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DESIGN AND SYNTHESIS OF NEW POTENTIAL PHOTOAFFINITY LABELS FOR MAMMALIAN SQUALENE EPOXIDASE

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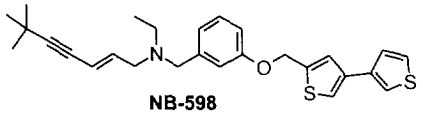
Abstract: Aromatic azides derived from known inhibitors have been prepared as potential photoaffinity labels for mammalian squalene epoxidase. Three of them (compounds **5b**, **5c** and **6**) were found to inhibit pig liver SE with submicromolar affinities and to block cholesterol biosynthesis in HepG2 cells. © 1997 Elsevier Science Ltd.

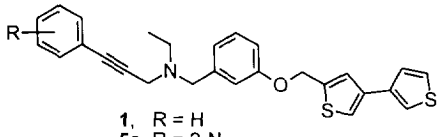
Squalene epoxidase (SE) (EC 1.14.99.7) catalyzes the conversion of squalene to (3*S*)-2,3-oxidosqualene. This enzymatic reaction is known to be the first step among oxygenase catalyzed reactions in cholesterol biosynthesis requiring O_2, NADPH and FAD for full activity[1].

Banyu Pharmaceutical has reported NB-598 as a highly potent specific inhibitor of SE with promising hypocholesterolemic properties[2].



NB-598

New 3,3'-bisthienyl derivatives of type **1** (analogs of NB-598) have been found by Yamanouchi to be potent SE inhibitors[3], and, as part of our program directed toward the discovery of new cholesterol lowering drugs, we have identified a new series of aryloxymethylsilane derivatives of type **2** as potent, orally active SE inhibitors.[4]



1, R = H
5a, R = 2-N_3
5b, R = 3-N_3
5c, R = 4-N_3

2, R = CN
6, R = N_3

In order to have a better understanding of the binding sites of inhibitors such as **1** or **2** for mammalian SE, we initiated a program to develop new photoaffinity labeling reagents[5] which could provide important information concerning the amino-acid residues of the enzyme involved in inhibitors recognition.[6]

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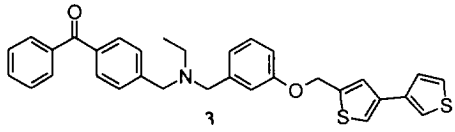
Attempts have been made by the group of Prestwich[7] to design photoprobes based on the structure of NB-598 using a benzophenone moiety of type **3** or a diazirine moiety of type **4** as the photoreactive modules but their inhibitory capacity (IC_{50}>500 μM) was considerably decreased compared to NB-598 and too low to conduct further photolabeling experiments.



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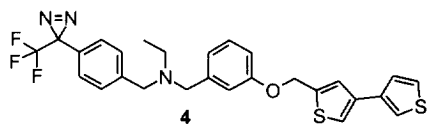

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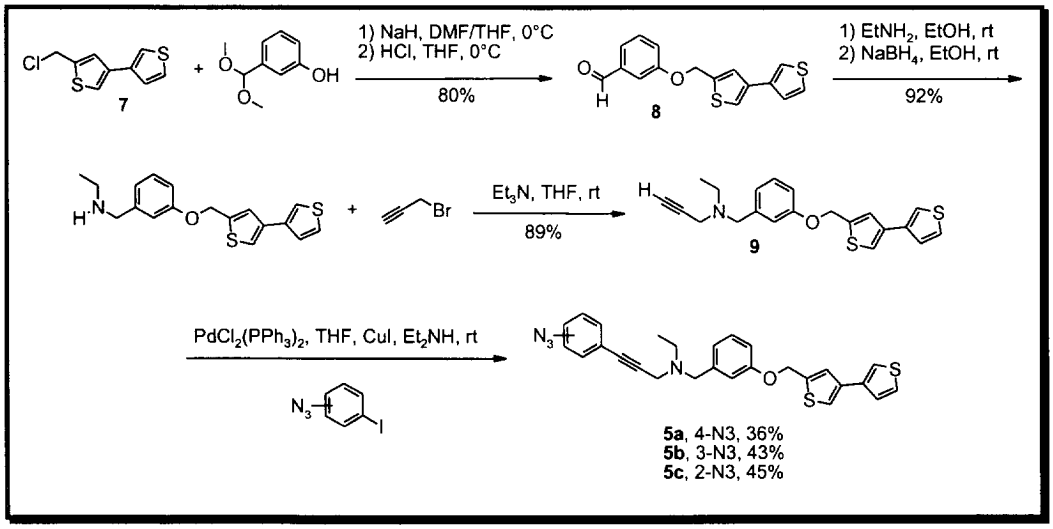
We focused our attention on the use of aryl azide photoprobes based on the backbones of compound **1** or compound **2** hoping that the small azide group would give us the maximum analogy with the parent derivatives. Furthermore, we expected that photoactivation of the azide group in compounds **5a-c** and **6** would hopefully interact with different amino acid residues of the binding site and therefore give interesting topological information concerning the binding mode of such inhibitors. We report here the synthesis of aromatic azides **5a-c** and **6** as well as their preliminary biological properties.

