Bioorganic & Medicinal Chemistry Letters 19 (2009) 6869–6871

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Development of photoaffinity probes for γ-secretase equipped with a nitrobenzenesulfonamide-type cleavable linker

Satoshi Yokoshima [a,b], Yuzo Abe [a], Naoto Watanabe [a], Yoichi Kita [a], Toshiyuki Kan [c], Takeshi Iwatsubo [a,b,d], Taisuke Tomita [a,b], Tohru Fukuyama [a,b,*]

[a] Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[b] Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[c] School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[d] Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 18 September 2009
Revised 19 October 2009
Accepted 20 October 2009
Available online 23 October 2009

Keywords:
γ-Secretase
Photoaffinity labeling
Probe
Cleavable linker

ABSTRACT

We have developed photoaffinity probes for γ-secretase with a nitrobenzenesulfonamide-type linker that can be cleaved with 2-mercaptoethanol under physiological conditions.

Photoaffinity labeling is an efficient method for studying the interactions between biologically active compounds (ligands) and their target molecules.[1] The method facilitates the identification of both the target molecule and the binding domain. A photoaffinity probe is usually composed of a ligand, a photoreactive labeling group, and an indicator. A biotin tag is widely employed as an indicator because the biotinylated molecule can be easily detected by several immunological methods and isolated through binding to avidin beads.[2] One drawback to this approach is that harsh conditions are required to disrupt the strong interaction between the biotin and the avidin. Thus, to liberate the biotinylated molecule, the beads are typically boiled with detergents to denature the avidin. These conditions may result in degradation of the target molecules as well as contamination of the other proteins that bind to the beads with non-specific manner. To overcome this drawback, a variety of cleavable linkers, which can be cleaved under mild conditions to specifically release the labeled target molecules, have been developed.[3]

We have focused on the functional analysis of γ-secretase, which is one of the important therapeutic targets for Alzheimer's disease.[4] During the course of investigation of the potent γ-secretase inhibitor DAPT (**1**), we synthesized the photoaffinity probe DAP-BpB (**2**) and found that the major target of DAPT is presenilin 1 C-terminal fragment (PS1 CTF) (Fig. 1).[5] However, further detailed examinations of the DAPT-PS1 CTF interaction could not be conducted because of the low purity of the labeled proteins, which is caused by contaminated proteins bound to the avidin beads. We have prepared the other photoaffinity probes with disulfide moieties as cleavable linkers. However, they failed to effectively probe the PS1 CTF (data not shown). These results prompted us to develop photoaffinity probes equipped with a novel cleavable linker.

We envisioned that 2-nitrobenzensulfonamide would be an effective cleavable linker because this molecule can be cleaved under mild conditions using thiolate to generate an amine unit and a

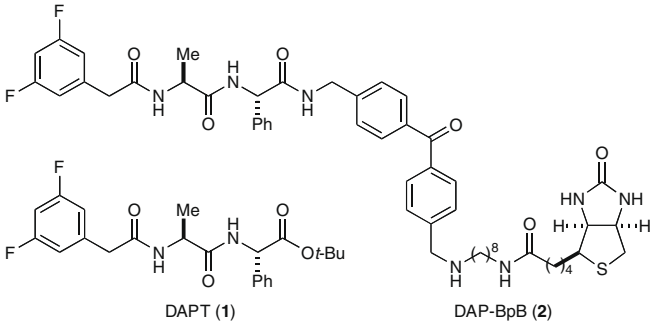
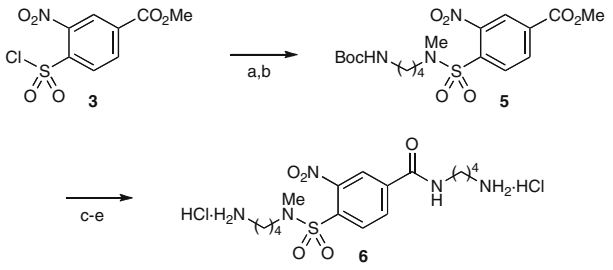


Figure 1. Structures of DAPT and DAP-BpB.

