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Synthesis of biotinylated photoaffinity probes based on arylsulfonamide γ-secretase inhibitors

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Abstract—Synthesis and biological evaluation of an arylsulfonamide class of γ-secretase inhibitors are described. Design, synthesis, and biological evaluation of multifunctional molecular probes harboring a benzophenone photophore as a cross-linking group and a biotin tag are also reported.

Alzheimer's disease (AD) is a most common neurodegenerative disorder currently being a serious public health problem in the aging society. The accumulation of 40–42 residue amyloid β-protein (Aβ) in brain regions serving memory and cognition is a central pathogenic feature of AD. Aβ is generated through proteolysis of amyloid precursor protein (APP)[1] by two types of membrane associated aspartic proteases termed β- and γ-secretase, both of which have been significant therapeutic targets toward prevention and treatment of AD.[2] γ-Secretase, a macromolecular complex comprised of Presenilin-1 (PS1), Pen-2, Aph-1, and Nicastrin, is known to endoproteolyze several transmembrane proteins other than APP. Pharmacological regulation of the substrate selectivity of the proteolytic processing mediated by γ-secretase is, therefore, an important issue toward the development of γ-secretase-targeted therapeutics without any severe adverse effects. To date, a number of small-molecule γ-secretase inhibitors, including BMS-299,897 (**1**) and related arylsulfonamides[3] (Fig. 1), have been discovered. Recent in vivo studies using transgenic mice have shown that BMS-299,897 effectively reduced brain Aβ levels without causing Notch mediated toxicity,[4] which is the major plausible adverse effect of other dipeptidic inhibitors such as LY411575 (*N*^{2}-[(2*S*)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-*N*^{1}-[(7*S*)-5-methyl-6-oxo-6,7-dihydro-5*H*-dibenzo[*b*,*d*]azepin-7-yl]-L-alaninamide) and related compounds.[5] These intriguing results combined with the distinct structural feature suggested that the biological mode of action(s) of the arylsulfonamide inhibitors would be different from those of the previously known dipeptidic class of derivatives. However, using a photoaffinity probe DAP-BpB (**4**) designed based on DAPT (**3**: *N*-[*N*-(3,5-difluorophenylacetyl)-L-alanyl]-(*S*)-phenylglycine *tert*-butylester),[6,7] our recent studies have revealed that an analogue of **1** competitively inhibits labeling of the C-terminal fragment of PS1 (PS1-CTF) by DAP-BpB in a dose-dependent manner.[7] Our finding implies the possibility that the arylsulfonamides and DAPT derivatives interact with the same region of PS1-CTF. However, we cannot rule out another possibility that the arylsulfonamides affect allosterically the binding of DAPT to PS1-CTF. To clearly address this complicated issue, we set out to synthesize arylsulfonamide-based photoaffinity probes, which will provide direct evidence for the molecular target(s) and mode of action(s) of the arylsulfonamide inhibitors.[8] Herein we report our investigation of structure–activity relationships of **1** and related analogues, and the design and synthesis of molecular probes based on **1**.

Keywords: Structure–activity relationships; Photoaffinity probes; Arylsulfonamides; γ-Secretase inhibitors.
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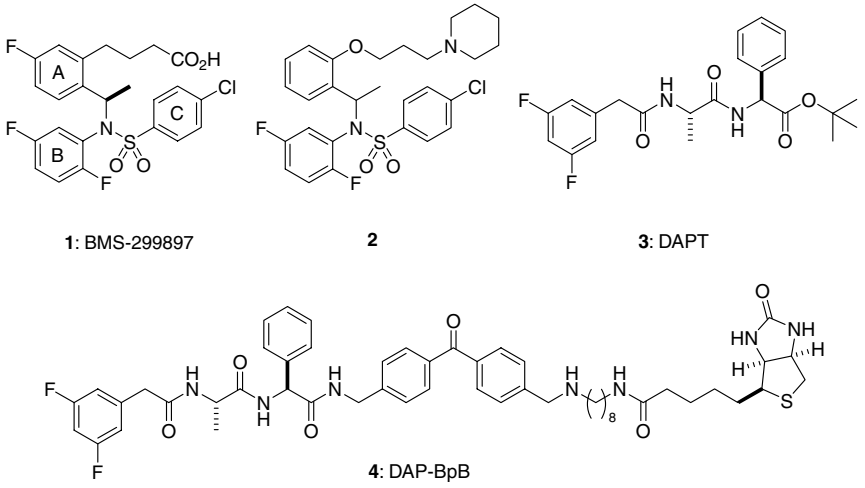
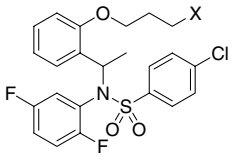


