Synthesis and Physical Nature of Fluorescent Photoaffinity Probe for the Bioorganic Studies on Tautomycin, a Protein Phosphatase Type 1 Selective Inhibitor

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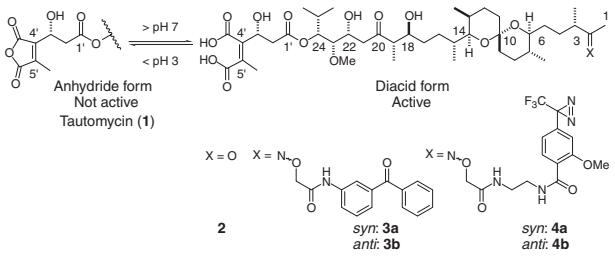
Fluorescent photoaffinity probe, which possesses a dansyl amide functional group on the maleic moiety of tautomycin, was prepared in order to detect the trace amount of labeled peptides. The parent compound dramatically showed fluorescence quenching before the photoreaction due to the exciplex formation on the basis of the folded conformation. Several benzhydrol analogs, on the other hand, recovered the strong fluorescence; thus, this system could be employed for the photoaffinity labeling studies.

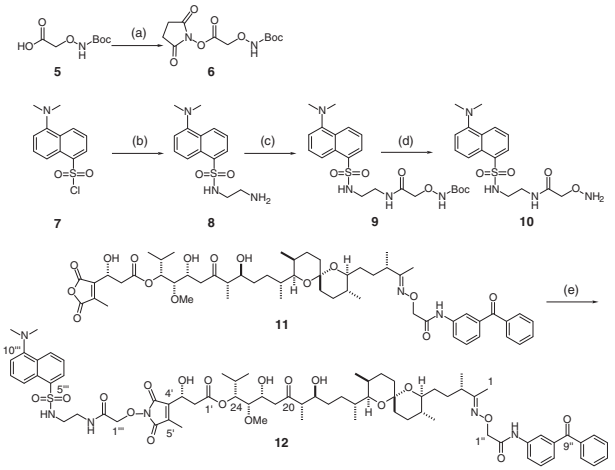
Tautomycin (TTM, **1**) has been known as protein phosphatase type 1 (PP1) selective inhibitor,[1,2] and the active inhibitor is not the anhydride (**1**) but the dicarboxylic acid form (**2**).[3] While okadaic acid largely inhibits PP2A and weakly does PP1, and calyculin and microcystin-LR are inhibitors to both phosphatases.[2] In 1995 the X-ray crystallographic analysis of PP1–microcystin-LR complex provided the details of molecular interaction between the macro molecule and the toxin.[4] Successively the X-ray structures were reported on PP1 complexes with okadaic acid (2001)[5] and with calyculin (2002).[6] PP1–TTM complex, however, has not yet crystallized as a nature of TTM. We planned, therefore, photoaffinity experiments to study the molecular interaction.



Scheme 1. Tautomycin and photoaffinity probes.

Recently, we have accomplished the synthesis of two types of photoaffinity probes (**3**, **4**), which possesses a benzophenone or a diazirine photophore linking at the 2 position of TTM in order to study the binding site of PP1.[7] Further, we designed to introduce a fluorescent unit into tautomycin moiety in order to detect the peptides after the photoreaction. The principles are: (i) photoaffinity labeling, (ii) tryptic digestion, (iii) acidic transformation into the maleic anhydride, and (iv) the fluorescent unit introduction into the anhydride moiety. On the basis of this plan, we describe the synthesis of a fluorescent photoaffinity probe to detect the labeled peptides using HPLC–(UV–FL)–ESI–Q–TOF–MS.

