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NOVEL NON-SQUALENOID PHOTOAFFINITY LABELS FOR MAMMALIAN SQUALENE EPOXIDASE

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Abstract. Specific affinity labeling of the squalene binding site of squalene epoxidase remains an elusive goal. We wish to report here the design and synthesis of six novel, non-squalenoid, photoaffinity labels for squalene epoxidase, based on the potent inhibitor NB-598 (**3**).

Squalene epoxidase (SE) (EC 1.14.99.7) catalyzes the conversion of squalene (**1**) to (3*S*)-2,3-oxidosqualene.[1] SE is a flavoprotein monoxygenase and requires FAD, NADPH-cytochrome P-450 reductase (EC 1.6.2.4), NADPH, oxygen, and either detergent or a soluble protein factor for full enzymatic activity.[2,3] SE has been the focus of efforts to develop hypocholesterolemic, herbicidal and antifungal agents.[4,5] Recently, Banyu Pharmaceuticals reported that NB-598 (**3**) was a highly-potent, competitive, and specific inhibitor of mammalian SE.[6] The structure of NB-598 was derived by structure-activity modifications based on the allylamine[5] terbinafine (**2**), a potent inhibitor of fungal SE. Based on these structural observations and as a continuation of our program to prepare affinity labels for the squalene binding site of SE[7-9], we have synthesized a series of six novel non-squalenoid photoprobes.

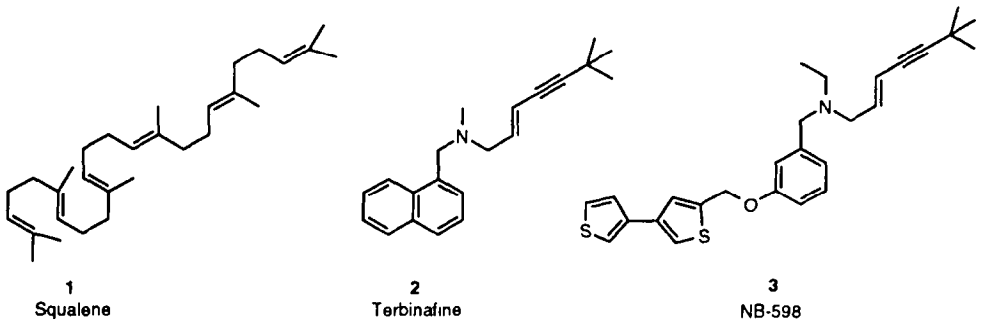


Figure 1. Structures of the substrate (**1**) and inhibitors of fungal (**2**) and mammalian (**3**) SE.

Design

The characteristics of these potent allylamine-type SE inhibitors and subsequent SAR studies[10] guided the design of our probes (Figure 2). Analysis of the structure of the antifungal allylamines or benzylamines[11,28] and the NB-598 class of vertebrate inhibitors[12] seems to support a dissection of these inhibitors into three subunits (Figure 2a).