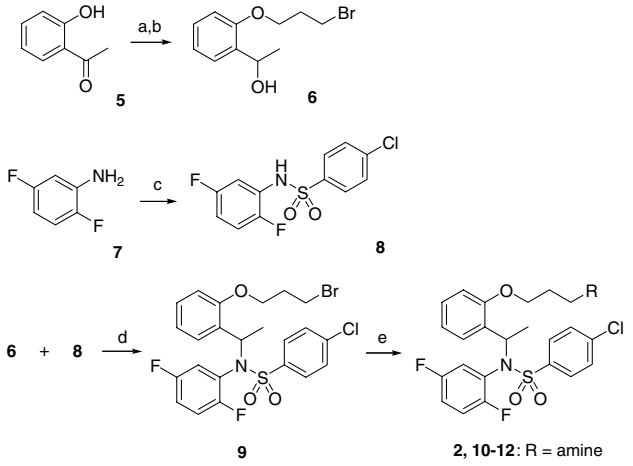
Figure 1. Structures of BMS-299897 (**1**), its analogue **2**, DAPT (**3**), and DAP-BpB (**4**).

To design probes maintaining sufficient potency, the structure–activity relationships of the arylsulfonamide inhibitors had to be first established. Since our preliminary attempts to modify **1** into the corresponding amide derivatives resulted in complete loss of potency, compound **2** was instead selected as a starting point. To probe structure–activity relationships of the appendage of the A ring,[9] a series of analogues were investigated, which could be prepared conveniently from 2-hydroxyacetophenone **5** (Scheme 1). Thus, Mitsunobu reaction[10] of **5** with 3-bromopropanol followed by reduction afforded alcohol **6**. Sulfonamide **8** prepared from 2,5-difluoroaniline **7** was alkylated with **6** under the Mitsunobu conditions to give rise to bromide **9**. A set of side chain modified analogues could be readily accessed from **9**; treatment of **9** with various amines led to the tertiary amines **2** and **10**–**12**. Their ability to inhibit Aβ production was evaluated by the cell-free in vitro assay using recombinant C-terminal fragment of APP as a substrate,[11] and the results are summarized in Table 1. Tertiary amines **2** and **10**–**12** showed good inhibitory activity against Aβ40 production with IC_{50} values around the micromolar range. In contrast, other derivatives such as amides, nitriles, halides, azides and carboxylic acids (not shown) were only weakly active or inactive at 10 μM, suggesting the importance of a basic nitrogen for potency and the difficulty of drastic structural modification such as installation of a cross-linking group and/or a biotin tag at this position. Since the pyrrolidine derivative **10** (previously described as HF14057)[7d] was easily accessible from **5** in just five steps with high overall yield and was almost equipotent to the racemic **1** (IC_{50} 0.86 μM),[12] we selected **10** as a lead for further examination of structure–activity relationships.

Table 1. Aβ40 production inhibitory activity for compounds **2** and **10**–**12**

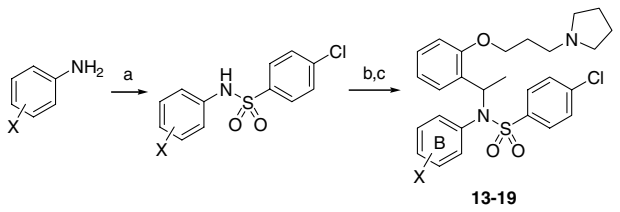


Compound	X	Aβ40 IC_{50} (μM)
2		2.1
10		2.0
11		4.4
12		1.0



Scheme 1. Reagents and conditions: (a) 3-bromopropanol, PPh_3, DEAD, toluene, 60 °C; (b) $NaBH_4$, $MeOH/CH_2Cl_2$, 0 °C, 50% (two steps); (c) *p*-$ClC_6H_4SO_2Cl$, pyridine, CH_2Cl_2, rt, 80%; (d) PPh_3, DEAD, toluene, 0 °C to rt, 74%; (e) secondary amine, rt, 76–95%.