* Corresponding author. Tel.: +81 3 5841 4777; fax: +81 3 5802 8694.
E-mail address: fukuyama@mol.f.u-tokyo.ac.jp (T. Fukuyama).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.086



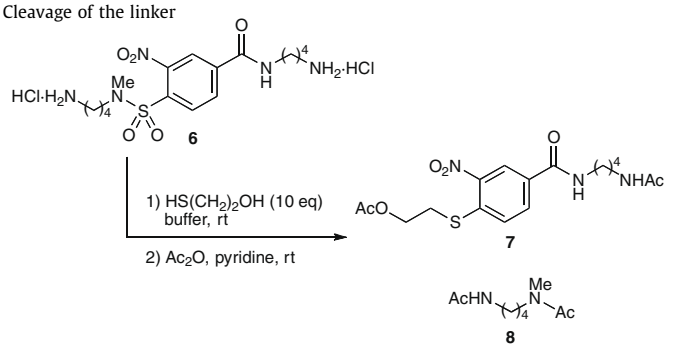
Scheme 1. Reagents and conditions: (a) BocHN$(CH_2)_4NH_2$ (**4**), Et_3N, CH_2Cl_2, rt; (b) MeI, Cs_2CO_3, DMF, reflux; (c) LiOH·H_2O, THF–MeOH, rt; (d) BocHN$(CH_2)_4NH_2$ (**4**), WSCD·HCl, HOBt, DMF, rt, 68% (four steps); (e) $SOCl_2$, MeOH, rt.

3-nitorobenzene unit.[6] It has been reported that 3-nitro-4-sulfamoylbenzamides can be used as linkers for solid-phase synthesis.[7] To examine whether this unit can be used for photoaffinity labeling, we synthesized a model compound bearing 3-nitro-4-sulfamoylbenzamide and evaluated its cleavability under physiological conditions.

To access the model compound, known sulfonyl chloride **3**[7] was treated with *N*-Boc-butanediamine (**4**) and the resulting sulfonamide unit was methylated to afford **5** (Scheme 1). Hydrolysis of the methyl ester in **5**, coupling of the resultant carboxylic acid with amine **4**, and final deprotection of the Boc groups furnished the requisite model compound **6**.

With model sulfonamide **6** in hand, we attempted to cleave the sulfonamide unit under physiological conditions. Sulfonamide **6** was treated with 10 equiv of 2-mercaptoethanol in two buffers. The reaction progress was determined by isolation of **7** after acetylation of the reaction mixture. As shown in Table 1, the cleavage reactions proceeded smoothly in aqueous media to afford **7** in excellent yield. The reaction rates depended on the concentrations of both the substrate and 2-mercaptoethanol. The cleavage reaction proceeded even in almost neutral phosphate buffer (pH 7.4) although the time needed for completion of the reaction was longer than the reaction time required at higher pH. Both of the reaction conditions were much milder than the conditions required for breaking the avidin–biotin interaction.

Table 1
Cleavage of the linker

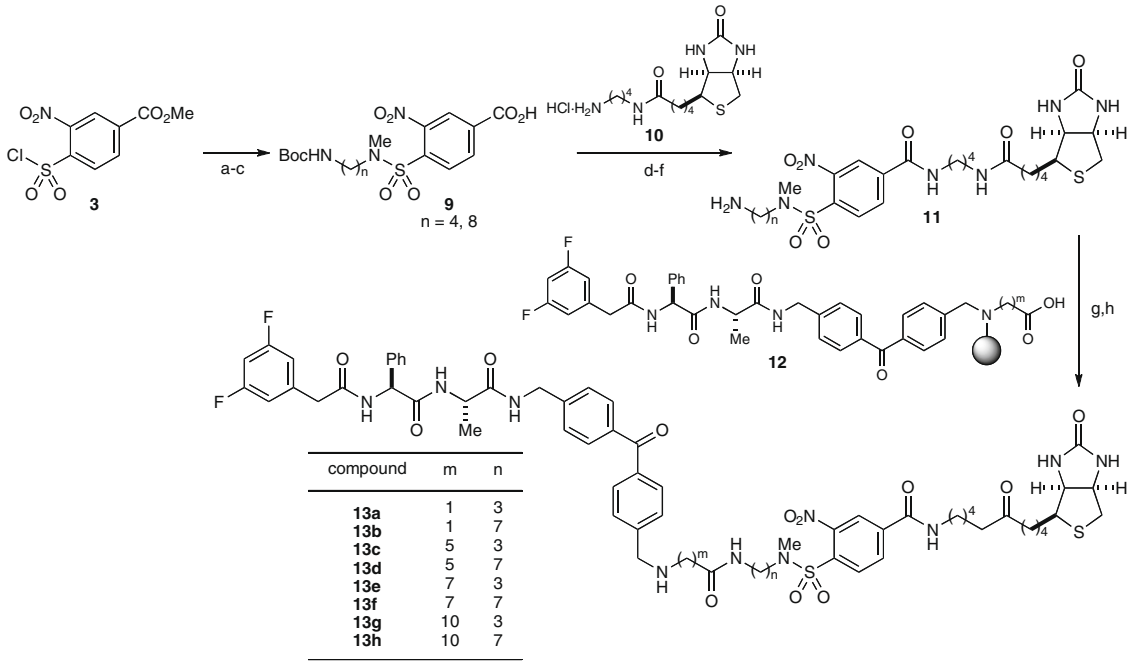


Buffer	pH	Concentration of **6** (mM)	Time (h)	Yield (%, **7**)
$NaHCO_3$–	9.2	3.0	7	93
Na_2CO_3		1.5	12	97
KH_2PO_4–	7.4	3.0	24	93
$NaHPO_4$		1.5	33	100