The synthesis of compounds **5a-c** was accomplished according to scheme 1 and started from bisthienyl derivative **7** which preparation was described in a previous paper.[8] Treatment of the dimethylacetal derived from 3-hydroxybenzaldehyde with chloride **7** in the presence of NaH followed by deprotection with dilute hydrochloric acid afforded aldehyde **8** in 80% overall yield. A two steps reductive amination sequence and alkylation of the so-formed amine by propargyl bromide ended the preparation of key derivative **9** in 80% yield. Sonogashira coupling[9] between **9** and the appropriate iodophenylazide[10] *via* a palladium catalyzed reaction furnished the expected compounds **5a-c** in moderate yields.[11] Altogether, the route described here to produce derivatives **5a-c** proved to be an efficient way of preparing potential photoactive analogs of compound **1**.

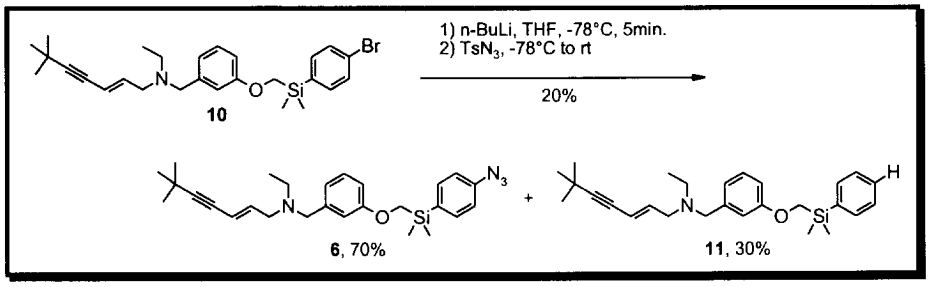
Scheme 1



The preparation of azide **6** was first envisioned starting from the previously reported[4] arylbromide **10** by a straightforward strategy i.e. low temperature bromine-lithium exchange followed by treatment of the formed anion with tosylazide[12] to generate either a triazene or directly the azido group.[13] However, under these experimental conditions (scheme 2), a mixture of compound **6** and **11** was obtained in low yield, separation being only possible by semi-preparative HPLC.