We next investigated the effect of modification of the B ring of **10**. We elaborated compounds **13**–**19** from the respective anilines by successive sulfonylation, Mitsunobu alkylation with alcohol **4**, and treatment with pyrrolidine (Scheme 2). Their inhibitory activities are shown in Table 2. Interestingly, the unsubstituted



Scheme 2. Reagents and conditions: (a) *p*-$ClC_6H_4SO_2Cl$, pyridine, CH_2Cl_2, rt, 67–93%; (b) compound **6**, PPh_3, DEAD, toluene, 0 °C to rt, 62–87%; (c) pyrrolidine, rt, 64–94%.

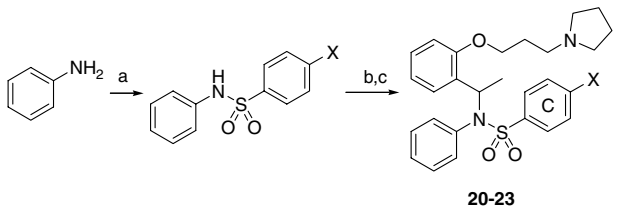
Table 2. Aβ40 production inhibitory activity for compounds **10** and **13–19**

Compound	X	Aβ40 IC_{50} (μM)	Relative potency[a]
10	2,5-F_2	2.0	1
13	*o*-Cl	0.49	4.0
14	*m*-Cl	0.53	3.8
15	*p*-Cl	1.3	1.5
16	*o*-OMe	0.47	4.3
17	*m*-OMe	0.53	3.8
18	*p*-OMe	1.1	1.8
19	H	0.01	200

[a] Values are calculated by dividing the IC_{50} value of compound **10** with that of each compound.

derivative **19** displayed dramatically enhanced inhibitory potency, being approximately 100-fold active compared to the parent **10**. Also noteworthy is that compounds **13–18** exhibited potent inhibitory activity, suggesting that the modification of the substituents of the B ring would be well tolerated.

Structure–activity relationships of the C ring substituent were briefly investigated. Alcohol **6** could readily be alkylated with a series of sulfonamides under the standard Mitsunobu conditions, and ensuing treatment with pyrrolidine afforded compounds **20–23** (Scheme 3). Their inhibitory activities were found to be 10- to 100-fold less potent than that of **19** (Table 3). It seems likely



Scheme 3. Reagents and conditions: (a) arylsulfonyl chloride, pyridine, CH_2Cl_2, rt, 82–100%; (b) compound **6**, PPh_3, DEAD, toluene, 0 °C to rt, 46–60%; (c) pyrrolidine, rt, 64–86%.

Table 3. Aβ40 production inhibitory activity for compounds **19–23**

Compound	X	Aβ40 IC_{50} (μM)	Relative potency[a]
19	Cl	0.01	1
20	F	0.35	0.03
21	CF_3	0.40	0.03
22	Br	1.9	0.005
23	H	0.69	0.01

[a] Values are calculated by dividing the IC_{50} value of compound **19** with that of each compound.

that the modification of the C ring moiety induces loss of activity.

With the preliminary structure–activity relationships in mind, we set out on the design and synthesis of multifunctional molecular probes based on arylsulfonamides. We planned to utilize a benzophenone group as a photophore. To establish a suitable modification position for the incorporation of a photophore, we planned to synthesize five compounds (**24–28**), each of which has a benzoyl group at the A or B ring (Fig. 2).