With a suitable cleavable unit in hand, we focused on incorporation of the linker unit into photoaffinity probes (Scheme 2). Two variants of carboxylic acids **9** with different chain lengths (n = 4 or 8) were prepared following the same procedures described in the synthesis of the model compound. Condensation of **9** with biotin unit **10** proceeded smoothly. After removal of the Boc group with methanolic hydrochloric acid, the resulting salt was liberated with Amberlite IR-400 (OMe form). Condensation of the resulting amines **11** (n = 4, 8) with polymer-bound carboxylic acid units **12** (m = 1, 5, 7, 10),[8] followed by acidic treatment, afforded photoaffinity probes **13a**–**h** with different lengths.

Using an in vitro γ-secretase assay, we found that these probes had inhibitory activities at almost equal levels to that of DAPT (Table 2).

We next investigated the labeling abilities of probes **13a**–**h**. CHAPSO-solubilized lysates of HeLa cells (~150 μg) mixed with the



compound	m	n
13a	1	3
13b	1	7
13c	5	3
13d	5	7
13e	7	3
13f	7	7
13g	10	3
13h	10	7

Scheme 2. Reagents and conditions: (a) BocHN$(CH_2)_nNH_2$, Et_3N, CH_2Cl_2, rt; (b) MeI, Cs_2CO_3, MeCN, reflux; (c) LiOH·H_2O, THF–MeOH–H_2O, rt; (d) **9**, PyBoP, HOBt, DMF, rt; (e) $SOCl_2$, MeOH, rt; (f) Amberlite IR-400 (OMe form), MeOH; (g) **11**, WSCD·HCl, HOBt, *i*-Pr_2NEt, NMP, rt; (h) TFA, CH_2Cl_2, rt.

Table 2
Inhibitory activities of probes **13a**–**h** for Aβ40 and 42[a]

Compound	Aβ40 (%)	Aβ42 (%)
13a	81.9	75.9
13b	81.9	77.0
13c	54.9	60.2
13d	78.2	59.3
13e	62.8	66.2
13f	62.3	89.3
13g	74.5	86.3
13h	55.0	69.2
DAPT (**1**)	77.4	76.3

[a] Percentages of inhibition of the Aβ generation in the presence of 1 μM of compounds (DMSO = 0%). Detailed experimental conditions are described in Ref. 3b.

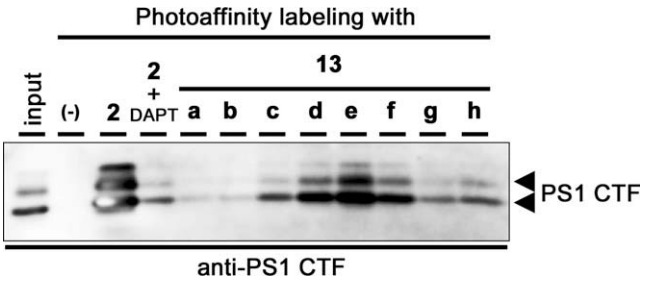


Figure 2. Photoaffinity labeling with probes **13a**–**h**.

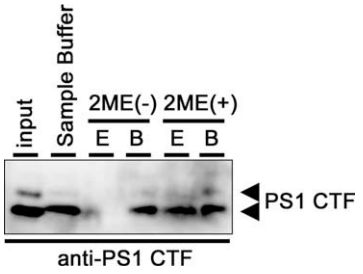


Figure 3. Photoaffinity labeling with probe **13e**. 2ME: 2-mercartoethanol. E and B indicate the eluted proteins and the material remaining on the beads, respectively.

probes (**2** or **13a**–**h**, 0.1 μM) were irradiated with long-wave near-UV light for 1 h. The biotinylated proteins were then captured with streptavidin sepharose beads. The beads were boiled in buffer containing 2% SDS and 1% 2-mercaptoethanol to liberate the total biotinylated PS1 CTF separated by SDS–PAGE using 12.5% Tris–Glycine gel. Proteins were then transferred on nitrocellulose membrane and detected by western blotting using chemiluminescence method as previously described.[5] The probes **13a**–**h** labeled PS1 CTF in a similar manner to that by DAP-BpB, while the levels of the biotinylated proteins varied (Fig. 2). No clear relationship between the labeling and the inhibitory activities of the probes was observed. Among them, probe **13e** showed the best labeling ability and was used in further investigations.