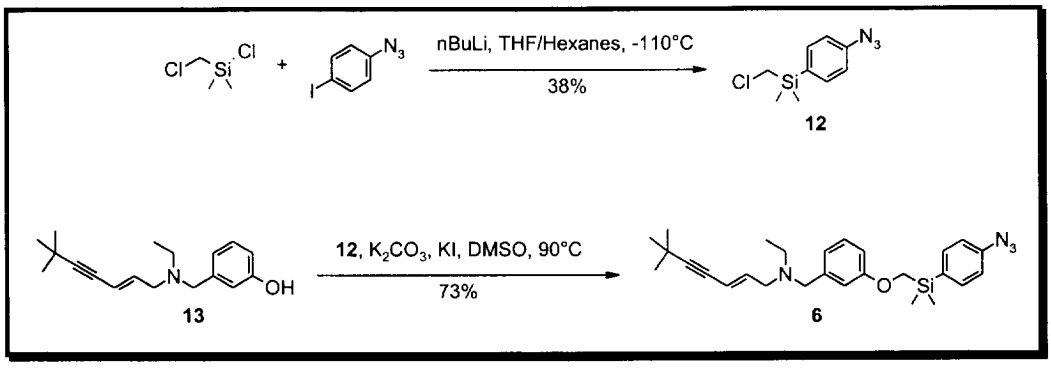
Our efforts to circumvent the production of **11** i.e. use of t-BuLi (1 or 2 equiv.), very low temperature experiments (< -100°C) or one-pot treatment to avoid a possible prototropy within the molecule were disappointing and led us to follow a more successful back-up strategy.

Scheme 2



Compound **6** could thus be obtained by a convergent approach as described in scheme 3. The crucial step was the preparation of azide **12** which could be reached by the one-pot treatment of 4-iodophenylazide with n-butyllithium at -110°C in the presence of chloromethyldimethylchlorosilane. Alkylation of phenol **13** with azide **12** was then conducted as described earlier[4] to provide cleanly **6** in 70 % yield.[14]

Scheme 3



The potential SE inhibitors **5a-c** and **6** have been evaluated as *in vitro* inhibitor of pig liver microsomal SE[15]and as cholesterol biosynthesis inhibitors in human HepG2 cells by measuring the extend of cholesterol radiolabeling after incubation with ^{14}C-mevalonate.[2] The results obtained are summarized in table 1.

Table 1

	Pig liver S.E. IC_{50} (μM)	$HepG_2$ IC_{50} (μM)
5a	>100	>100
5b	0.27	0.54
5c	0.96	2.10
6	0.52	1.20
1	0.10	0.07
2	0.02	0.06

The azide derivatives **5b** and **6** although less potent than their parent derivatives **1** and **2**, are good SE inhibitors (IC_{50}~0.5μM) and, moreover, both of them are able to control cholesterol biosynthesis in HepG2 cells. Nevertheless, the decrease in affinity observed by introducing an azidogroup or replacing a nitrile residue by an azido function shows that SE is very sensitive to even small structural modifications thus rendering the design of efficient photoprobes extremely limited as reported earlier.[7] Interestingly enough, SE is very sensitive to the position of the azide functionality in inhibitors of type **5** since compound **5b** is a potent inhibitor of SE, **5c** is slightly less active while its regioisomer **5a** is almost inactive.

In conclusion, successful synthetic routes have been designed to prepare compounds of type **5** and **6** allowing an easy introduction of the arylazide photoprobe. Compounds **5b** and **6** are potential mammalian SE photoaffinity labels especially in view of their good affinity for pig liver SE and work is in progress to introduce a tritium atom on these molecules.

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References and Notes :