Synthesis of compounds **24** and **25** was commenced with commercially available acid **29** (Scheme 4). Conversion of **29** to the corresponding amide **30** was followed by

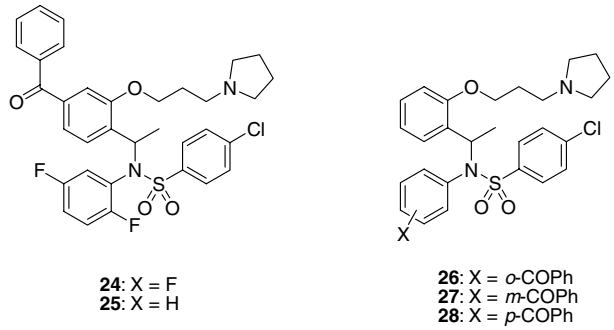
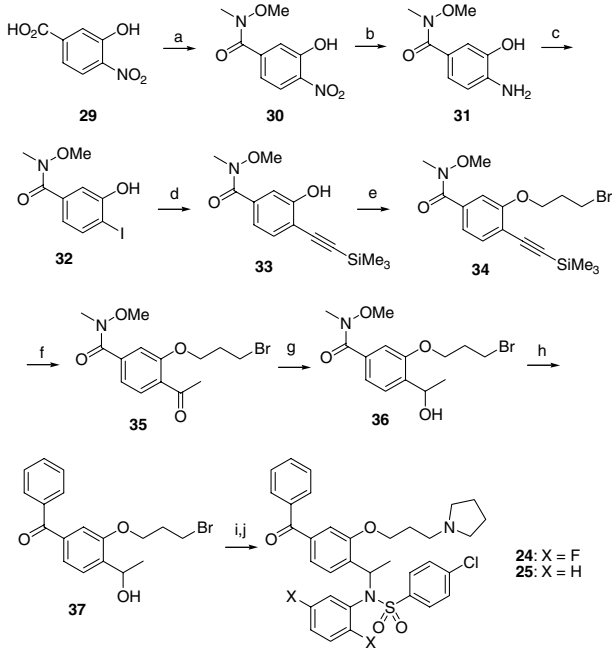


Figure 2. Structures of benzophenone-embedded analogues **24–28**.



Scheme 4. Reagents and conditions: (a) MeONHMe·HCl, EDCI·HCl, HOBt·H_2O, Et_3N, CH_2Cl_2, rt, 90%; (b) H_2, 5% Pd/C, MeOH, rt, 99%; (c) $NaNO_2$, aq HCl, 0 °C then KI, rt, 68%; (d) trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, PPh_3, CuI, Et_3N, THF, rt, 98%; (e) 3-bromopropanol, PPh_3, DEAD, toluene, 0 °C to rt, 89%; (f) HCO_2H, 60 °C, 70%; (g) $NaBH_4$, MeOH, 0 °C, 97%; (h) PhMgBr, THF, −78 °C to rt, 98%; (i) compound **8** or *N*-phenyl-*p*-chlorobenzenesulfonamide, PPh_3, DEAD, toluene, 0 °C to rt; (j) pyrrolidine, rt, 80% (**24**), 78% (**25**).

reduction of the nitro group to give amine **31**, which was transformed to the iodide **32** via diazotization. Under modified Sonogashira conditions,[13,14] iodide **32** was coupled with trimethylsilylacetylene to afford the coupling product **33**. Mitsunobu alkylation of **33** with 3-bromopropanol led to alkyne **34**, which was hydrated with formic acid to furnish acetophenone **35**. Reduction of **35** with $NaBH_4$ afforded alcohol **36**, which was reacted with PhMgBr to give rise to alcohol **37**. Mitsunobu coupling of **37** with appropriate sulfonamide followed by treatment with pyrrolidine furnished **24** and **25**. On the other hand, compounds **26**–**28** were prepared from the respective aminobenzophenones as described for the other B ring analogues. Evaluation of these benzophenone analogues revealed that incorporation of a benzoyl group to the A ring was unexpectedly favorable for increasing potency, as compound **24** displayed ca. 10-fold potent inhibitory activity compared to the parent **10** (IC_{50} 0.13 μM), while the B ring modified analogues **26**–**28** were almost equipotent to **10** (Table 4). Interestingly, in the case of these benzophenone analogues, elimination of two fluorine atoms from the B ring was detrimental for potency. Therefore, incorporation of (+)-biotin as a reporter group to **24** was next explored.

