Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3306–3309
Bioorganic & Medicinal Chemistry Letters

Design and synthesis of a biotin-tagged photoaffinity probe of paeoniflorin

Wen-Wei Qiu, Jie Xu, Da-Zhi Liu, Jing-Ya Li, Yang Ye, Xing-Zu Zhu, Jia Li* and Fa-Jun Nan*

Chinese National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, 189 Guo-Shou-Jing Road, Shanghai 201203, China

Received 13 February 2006; revised 4 March 2006; accepted 9 March 2006
Available online 18 April 2006

Abstract—A trifunctional probe (binding element–photoreactive group–affinity tag) of natural product paeoniflorin was designed and synthesized based on the previous primary structure-activity relationship. This new probe is a potential tool for labeling, purification, and identification of the target proteins.

Paeoniae alba radix (red peony root; Chi shao Yao), the dried, peeled root of *Paeonia lactiflora* Pall or *Paeonia veitchii* Lynch, is a traditional Chinese crude drug. It has been used as a spasmolytic and pain-relieving agent to treat certain types of dementia and relieve abdominal spasmodic pain and muscle stiffness. Paeoniflorin (PF; Fig. 1), the main monoterpene glucoside isolated from the root of *P. lactiflora* in 1963,[1] is one of the bioactive components of *Paeoniae radix*. It has been reported to exert antihyperglycemic,[2] antithrombotic,[3] and anticonvulsant effects,[4] stimulate the release of noradrenaline,[5] enhance cognitive function,[6–8] and to have antihypotensive effects via activation of the adenosine A_1 receptor.[9] Recently, Liu et al.[10] reported that PF has a neuroprotective effect without cardiovascular side effects, which might be mediated by the activation of the adenosine A_1 receptor in a manner different to that of classical adenosine A_1 receptor agonists, and therefore suggested that the adenosine A_1 receptor may be a potential molecular target of PF. However, it is difficult to explain many of the pharmacological effects of PF observed in various disease models based on only one target protein. Efforts for the further optimization based on PF and structure–activity relationship studies have been hindered by the lack of knowledge of its target protein(s). We designed and synthesized a trifunctional biotin-tagged photoaffinity probe for PF based on the results of previous primary structure–activity relationship studies. The probe could be used as a chemical tool not only to label its target proteins, but also to purify and identify the target after photo-cross-linking.

Photoaffinity labeling technology is emerging as a very useful tool for the identification and localization of proteins and their active sites in drug-discovery studies.[11–14] Not only do photoprobes enable the target protein to be identified but they also furnish structural information about the receptor-binding domain by forming a covalent bond between the ligand and the specific reactive protein. Various photophores, such as phenyldiazirine, arylazide, and benzophenone, have been used. The 3-aryl-3-trifluoromethyldiazirines appear to come closest to satisfying the chemical and biological criteria required for useful photophores.[15] A combination of this technology with the widely used avidin–biotin method has emerged as a powerful tool in many fields, especially in the design of molecular probes[16–20] and their utilization in proteomic studies. In our study, a trifunctional biotin-tagged photoaffinity probe of PF (Fig. 1) was designed and synthesized. The probe comprises a binding element, a photoreactive group, and an affinity tag. The binding element group ferries the probe to the enzyme active site. Upon UV irradiation, the photolabile group

Keywords: Synthesis; Photoaffinity probe; Paeoniflorin.
* Corresponding authors. Tel.: +86 21 50800954; fax: +86 21 50800721 (F.-J.N.); tel.: +86 21 50801313x132; fax: +86 21 50801552 (J.-L); e-mail addresses: jli@mail.shcnc.ac.cn; fjnan@mail.shcnc.ac.cn

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.03.021

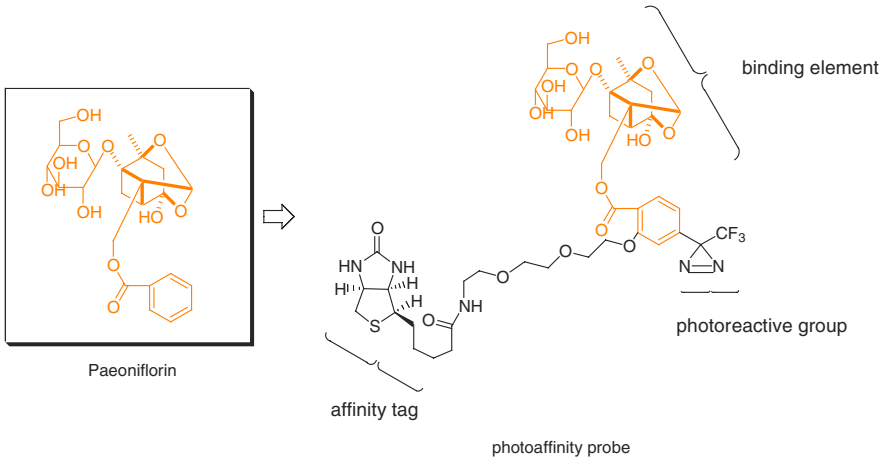


Figure 1. Structure of paeoniflorin and its photoaffinity probe.

covalently modifies the enzyme to form a covalent enzyme–probe adduct, and the tag renders the modified enzyme distinguishable from unlabeled proteins for subsequent purification, digestion, and sequencing.

