.45 (t, J = 4.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 37.9, 41.9, 55.3, 62.6, 110.0, 115.7, 124.5, 125.0, 127.3, 128.2, 128.5 (3C), 129.1 (2C), 141.9, 150.0, 153.0. MS (ESI) m/z: 326 $[M + H]^+$.

2-Oxo-*N*-[4-(2-thienyl)butyl]-1,3-benzoxazole-3-carboxamide (65). The reaction was carried out following method C, using **50** (70 mg, 0.518 mmol) and amine **67d** (150 mg, 0.782 mmol). White solid (83 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.84 (m, 4H), 2.89 (t, J = 7.2 Hz, 2H), 3.46 (q, J = 6.7 Hz, 2H), 6.80 (d, J = 3.3 Hz, 1H), 6.91 (dd, J = 5.1, 3.4 Hz, 1H), 7.11 (dd, J = 5.1, 1.2 Hz, 1H), 7.22−7.29 (m, 3H), 8.04−8.10 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 29.0, 29.1, 29.6, 40.1, 110.0, 115.7, 123.2, 124.5, 124.7, 125.1, 126.9, 128.1, 141.9, 144.8, 150.0, 153.3. MS (ESI) m/z: 317 $[M + H]^+$, 334 $[M + NH_4]^+$.

Pharmacology. Preparation of Enzyme-Enriched Lysate. Cells were suspended in 20 mM Tris-HCl (pH 7.5) with 0.32 M sucrose, sonicated, and centrifuged at 800*g* for 30 min at 4 °C. Supernatants were then centrifuged at 12.000*g* for 30 min at 4 °C. Pellets were resuspended in PBS buffer (pH 7.4) and subjected to three freeze−thaw cycles at −80 °C. The suspension was finally centrifuged at 105.000*g* for 1 h at 4 °C and protein concentration was measured in the supernatant with bicinchoninic acid based protein assay.

Fluorescence-Based in Vitro Assay. Compounds were assessed for their ability to inhibit hydrolysis of the fluorogenic substrate, *N*-[(1S,2R)-2-hydroxy-1-(hydroxymethyl)-4-(2-oxochromen-7-yl)-oxybutyl]dodecanamide, which is converted to umbelliferone by AC in the fluorescence-based in vitro assay, as previously described.[12,18] Lysosomal lysate, enriched with AC, was prepared from Hek293 cells stably expressing human AC and preincubated with test compounds and a positive control (diluted 20× from DMSO stock solutions at different concentrations) for 10 min. Then, the fluorogenic substrate (diluted 40× from EtOH stock solution, final concentration 5 μM) was added and the mixture was incubated for 3 h at 37 °C, stopped with MeOH, and treated with $NaIO_4$ (fresh solution in 100 mM glycine/NaOH buffer pH 10.6) followed by a 2 h incubation at 37 °C in the dark. Fluorescence intensities were measured (excitation/emission: 355/460 nm) and plotted as a function of compound concentrations. IC_{50} values were calculated by nonlinear regression analysis using GraphPad Prism 5 (GraphPad Software Inc., CA, USA) applying a standard slope curve fitting.

PBS Stability Assay.[12] Compounds were incubated at 10 μM in phosphate-buffered saline (pH 7.4; 1% DMSO) at 37 °C under shaking. Compounds were sampled at various time points and analyzed on a Xevo triple-quad UPLC system using a BEH C18 reversed phase column and a linear gradient of MeCN in water. Stability was evaluated from the corresponding MRM (multiple reaction monitoring) peak areas plotted versus time. The corresponding decay profile was fitted with Prism to derive the corresponding half-life values.

Animals and Treatments. Male C57BL/6 mice (20−35 g, Charles River) were group-housed at rt on a 12 h light/dark cycle. Water and standard chow pellets were freely available. Drugs were dissolved in 15% polyethylene glycol, 15% Tween-80, and 70% saline (injection volume, 10 mL/kg; ip). Animals were sacrificed 30 min, 3 h, 6 h, 12 h, 16 h, and 24 h after drug administration; tissues were collected, frozen in liquid nitrogen, and stored at −80 °C. All procedures were performed in accordance with the Italian regulations on the protection of animals used for experimental and other scientific purposes (D.M. 116192) and with European Economic Community regulations (O.J. of E.C. L 358/1 12/18/1986).