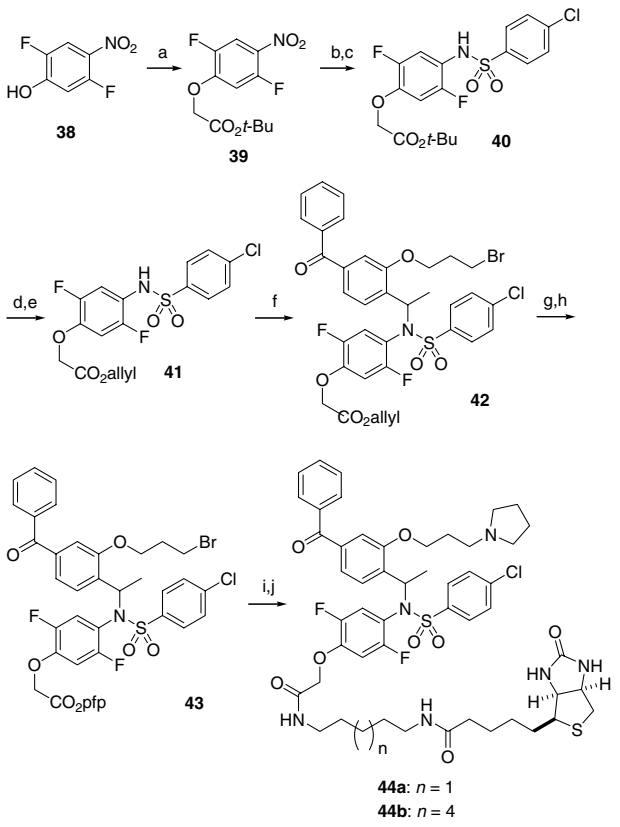
We designed two types of biotinylated photoaffinity probes, in which (+)-biotin was attached at the B ring (Scheme 5, **44a**,**b**) or at the end of the benzoyl group (Scheme 6, **49a**,**b**). The synthesis of **44a**,**b** is illustrated in Scheme 5. The known phenol **38**[15] was alkylated with *tert*-butyl bromoacetate to give **39**. The nitro group was reduced and subsequent sulfonylation of the derived aromatic amine furnished sulfonamide **40**. At this stage, the *tert*-butyl group was removed and the resultant acid was converted to allyl ester **41**. Mitsunobu alkylation of **41** with **37** afforded the coupling product **42**. Deprotection of the allyl ester of **42** [$Pd(PPh_3)_4$/pyrrolidine] was followed by condensation with pentafluorophenol (PfpOH) to afford the activated pfp ester **43**. Coupling of **43** with appropriate biotinylated amines[16,17] followed by treatment with pyrrolidine afforded the targeted probes **44a**,**b**.

On the other hand, the synthesis of **49a**,**b** is summarized in Scheme 6. The benzophenone moiety was efficiently constructed by treatment of iodoarene **45**[18] with *i*-PrMgBr[19] followed by coupling with the Weinreb amide **36** to furnish benzophenone **46** in 71% yield. After coupling with **8** under Mitsunobu conditions to give **47**, the silyl group was removed by aqueous HF to afford **48**. Activation of the resultant hydroxyl group as the corresponding mixed

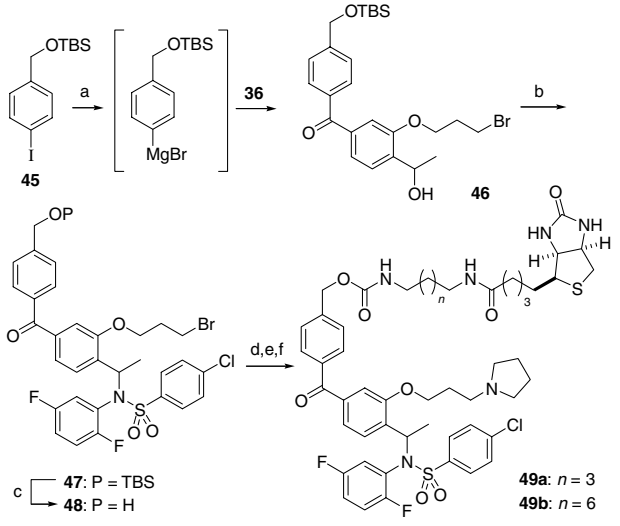
Table 4. Aβ40 production inhibitory activity for compounds **10** and **24**–**28**