In designing our trifunctional probe, we took into consideration previous reports that indicated that the benzoyl group of PF has little effect on its activity.[2] Consequently, we modified the photolabile unit and the tag at the phenyl group by inserting a flexible hydrophilic polyether spacer between the tag and the phenyl group (Fig. 1) to reduce the effect of the modification on the activity of PF. A competitive experiment was conducted (Fig. 2) to verify that the action mode of the photoaffinity probe was similar to that of its parent compound, PF.

The synthesis of the probe followed that shown in Scheme 1. First, two of the four hydroxyl groups of PF (PF was obtained from *P. alba radix* by isolation, purity >98%) were protected with TBSCl in the presence of imidazole in DMF to obtain compound **1**. The other two hydroxyl groups in the carbohydrates could not be protected easily and have low reactivity. Compound **2** was obtained by hydrolyzing compound **1** with LiOH in methanol to remove the benzoyl group. Condensation of compound **2** with compound **5**[21] using DCC and DMAP in dichloromethane produced compound **3**. Deprotection of compound **3** using aqueous tetrabutylammonium fluoride in THF yielded the trifunctional photoaffinity probe, compound **4**.[22]

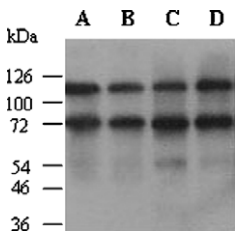
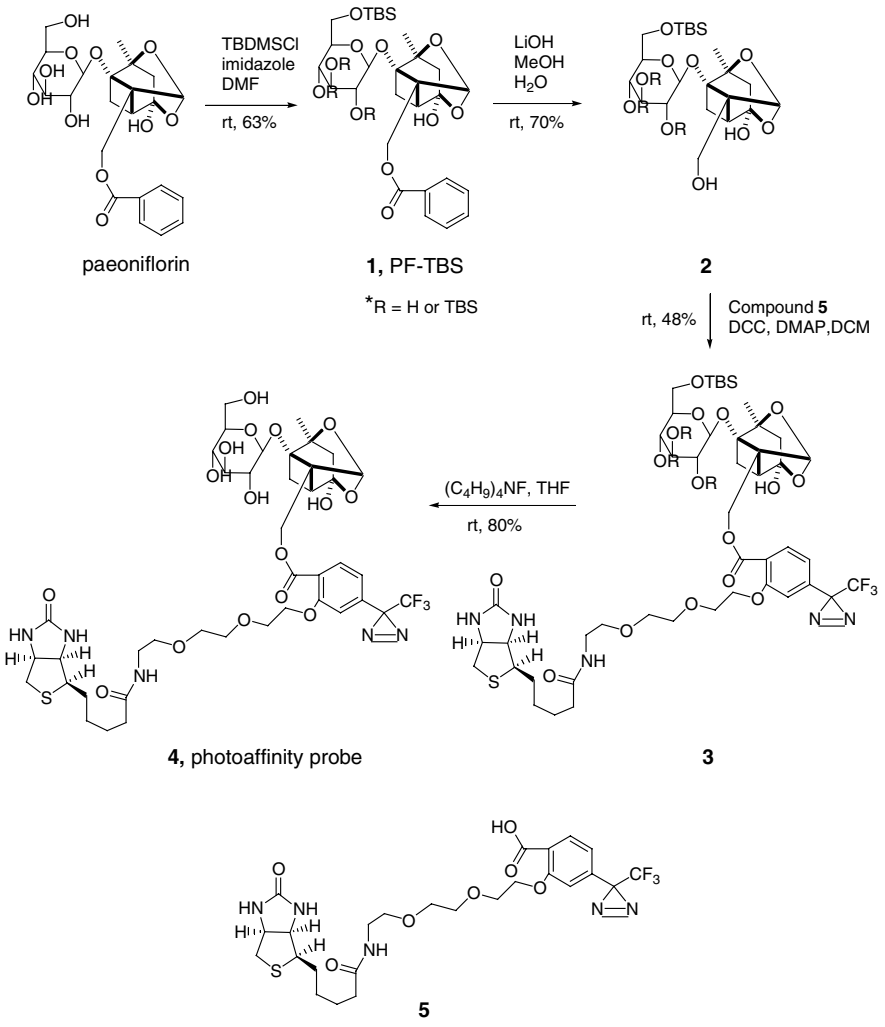