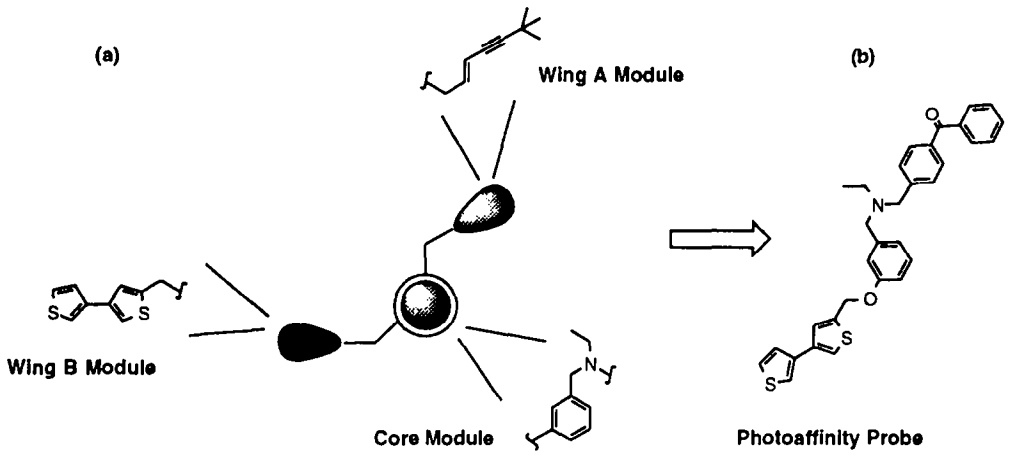
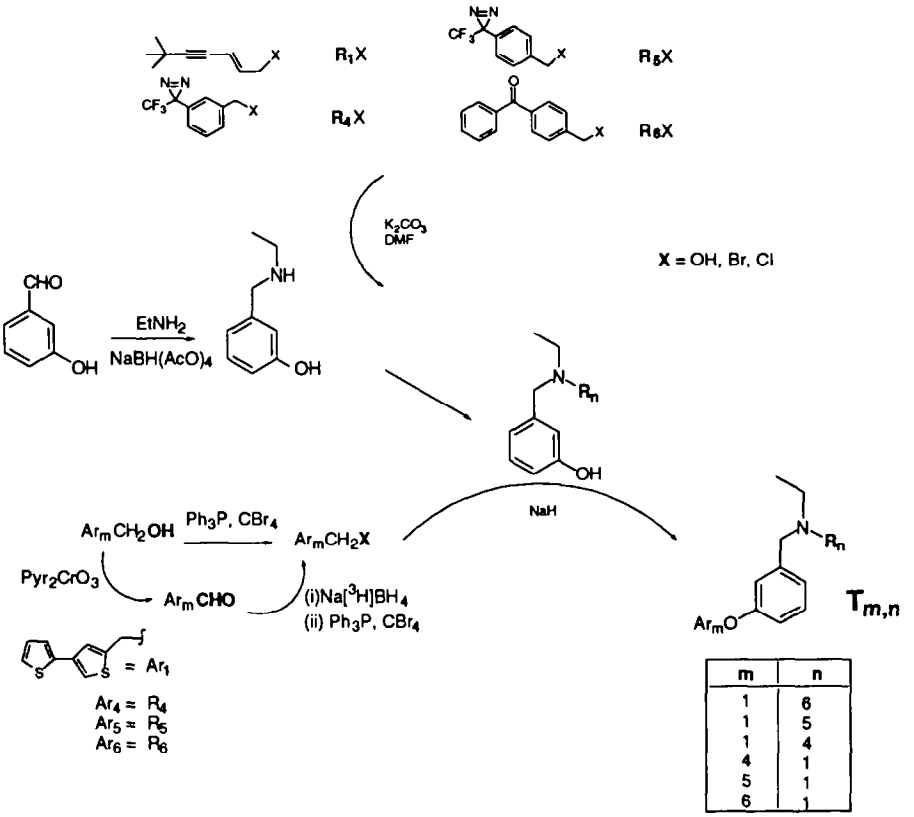


Figure 2. (a) Modular dissection of NB-598 into three subunits representing the different pharmacophoric regions. (b) One of the six photoaffinity probes ($T_{1,6}$).

Following this retrosynthetic analysis, six novel probes were designed and synthesized (Figure 3) with the hope that they could help in the purification and characterization of the binding site of pig and rat SE. To this end, each of the wing modules was in turn replaced with one of three photoreactive modules bearing either a diazirine moiety (R_4 and R_5, Figure 3) or a benzophenone moiety (R_6, Figure 3); the complementary wing was kept as in NB-598 (either R_1 or Ar_1, Figure 3). The choice of probes reflected the need for high cross-linking selectivity and efficiency, combined with maximum analogy to known potent inhibitors of the allylamine or benzylamine family.[11,28] The benzophenone moiety was expected to be a superior probe[13,14] in terms of its preparation, stability to reagents and ambient light, cross-linking efficiency, and mild conditions for photoactivation.



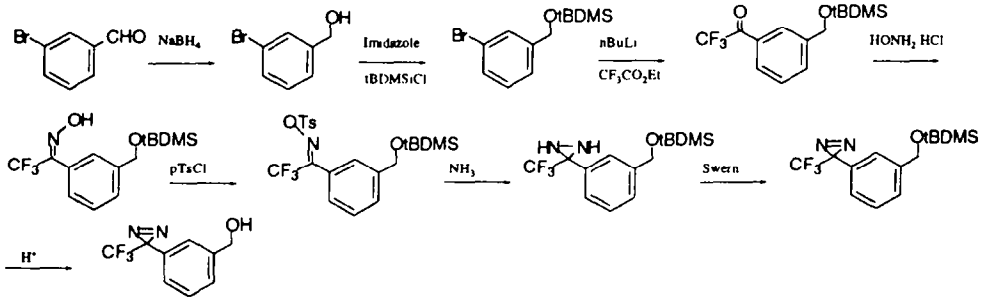
m	n
1	6
1	5
1	4
4	1
5	1
6	1

Figure 3. Convergent synthesis of the tritium-labeled photoaffinity labels.

Synthesis

The core and the wing modules were prepared independently and then assembled as shown in Figure 3. The convergent synthesis allowed facile introduction of the radiolabeled wing module at the last step.