Compound	Aβ40 IC_{50} (μM)	Relative potency[a]
10	2.0	1
24	0.13	15
25	17.0	0.1
26	1.2	1.7
27	3.0	0.7
28	3.2	0.6

[a] Values are calculated by dividing the IC_{50} value of compound **10** with that of each compound.



Scheme 5. Reagents and conditions: (a) $BrCH_2CO_2t$-Bu, K_2CO_3, DMF, rt, 96%; (b) H_2, 5% Pd/C, MeOH, rt; (c) *p*-$ClC_6H_4SO_2Cl$, pyridine, CH_2Cl_2, rt, 86%; (d) TFA, CH_2Cl_2, 0 °C to rt; (e) allyl alcohol, EDCI·HCl, HOBt·H_2O, Et_3N, DMF/THF, rt, 85%; (f) compound **37**, PPh_3, DEAD, toluene, 0 °C to rt, 81%; (g) $Pd(PPh_3)_4$, pyrrolidine, THF, rt; (h) pentafluorophenol, EDCI·HCl, CH_2Cl_2, rt, 54%; (i) (5-biotinamido)pentylamine or (8-biotinamido)octylamine, Et_3N, DMF, rt; (j) pyrrolidine, rt, 35% for **44a** (two steps), 33% for **44b** (two steps).



Scheme 6. Reagents and conditions: (a) *i*-PrMgBr, THF, −20 °C; then **36**, −78 to 0 °C, 71%; (b) compound **8**, PPh_3, DEAD, toluene, 0 °C to rt, 82%; (c) HF, CH_3CN/H_2O, 0 °C, 95%; (d) *p*-$NO_2C_6H_4OCOCl$, pyridine, THF/CH_3CN, rt, 97%; (e) (5-biotinamido)pentylamine or (8-biotinamido)octylamine, Et_3N, DMF, rt; (f) pyrrolidine, rt, 61% for **49a** (two steps), 53% for **49b** (two steps).

Table 5. Aβ40 production inhibitory activity for compounds **10**, **44a**,**b**, and **49a**,**b**

Compound	Aβ40 IC_{50} (μM)	Relative potency[a]
10	2.0	1
44a	0.01	200
44b	0.02	100
49a	0.0029	690
49b	0.0061	328

[a] Values are calculated by dividing the IC_{50} value of compound **10** with that of each compound.

carbonate (*p*-nitrophenyl chloroformate, and pyridine), coupling with appropriate biotinylated amine, and ensuing displacement of the bromide with pyrrolidine furnished the targeted probe **49a**,**b**.

The biotinylated photoaffinity probes **44a**,**b** and **49a**,**b** thus generated were evaluated and the results are summarized in Table 5. To our delight, all compounds displayed significant inhibitory activities against Aβ40 production, suggesting that these photoprobes maintain sufficient affinity toward γ-secretase.

In conclusion, we have investigated structure–activity relationships of an arylsulfonamide class of γ-secretase inhibitors. Examination of the appendage of the A ring revealed that the readily accessible tertiary amine derivatives (**2** and **10**–**12**) were almost equipotent to the parent compound **1**. Modification of the substituents of the B ring was well tolerated, but replacement of the chlorine atom of the C ring with another group was detrimental to potency. Introduction of a benzoyl group to the appropriate position of the A ring was unexpectedly favorable for increasing potency. The preliminary structure–activity relationships led us to the design and synthesis of biotinylated photoaffinity probes **44a**,**b** and **49a**,**b** that were proved to display excellent inhibitory activity against Aβ production in our cell-free in vitro assay. Photoaffinity labeling experiment using **44a**,**b** and **49a**,**b** to elucidate molecular target(s) of arylsulfonamide γ-secretase inhibitors is ongoing in our laboratories and the results will be reported shortly.

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