AC Activity in Lungs and Cerebral Cortex. AC activity in lung and cerebral cortex lysosomal extracts was measured by LC−MS as previously described.[19] Briefly, tissues (10−20 mg) were suspended in 20 mM Tris-HCl (pH 7.5) with 0.32 M sucrose, sonicated, and centrifuged at 800*g* for 15 min at 4 °C. Supernatants were then centrifuged at 12.000*g* for 30 min at 4 °C. Pellets were resuspended in PBS buffer (pH 7.4) and subjected to two freeze−thaw cycles at −80 °C. The suspension was finally centrifuged at 105.000*g* for 1 h at 4 °C, and protein concentration was measured in the supernatant with bicinchoninic acid based protein assay. Lysosomal preparations from tissues were diluted in assay buffer (100 mM sodium phosphate, 0.1% Nonidet P-40, 150 mM NaCl, 3 mM DTT, 100 mM sodium citrate, pH 4.5). Reactions were started by the addition of 50 μM C12-ceramide (Nu-Chek Prep, Elysian, MN) and carried on for 1 h at 37 °C. Reactions were stopped by addition of a mixture of chloroform/MeOH (2:1) containing 1 nmol of 11-lauroleic acid (NuChek Prep). The organic phases were collected, dried under nitrogen, and analyzed by UPLC−MS (Acquity, Waters) in the negative-ion mode monitoring the reaction product (lauric acid, m/z: 199) using 11-lauroleic acid as internal standard. Lipids were eluted on an Acquity UPLC BEH C18 column (50 mm length, 2.1 mm i.d., 1.7 μm pore size, Waters) at 0.5 mL min^{-1} for 1.5 min with a gradient of MeCN and water, both containing 0.25% acetic acid and 5 mM ammonium acetate (70−100% MeCN in 0.5 min, 100% MeCN for 0.5 min, 70% MeCN for 0.4 min). The column temperature was 40 °C. Electrospray ionization (ESI) was in the negative mode, capillary voltage was 1 kV, and cone voltage was 50 V. N_2 was used as drying gas at a flow rate of 500 L/h and at a temperature of 400 °C. The $[M - H]^-$ ion was monitored in the selected-ion monitoring mode (m/z values: lauric acid 199, 11-lauroleic acid 197.35). Calibration curves were generated with authentic lauric acid (Nu Check Prep).

Sphingolipid Levels Measurement. Sphingolipids (namely, dihydroceramide d18:0/16:0, ceramide 18:1/16:0, sphingosine, and sphingosine 1-phosphate) were quantified by LC−MS analysis according to the method previously described.[20]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01188.

Detailed experimental procedures of intermediates **37a,c,d,g,l−p**; **39a−g**; **40a,b**; **41a,b**; **42**; **44**; **45a−c**; **46a−c**; **47a−c**; **48**; **49**; **51a,b**; **52a,b**; **67a−j**; **70a−j** (PDF)

Molecular formula strings (CSV)

DOI: 10.1021/acs.jmedchem.5b01188
J. Med. Chem. 2015, 58, 9258−9272

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Notes

The authors declare the following competing financial interest(s): Two patent applications protecting the class of compounds disclosed in this paper were filed by the following authors: Piomelli, D.; Pizzirani, D.; Bach, A.; Realini, N.; Melzig, L.; Scarpelli, R.

ACKNOWLEDGMENTS

The authors thank Luca Goldoni for NMR technical support, Andrea Armirotti and Giuliana Ottonello for analytical stability data, Abdul Basit for sphingolipid levels measurement method, and Silvia Venzano for handling compounds. A.B. was generously supported by a Carlsberg Foundation fellowship.

ABBREVIATIONS USED

AC, acid ceramidase; $AlCl_3$, aluminum chloride; DMAP, 4-dimethylaminopyridine; DMSO, dimethyl sulfoxide; h-AC, human acid ceramidase

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