The core module was prepared by reductive amination of 3-hydroxybenzaldehyde.[15] The synthesis of the allylic[16] (R_1, X = OH) and benzophenone[17] (R_6, X = Cl) modules was accomplished following literature procedures. In addition, the halo-benzophenone (R_6, X = Cl) was hydrolyzed to the corresponding hydroxy derivative[18], in order to introduce the radiolabel after preliminary oxidation. The preparation of the diazirine modules (R_4 and R_5, X = OH) followed a combination of literature methods (Scheme 1). In particular, the oxidation of diaziridines to diazirines was accomplished by the Swern oxidation method.[23] This method was found most reliable compared to the classical route[20] for our compounds.



Scheme 1. Synthesis of the diazirine R_4.

Each of the wing modules (X = OH) was subsequently transformed into the corresponding halo-derivative[24,25] before coupling to the core module. In each case, the radioactive label was incorporated into the B-wing module via reduction of the aldehyde derivative with tritiated sodium borohydride. In this way, the manipulation of both radioactive and light-sensitive compounds was reduced to a minimum, since the B-wing module was introduced in the last step of the synthesis.

These photolabels[29] are currently being used in our laboratory for the labeling of pig and rat SE. However, their inhibitory capacity was considerably decreased compared to their parent molecule (NB-598). All photoprobes showed IC_{50} values[27] exceeding 500 μM for the inhibition of partially-purified pig SE.[26]

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(28) Note added to revision: Nussbaumer et al. (*J. Med. Chem.* **1994**, *37*, 610-615) independently confirmed the SAR dissection used for the design of allylamine or benzylamine photoaffinity analogs in this work.

(29) Spectroscopic data.

$T_{4,1}$: ^{1}H NMR ($CDCl_3$) δ: 7.52-6.81 (8 H), 6.06 (1 H, dt, J_{vinyl} = 15.9 Hz, J_{allyl} = 6.4 Hz), 5.65 (1 H, d, J_{vinyl} = 15.9 Hz, H2'), 5.05 (2 H, s), 3.53 (2 H, s), 3.09 (2 H, d, J_{allyl} = 6.3 Hz), 2.51 (2 H, q, J = 6.9 Hz), 1.24 (9 H, s), 1.03 (3 H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 11.96 (q); 27.83 (s); 31.01 (q; 47.32 (t); 55.15 (t); 57.69 (t); 69.35 (t); 77.35 (s); 98.34 (s); 112.37 (d); 113.46 (d); 115.07 (d); 121.71 (d); 122.17 (q, $^2J_{CF}$ = 275 Hz); 125.37 (d); 126.05 (d); 128.66 (d); 129.18 (d); 129.10 (d); 129.50 (s); 138.44 (d); 139.43 (d); 141.64 (s); 158.64 (s). MS $C_{27}H_{30}N_3OF_3$: calcd. 469.2341, found 469.2340, dev -0.1 ppm.

$T_{5,1}$: ^{1}H NMR ($CDCl_3$) δ: 7.48 (2 H, d, J = 8.1 Hz), 7.22 (3 H, d and dd), 6.98 (1 H, s), 7.00 (1 H, d, J = 7.4 Hz), 6.81 (1 H, d, J = 7.9 Hz), 6.09 (1 H, dt, J_{vinyl} = 15.9 Hz, J_{allyl} = 6.4 Hz), 5.65 (1 H, d, J_{vinyl} = 15.9 Hz, H2'), 5.09 (2 H, s), 3.55 (2 H, s), 3.10 (2 H, d, J_{allyl} = 6.4 Hz), 2.51 (2 H, q, J = 7.2 Hz), 1.26 (9 H, s), 1.04 (3 H, t, J 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.01 (q); 27.92 (s); 31.05 (q); 47.33 (t); 55.15 (t); 57.67 (t); 69.15 (t); 112.31 (d); 113.33 (d); 115.03 (d); 121.65 (d); 126.77 (d); 127.72 (d); 128.66 (s); 129.20 (d); 139.20 (d); 139.54 (s); 141.71 (s); 158.64 (s). MS $C_{27}H_{30}N_3OF_3$: calcd. 469.2341, found 469.2354, dev 2.8 ppm.