Synthesis of the fluorescent photoaffinity probe (**12**) is summarized in Scheme 2. Dansyl chloride (**7**) was connected as an efficient fluorophore with the ethylenediamine to provide the amine **8**, which was successively connected with the activated ester **6** in the presence of triethylamine (Et_3N) to furnish the compound **9**. The protective *t*-butoxycarbonyl (Boc) group of **9** was removed with TFA in CH_2Cl_2 to afford the fluorescent unit **10** as a green oil.[8] The coupling reaction was performed with **11**, one of the synthesized photoaffinity probes.[7] The maleimidation of **11** with **10** was carried out in 70% *N*,*N*′-dimethylacetamide (DMA)/H_2O at pH 4, which was successively purified by HPLC [ODS-UG-5; 250 × 10.0 mm i.d., 90% CH_3CN/H_2O, 4.0 mL/min, $T_r = 3.5$ (**10**), 11.5 (**11**), and 13.5 min (**12**)] to furnish the pure fluorescent photoaffinity probe (**12**) as a light yellow oil in 83% yield.[9] The excess **10** was recovered in 82% yield. No oxime formation was observed at the 20-ketone position under this condition presumably due to the steric congestion.



Scheme 2. Synthesis of fluorescent photoaffinity probe: (a) *N*-hydroxysuccinimide, EDC·HCl, DMF, rt, 36 h, 78%; (b) ethylenediamine, Et_3N, CH_2Cl_2, rt, quant.; (c) **6**, Et_3N, DMF, rt, 1 h, 68%; (d) TFA/CH_2Cl_2 (1:1), 0 °C, 1 h, 87%; (e) **10**, 70% DMA/H_2O, pH 4, rt, 16 h, then purification by HPLC, 83%.

The fluorescent photoaffinity probe (**12**) surprisingly showed a dramatic fluorescence quenching (Figure 1a); thus, the ratio of the fluorescent intensity between **9** and **12** being 9:1. Figure 1b shows the UV–vis absorption of **9**, **11**, and **12**. The band at around 306 nm of **11** is the absorption of the benzophenone moiety, and the band at 340 nm of **9** is the dansyl amide moiety. The fluorescent emission wavelength of dansyl amide moiety does not overlap with the absorption bands of benzophenone and dansyl amide moieties. No energy transfer process would thus be possible. There might be some orbital interaction at the excited states between the 2 chromophores. The lower intensity of the fluorescence might be due to an exciplex formation.

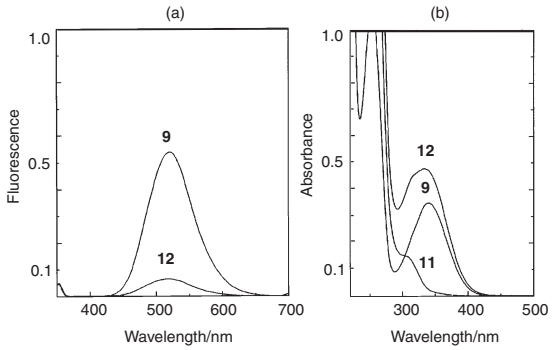


Figure 1. (a) Fluorescence emission spectra of **9** and **12** in acetonitrile (0.2×10^{-4} M) at room temperature. (b) UV–vis absorption spectra of **9**, **11**, and **12** in acetonitrile (1.0×10^{-4} M) at room temperature.

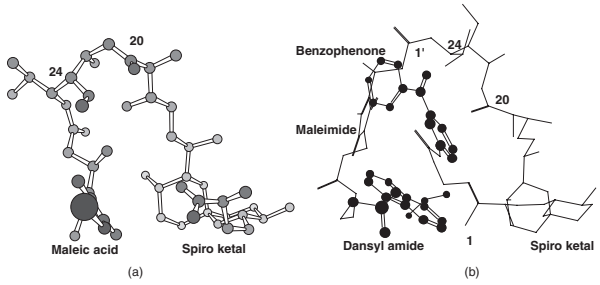


Figure 2. Proposed 3-D structures of **2** and **12**; (a) One of the stable conformations of **2**; (b) One of the energy-minimized conformations of **12** on the basis of **2**.