After capturing the biotinylated proteins with probe **13e**, the streptavidin sepharose beads were treated in carbonate buffer (pH 9.2) containing 2% SDS in the presence or absence of 3% 2-mercaptoethanol. To reduce non-specific degradation and/or aggregation of the peptide, the cleavage reaction was performed for 4 h at 20 °C. The eluates were analyzed by western blotting (E in Fig. 3). To evaluate the efficiency of the cleavage, the residual beads were subsequently boiled and the biotinylated PS1 CTFs remained on the beads was analyzed (B in Fig. 3). In the presence of 2-mercaptoethanol, the linker was cleaved to elute PS1 CTF from the beads, while no PS1 CTF was detected in the eluates without 2-mercaptoethanol. However, some amount of the biotinylated proteins still remained on the beads. Although the cleavage was expected to occur more efficiently at higher pH, amount of the captured PS1 CTF was lower under these conditions (data not shown). Efforts to optimize the cleavage reaction (i.e., the reaction times, the type of buffer, and the linker length) are currently underway.

In summary, we found that a nitrobenzenesulfonamide-type linker could be cleaved under physiological conditions in the presence of 2-mercaptoethanol. Using this linker unit, we successfully developed photoaffinity probes for the γ-secretase.

Acknowledgements

This work was financially supported in part by a Grant for the 21th Century COE Program, Grants-in-Aid for Young Scientists (S) from Japan Society for the Promotion of Science (JSPS) (T.T.), by the Targeted Proteins Research Program of the Japan Science and Technology Corporation, and Scientific Research on Priority Areas from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. N.W. was a research fellow of JSPS.

References and notes

1. For reviews, see: (a) Dorman, G.; Prestwich, G. D. *Biochemistry* **1994**, *33*, 5661; (b) Kotzyba-Hirbrt, F.; Kapfer, I.; Goeldner, M. *Angew. Chem., Int. Ed.* **1995**, *34*, 1296; (c) Tomohiro, T.; Hashimoto, M.; Hatanaka, Y. *Chem. Rec.* **2005**, *5*, 385.
2. Hofmann, K.; Kiso, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3516.
3. (a) Herman, T.; Levy, J.; Shimkus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2593; (b) Olejnik, J.; Sonar, S.; Krzymanska-Olejnik, E.; Rothschild, K. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7590; (c) Fang, K.; Hashimoto, M.; Jockusch, S.; Turo, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1998**, *120*, 8543; (d) Li, H.; Liu, Y.; Fang, K.; Nakanishi, K. *Chem. Commun.* **1999**, 365; (e) Fang, S.; Bergstrom, D. *Nucleic Acid Res.* **2003**, *31*, 708; (f) Park, J.; Sadakane, Y.; Masuda, K.; Tomihiro, T.; Nakano, T.; Hatanaka, Y. *ChemBioChem* **2005**, *6*, 814; (g) Verhelst, S. H. L.; Fonovic, M.; Bogyo, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1284; (h) Bongo, N. B.; Tomohiro, T.; Hatanaka, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 80.
4. Tomita, T. *Expert Rev. Neurother.* **2009**, *9*, 661.
5. (a) Kan, T.; Tominari, Y.; Morohashi, Y.; Natsugari, H.; Tomita, T.; Iwatsubo, T.; Fukuyama, T. *Chem. Commun.* **2003**, 2244; (b) Morohashi, Y.; Kan, T.; Tominari, Y.; Fuwa, H.; Okamura, Y.; Watanabe, N.; Natsugari, H.; Fukuyama, T.; Iwatsubo, T.; Tomita, T. *J. Biol. Chem.* **2006**, *281*, 14670.
6. (a) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373; (b) Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831; (c) Kan, T.; Fukuyama, T. *J. Synth. Org. Chem. Jpn.* **2001**, *59*, 779; (d) Kan, T.; Fukuyama, T. *Chem. Commun.* **2004**, 353.
7. (a) Kay, C.; Murray, P. J.; Sandow, L.; Holmes, A. B. *Tetrahedron Lett.* **1997**, *38*, 6941; (b) Congreve, M. S.; Kay, C.; Scicinski, J. J.; Ley, S. V.; Williams, G.; Murray, P. J.; McKeown, S. C.; Watson, S. P. *Tetrahedron Lett.* **2003**, *44*, 4153.
8. Kan, T.; Kita, Y.; Morohashi, Y.; Tominari, Y.; Hosoda, S.; Tomita, T.; Natsugari, H.; Iwatsubo, T.; Fukuyama, T. *Org. Lett.* **2007**, *9*, 2055.