$T_{6,1}$: ^{1}H NMR ($CDCl_3$) δ: 7.87-7.48 (9 H, m), 7.25 (1 H, dd, J = 7.8 Hz), 7.05 (1 H, s), 6.96 (1 H, d, J = 7.5 Hz), 6.88 (1 H, d, J = 8.1 Hz), 6.10 (1 H, dt, J_{vinyl} = 15.9 Hz, J_{allyl} = 6.3 Hz), 5.68 (1 H, d, J_{vinyl} = 15.9 Hz), 5.18 (2 H, s), 3.57 (2 H, s), 3.12 (2 H, d, J_{allyl} = 6.6 Hz), 2.53 (2 H, q, J = 6.9 Hz), 1.26 (9 H, s), 1.05 (3 H, t, J = 6.9 Hz). ^{13}C NMR ($CDCl_3$) δ: 12.07 (q); 27.96 (s); 30.10 (q); 47.32 (t); 55.14 (t); 57.62 (t); 69.32 (t); 77.31 (s); 98.33 (s); 112.34 (d); 113.29 (d); 115.01 (d); 121.67 (d);127.05; 128.35; 129.29; 130.08; 130.43; 132.48; 137.09; 137.65; 139.63; 141.67; 141.98; 158.64 (s); 196.38 (s). MS $C_{32}H_{35}N_2O$: calcd. 465.2668; found 469.2677; dev 2.0 ppm.

$T_{1,4}$: ^{1}H NMR ($CDCl_3$) δ: 6.86-7.40 (13 H, m), 5.21 (2 H, s), 3.55 (2 H, s) 3.53 (2 H, s), 2.47 (2 H, q, J = 7.0 Hz,), 1.05 (3 H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 11.91 (q); 47.37 (t); 55.42 (t); 57.75 (t); 65.08 (t); 113.60 (d); 115.09 (d); 119.80; 120.27; 121.69 (d); 122.21 (q, $^2J_{CF}$ = 275 Hz); 124.86; 125.90; 126.07; 126.176; 126.44; 128.66; 129.02; 129.22; 129.83; 136.97 (s); 137.14 (s); 140.20 (s); 141.22 (s), 141.52 (s), 158.64 (s). ^{19}F NMR ($^{15}NH_4Cl$) δ: 54.51. MS $C_{27}H_{24}F_3N_3OS_2$: calcd. 527.1313, found 527.1313, dev -0.1 ppm.

$T_{1,5}$: ^{1}H NMR ($CDCl_3$) δ: 7.39 (2 H, d, J = 7.8 Hz), 7.30-7.35 (5 H, m), 7.24 (1 H, dd, J = 7.8 Hz), 7.12 (2 H, d, J = 8.4 Hz), 7.04 (1 H, s), 6.96 (1 H, d, J = 7.8 Hz), 6.88 (1 H, d, J = 8.4 Hz), 5.22 (2 H, s), 3.56 (2 H, s), 3.53 (2 H, s), 2.48 (2 H, q, J = 7.2 Hz), 1.05 (3 H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 11.88 (q); 28.37 (q, $^1J_{CF}$= 40.3 Hz); 47.27 (t); 55.22 (t); 57.71 (t); 65.06 (t); 113.32 (d); 115.28 (d); 119.85 (d); 120.31; 121.68 (d); 122.17 (q, $^2J_{CF}$ = 275 Hz); 125.92 (d); 126.12 (d); 126.33 (d) 127.47 (s); 128.97 (d); 129.24 (d); 136.97 (s); 137.09 (s); 140.14 (s); 141.54 (s); 142.05 (s); 158.36 (s). ^{19}F NMR ($^{15}NH_4Cl$) δ: 54.3194. MS $C_{27}H_{24}F_3N_3OS_2$: calcd. 527.1313, found 527.1314, dev 0.3 ppm.

$T_{1,6}$: ^{1}H NMR ($CDCl_3$) δ: 7.20-7.35 (15 H, m), 7.07 (1 H, s), 6.98 (1 H, d, J = 7.5 Hz), 6.86 (1 H, d, J = 8.0 Hz), 5.21 (2 H, s), 3.63 (2 H, s), 3.57 (2 H, s), 2.52 (2 H, q, J = 7.1 Hz), 1.08 (3 H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 11.93 (q); 47.46 (t); 55.60 (t); 57.88 (t); 65.14 (t); 113.43 (d); 115.29 (d); 119.80 (d); 120.24 (d); 121.71 (d); 125.90 (d); 126.06 (d); 126.12 (d); 128.16 (d); 128.37 (d); 129.18 (d); 130.11 (d); 132.14 (d); 136.16 (s); 136.94 (s); 137.08 (s); 137.86 (s); 140.17 (s); 141.56 (s); 145.28 (s); 158.38 (s); 196.38 (s). MS $C_{32}H_{29}NO_2S_2$: cald. 523.1640, found 527.1637; dev -0.5 ppm.

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