Figure 2a shows one of the stable conformations of tautomycin diacid (**2**), which was reported previously through computer calculation with Biograf and NMRgraf programs using NOESY data.[3] On the basis of these data, we calculated for **12** to have energy-minimized conformer with a Macromodel (MMFF force field).[10] One of the resulting six conformers within 12.55 kJ/mol is shown in Figure 2b. The distance between benzophenone and dansyl amide moieties is 3.8–5.5 Å, which is short enough for the interaction. These results indicate that the fluorescence quenching occurred owing to the folded conformation of TTM. Moreover, reduction of **12** with sodium borohydride in MeOH led to two benzhydrol derivatives (**13**, **14**), which exhibited the usual fluorescence intensity.[11,12] Recovery of the fluorescence might be due to the absence of interacting chromophore. The ratio of fluorescent intensity between of **12** and **13** or **14** was about 1:10 by the fluorophotometer equipped on a HPLC. This means that the fluorescent photoaffinity labeled peptides should have the enough intensity, and could be detected by a fluorophotometer. Further studies are in progress in order to detect the labeled peptides through this strategy.

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References and Notes

1 For structure of tautomycin, see: a) M. Ubukata, X.-C. Cheng, and K. Isono, *J. Chem. Soc., Chem. Commun.*, **1990**, 244. b) X.-C. Cheng, M. Ubukata, and K. Isono, *J. Antibiot.*, **43**, 809 (1990). c) M. Ubukata, X.-C. Cheng, M. Isobe, and K. Isono, *J. Chem. Soc., Perkin Trans. 1*, **1993**, 617.

2 A. Takai, K. Sasaki, H. Nagai, G. Mieskes, M. Isobe, K. Isono, and T. Yasumoto, *Biochem. J.*, **306**, 657 (1995).

3 Y. Sugiyama, I. I. Ohtani, M. Isobe, A. Takai, M. Ubukata, and K. Isono, *Bioorg. Med. Chem. Lett.*, **6**, 3 (1996).

4 J. Goldberg, H. Huang, Y. Kwon, P. Greengard, A. C. Nairn, and J. Kuriyan, *Nature*, **376**, 745 (1995).

5 J. T. Maynes, K. S. Bateman, M. M. Cherney, A. K. Das, H. A. Luu, C. F. B. Holmes, and M. N. G. James, *J. Biol. Chem.*, **276**, 44078 (2001).

6 A. Kita, S. Matsunaga, A. Takai, H. Kataiwa, T. Wakimoto, N. Fusetani, M. Isobe, and K. Miki, *Structure*, **10**, 715 (2002).

7 M. Kurono, A. Shimomura, and M. Isobe, *Tetrahedron*, **60**, 1773 (2004).

8 Fluorescent unit **10**: UV–vis (CH_3CN) λ_{max} (ε) 340 nm (3509). IR (KBr) λ_{max} 3449, 2926, 1637 cm^{-1}. FL (CH_3CN) λ_{ex} 346 nm, λ_{em} 522 nm. ^1H NMR (400 MHz, $CDCl_3$) δ 2.89 (6H, s, $N(CH_3)_2$), 3.08 (2H, ddd, $J = 7.5$, 6.0, 4.0, $NHCH_2CH_2NH$), 3.39 (2H, ddd, $J = 7.5$, 6.0, 4.0, $NHCH_2CH_2NH$), 4.07 (2H, s, $COCH_2O$), 5.53 (1H, br t, $J = 6.0$, CH_2NH), 5.75 (2H, br s, ONH_2), 6.83 (1H, br s, $NHCH_2$), 7.19 (1H, br d, $J = 7.5$, Ar*H*), 7.52 (1H, dd, $J = 8.5$, 7.5, Ar*H*), 7.57 (1H, dd, $J = 8.5$, 7.5, Ar*H*), 8.23 (1H, dd, $J = 7.5$, 1.5, Ar*H*), 8.26 (1H, br d, $J = 8.5$, Ar*H*), 8.55 (1H, br d, $J = 8.5$, Ar*H*). ESI–Q–TOF–MS calcd for $C_{16}H_{23}N_4O_4S^+$ 367.1440 ($[M + H]^+$); found 367.1499.