1. Ono, T.; Bloch, K. *J. Biol. Chem.* **1975**, *250*, 1571-1579.
2. Horie, M.; Tsuchiya, Y.; Hayashi, M.; Iida, Y.; Iwasawa, Y.; Nagata, Y.; Sawasaki, Y.; Fukuzumi, H.; Kitani, K.; Kamei, T. *J. Biol. Chem.* **1990**, *265*, 18075-18078.
3. Suga, A.; Kazuta, K.; Morihira, K.; Matsuda, K.; Kakuta, H.; Moritani, H.; and Iizumi, Y. Yamanouchi Pharmaceutical, WO 9518121-A1 **1996**, CA 124/8603.
4. Gotteland, J.-P.; Brunel, I.; Gendre, F.; Désiré, J.; Delhon, A.; Junquéro, D.; Oms, P.; Halazy, S. *J. Med. Chem.* **1995**, *38*, 3207-3216.
5. Kotzyba-Hibert, F.; Kapfer, I.; Goeldner, M. *Angew. Chem. ,Int. Ed. Engl.* **1995**, *34*, 1296-1312.
6. Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479-12520.
7. Ceruso, M.; Prestwich, G. D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2179-2184.
8. Gotteland, J.-P.; Delhon, A.; Junquéro, D.; Oms, P.; Halazy, S. *Med. Chem. Res.* **1996**, *6*, 333-342.
9. a) Rossi, R.; Carpita, A.; Bellina, F. *Org. Prep. Proced. Int.* **1995**, 129-160. b) Alami, M.; Ferri, F.; Gaslain, Y. *Tetrahedron Lett.* **1996**, *37*, 57-58 and references therein.
10. Li, Y. Z.; Kirby, J. P.; George, M. W.; Poliakoff, M.; Schuster, G. B. *J. Am. Chem. Soc.* **1988**, *110*, 8092-8098.
11. Spectroscopic data for compound **5a**: ^{1}H-NMR ($CDCl_3$, 200MHz) δ ppm 7.43-6.96 (A_2B_2, 4H), 7.45-7.22 (m, 5H), 7.07-6.88 (m, 4H), 5.01 (s, 2H), 3.68 (s, 2H), 3.54 (s, 2H), 2.67 (q, J = 7.0Hz, 2H), 1.15 (t, J = 7.0Hz, 3H). Calculated for $C_{27}H_{24}N_4OS_2$: C; 66.91; H; 4.99; N; 11.56 Found: C; 66.84; H; 5.12; N; 11.35. **5b** : ^{1}H-NMR ($CDCl_3$, 200MHz) δ ppm 7.34-7.19 (m, 8H), 7.08 (brd, J = 6Hz, 1H), 7.01-6.87 (m, 4H), 5.21 (s, 2H), 3.67 (s, 2H), 3.54 (s, 2H), 2.66 (q, J = 7.1Hz, 2H), 1.14 (t, J = 7.1Hz, 3H). Calculated for $C_{27}H_{24}N_4OS_2$: C; 66.91; H; 4.99; N; 11.56 Found: C; 66.68; H; 4.96; N; 11.30. **5c** : ^{1}H-NMR ($CDCl_3$, 200MHz) δ ppm 7.45-7.41 (m, 1H), 7.34-7.21 (m, 6H), 7.13-7.08 (m, 4H), 7.02 (d, J = 7.8Hz, 1H), 6.90 (brd, J = 7.8Hz, 1H), 5.22 (s, 2H), 3.71 (s, 2H), 3.59 (s, 2H), 2.68 (q, J = 7.2Hz, 2H), 1.15 (t, J = 7.2Hz, 3H). Calculated for $C_{27}H_{24}N_4OS_2$: C; 66.91; H; 4.99; N; 11.56 Found: C; 67.19; H; 5.18; N; 11.24.
12. Curphey, T. J. *Org. Prep. Proced. Int.* **1980**, 112-115.
13. Wakefield, B. J. *Organolithium Methods*; Academic Press: London, 1988; p 127.
14. Spectroscopic data for compound **6** : ^{1}H-NMR ($CDCl_3$, 200MHz) δ 7.59-7.56 (A_2B_2, 2H), 7.18 (t, J = 7.7 Hz, 1H), 7.06-7.01 (A_2B_2, 2H), 6.95 (brs, 1H), 6.87 (m, 1H), 6.83 (m, 1H), 6.08 (td, J = 15.9, 6.0 Hz, 1H), 5.64 (td, J = 15.9, 1.2 Hz, 1H), 3.75 (s, 2H), 3.52 (s, 2H), 3.08 (dd, J = 6.0, 1.2 Hz, 2H), 2.50 (q, J = 7.0 Hz, 2H), 1.23 (s, 9H), 1.03 (t, J = 7.0 Hz, 3H), 0.42 (s, 6H). LRMS (DCI) for $C_{27}H_{36}N_4OSi$ 461 (M+1, 100), 435 (24), 315 (3), 166 (4).
15. The microsomal squalene epoxidase activity was assayed according to Bai, M.; Prestwich, G. D. *Arch. Biochem. Biophys.* **1992**, *293*, 305-313.

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