Figure 2. Photoaffinity labeling of rat cortex homogenate followed SDS–PAGE electrophoresis and then transfer onto PVDF membrane and detection with streptavidin–HRP. Samples were prepared by incubating 2.5 mg/mL cortex homogenate with 0 μM probe and exposed to UV light for 30 min (A), with 10 μM probe and exposed to UV light for 0 min (B), with 10 μM probe and exposed to UV light for 30 min (C), with 10 μM probe and 1 mM PF and then exposed to UV light for 30 min (D).

Photoaffinity labeling and streptavidin detection. The trifunctional probe was then evaluated for its ability to label target protein in homogenate preparation of rat cortex[23] in a preliminary labeling experiment.[24] The results showed that an approximate 54 kDa protein was specifically labeled by the probe (Fig. 2, lane C) and the cross-linked band could be decreased by adding PF competed with probe (Fig. 2, lane D). No specific labeling band could be detected if no probe was used (Fig. 2, lane A) or UV irradiation was omitted (Fig. 2, lane B). PF has been reported to exert a variety of pharmacological effects, suggestive of its binding to multiple protein targets, but to our knowledge, the exact mechanisms responsible for these effects and its molecular targets were unclear. This preliminary labeling experiment suggests that the present trifunctional probe (binding element–photoreactive group–affinity tag) for PF can be used to label its target proteins. By the way, the probe with an affinity group also provides the power to purify and identify the targets after photo-cross-linking.

A trifunctional probe (binding element–photoreactive group–affinity tag) for natural product Paeoniflorin was designed and synthesized. Preliminary labeling experiment suggests that this probe can be used to label its potential target protein specifically, and therefore provides a potential tool for purification and identification of the target protein, as well as for elucidation of the structural information of the binding site of target protein. Efforts to use this novel probe for further photoaffinity labeling and identification of the target protein are in progress.



Scheme 1. The synthesis of the probe.

Acknowledgments

The state key program of Basic Research of China (Grant 2004CB720300) and the Shanghai Commission of Science and technology (Grant 03dz19228), are appreciated for their financial support.

References and notes

1. Shibata, S.; Nakahara, M.; Aimi, N. *Chem. Pharm. Bull. (Tokyo)* **1963**, *11*, 372.
2. Hsu, F. L.; Lai, C. W.; Cheng, J. T. *Planta Med.* **1997**, *63*, 323.
3. Ye, J.; Duan, H.; Yang, X.; Yan, W.; Zheng, X. *Planta Med.* **2001**, *67*, 766.
4. Abdel-Hafez, A. A.; Meselhy, M. R.; Nakamura, N.; Hattori, M.; Watanabe, H.; Mohamed, T. A.; Mahfouz, N. M.; El-Gendy, M. A. *Chem. Pharm. Bull.* **1998**, *46*, 1486.
5. Liu, T. P.; Liu, M.; Tsai, C. C.; Lai, T. Y.; Hsu, F. L.; Cheng, J. T. *J. Pharm. Pharmacol.* **2002**, *54*, 81.
6. Ohta, H.; Ni, J. W.; Matsumoto, K.; Watanabe, H.; Shimizu, M. *Pharmacol. Biochem. Behav.* **1994**, *45*, 719.
7. Ohta, H.; Matsumoto, K.; Shimizu, M.; Watanabe, H. *Pharmacol. Biochem. Behav.* **1994**, *49*, 213.
8. Watanabe, H. *Behav. Brain Res.* **1997**, *83*, 135.
9. Cheng, J. T.; Wang, C. J.; Hsu, F. L. *Clin. Exp. Pharmacol. Physiol.* **1999**, *26*, 815.
10. Liu, D. Z.; Xie, K. Q.; Ji, X. Q.; Ye, Y.; Jiang, C. L.; Zhu, X. Z. *Br. J. Pharmacol.* **2005**, *146*, 604.
11. Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479.
12. Goeldner, M.; Kotzyba-Hibert, F.; Kapfer, I. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1296.
13. György, D.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64.
14. Hatanaka, Y.; Sadakane, Y. *Curr. Top. Med. Chem.* **2002**, *2*, 271.
15. Brunner, J.; Senn, H.; Richard, F. M. *J. Biol. Chem.* **1980**, *255*, 3313.
16. Hatanaka, Y.; Hashimoto, M.; Nishihara, S.; Narimatsu, H.; Kanaoka, Y. *Carbohydr. Res.* **1996**, *294*, 95.
17. Hatanaka, Y.; Kempin, U.; Jong-Jip, P. *J. Org. Chem.* **2000**, *65*, 5639.
18. Konoki, K.; Sugiyama, N.; Murata, M.; Tachibana, K.; Hatanaka, Y. *Tetrahedron* **2000**, *56*, 9003.