9 Fluorescent photoaffinity probe **12**: UV–vis (CH_3CN) λ_{max} (ε) 333 nm (4809). IR (KBr) λ_{max} 3421, 2927, 1728, 1663, 1540, 1320, 1098, 793 cm^{-1}. FL (CH_3CN) λ_{ex} 347 nm, λ_{em} 518 nm. ^1H NMR (600 MHz, $CDCl_3$) δ 0.76 (3H, d, $J = 6.8$, 7–CH_3), 0.85–1.70 (18H, m), 0.79 (3H, d, $J = 7.0$ Hz, 13-CH_3), 0.95 (3H, d, $J = 7.0$ Hz, 25-CH_3), 0.95 (3H, d, $J = 7.0$ Hz, H-26), 0.96 (3H, d, $J = 7.0$ Hz, 15-CH_3), 1.06 (3H, d, $J = 7.0$ Hz, 19-CH_3), 1.08 (3H, d, $J = 7.0$ Hz, 3-CH_3), 1.84 (1H, tt, $J = 13.0$, 5.0 Hz, H-12b), 1.92 (3H, s, H-1), 2.10 (1H, m, H-25), 2.18 (3H, s, 5′-CH_3), 2.45 (1H, m, H-3), 2.67 (1H, m, H-19), 2.71 (1H, dd, $J = 17.5$, 4.0 Hz, H-21a), 2.84 (1H, dd, $J = 16.0$, 9.0 Hz, H-2′a), 2.87 (6H, s, $N(CH_3)_2$), 2.91 (1H, dd, $J = 16.0$, 4.0 Hz, H-2′b), 2.96 (1H, dd, $J = 17.0$, 8.0 Hz, H-21b), 3.09 (2H, m, $NHCH_2CH_2NH$), 3.14 (1H, t, $J = 10.0$ Hz, H-6), 3.23 (1H, dd, $J = 10.0$, 2.0 Hz, H-14), 3.24 (1H, br s, 22-OH), 3.27 (1H, dd, $J = 6.0$, 2.0 Hz, H-23), 3.33–3.37 (3H, m, $NHCH_2CH_2NH$, 18-O*H*), 3.40 (3H, s, OCH_3), 3.70 (1H, m, H-18), 4.36 (1H, m, H-22), 4.37 (2H, s, H-1‴), 4.43 (1H, br s, 3′-OH), 4.56 (1H, d, $J = 16.0$ Hz, H-1″a), 4.60 (1H, d, $J = 16.0$ Hz, H-1″b), 5.08 (1H, t, $J = 6.0$ Hz, H-24), 5.18 (1H, m, H-3′), 5.63 (1H, t, $J = 6.0$, SO_2NHCH_2), 7.17 (1H, br d, $J = 7.5$, H-9‴), 7.44 (1H, t, $J = 8.0$, H-7″), 7.47–7.52 (4H, m, H-6″, 12″, 14″, 13‴), 7.54 (1H, dd, $J = 8.5$, 7.5, H-8‴), 7.60 (1H, t, $J = 7.5$ Hz, H-13″), 7.80 (3H, m, H-4″, 11″, 15″), 7.90 (1H, br t, $J = 6.0$, CH_2NHCO), 7.96 (1H, br d, $J = 8.0$ Hz, H-8″), 8.16 (1H, br s, CONHAr), 8.20 (1H, dd, $J = 7.0$, 1.0, H-12‴), 8.26 (1H, br d, $J = 8.5$, H-7‴), 8.50 (1H, br d, $J = 8.50$, H-14‴). ESI–Q–TOF–MS calcd for $C_{72}H_{99}N_6O_{18}S^+$ 1367.6737 ($[M + H]^+$); found 1367.6808.

10 Computer calculation was carried out on a Silicon Graphics Octan computer. Energy-minimization was performed on 10,000 initial starting conformations with global minimum search program using Macromodel version 7.1 with MMFF force filed. a) F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, and W. C. Still, *J. Comput. Chem.*, **11**, 440 (1990). b) T. A. Halgren, *J. Comput. Chem.*, **20**, 730 (1999), and references cited therein.

11 **13**: Reduced compound at C9″ and C20, ESI–Q–TOF–MS calcd for $C_{72}H_{104}N_6O_{18}S^{2+}$ 686.359 ($[M + 2H]^{2+}$); found 686.332.

12 **14**: Reduced compound at C9″, C20 and C2, ESI–Q–TOF–MS calcd for $C_{72}H_{106}N_6O_{18}S^{2+}$ 687.367 ($[M + 2H]^{2+}$); found 687.341.

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