19. Sugimoto, T.; Fujii, T.; Hatanaka, Y.; Yamamura, S.; Ueda, M. *Tetrahedron Lett.* **2002**, *43*, 6529.
20. Fujii, T.; Sugimoto, T.; Yamamura, S.; Ueda, M. *Tetrahedron Lett.* **2003**, *44*, 2497.
21. Hatanaka, Y.; Kanaoka, Y. *Heterocycles* **1998**, *47*, 625.
22. Compound **4**: white solid powder. ^{1}H NMR (600 MHz, CD_3OD): δ 1.39 (s, 3H), 1.43–1.76 (m, 6H), 1.83 (d, J = 12.6 Hz, 1H), 1.97 (d, J = 10.2 Hz, 1H), 2.20–2.24 (m, 3H), 2.49 (m, 1H), 2.60 (d, J = 7.2 Hz, 1H), 2.73 (d, J = 12.6 Hz, 1H), 2.89 (dd, J = 12.6 Hz, 1H), 3.22–3.28 (m, 4H), 3.38 (m, 2H), 3.58 (t, J = 5.4 Hz, 2H), 3.68 (m, 3H), 3.76 (m, 2H), 3.87 (d, J = 12 Hz, 1H), 3.94 (m, 3H), 4.27 (s, 2H), 4.32 (m, 1H), 4.51 (m, 2H), 4.68 (dd of AB system, J = 12 Hz, 2H), 5.46 (s, 1H), 6.90 (s, 1H), 7.01 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H); ESI-HRMS (m/z): $[M+Na]^+$ calcd for $C_{41}H_{54}F_3N_5NaO_{16}S$, 984.3136; found 984.3104.
23. *Homogenate preparation of rat cortex*. Rats were sacrificed by cervical dislocation and brains were removed and immediately placed in ice-cold saline. After dissection of the cortex, tissues were homogenized in 15 volumes of 0.32 mol/L sucrose using a glass/Teflon homogenizer. The homogenate was centrifuged at 1000*g* for 10 min, and the supernatant was centrifuged at 36,000*g* for 30 min and then centrifuged at 48,000*g* for 10 min. The precipitate was resuspended in 30 volumes of 50 mM Tris·HCl buffer (pH 7.4), and centrifuged at 48,000*g* for 10 min. Finally, precipitate was resuspended in 5 volumes of 50 mM Tris·HCl buffer (pH 7.4) and stored at −80 °C. The protein concentration of the suspension was measured according to Bradford method.
24. *Photoaffinity labeling and streptavidin detection.* The cortex homogenate was diluted to 2.5 mg/mL with 50 mM Tris·HCl buffer (pH 7.4). The labeling reaction was initiated by incubating homogenate with the probe (dissolved in DMSO) at 4 °C for 8 h, and then exposed to UV 365 nm (220v, 6 W, 365 nm) at a distance of 3 cm. The reaction mixture was centrifuged at 48,000*g* for 10 min, then the supernatant was removed and the precipitate was resuspended in lysis buffer (urea 480 mg/L, chaps 40 mg/L, Tris–base 4.8 mg/L, DTT 10 mg/L, Ampholate 50 ml/L, and bromophenol blue 0.002%) at 4 °C for 1 h and centrifuged at 18,000*g* for 2 h. The supernatant was dialyzed against 50 mM Tris·HCl buffer (pH 7.4). To detect the proteins photo-cross-linked by probe, the homogenate photolabeled with probe was subjected to SDS–PAGE electrophoresis and then transferred onto polyvinylidene fluoride membrane. The membrane was blocked with 1% BSA in PBS with 0.05% Tween 20 at room temperature for 1 h, washed using PBS with 0.05% Tween 20 for 5 min by two times, and then incubated with streptavidin–HRP polymer conjugate (Sigma, Cat.S 2438) diluted 1:10,000 in PBS with 0.05% Tween 20 at room temperature for 1 h. After six washes for 5 min each using PBS with 0.05% Tween 20, the membrane was treated with Enhanced ChemiLuminescence (ECL, Amersham) detection reagents. Biotinylated proteins were visualized by exposure to X-ray and imaging development.