Chinese Chemical Letters Vol. 15, No. 7, pp 771-773, 2004
http://www.imm.ac.cn/journal/ccl.html

Photoaffinity Probe Candidates for Gamma-aminobutyric Acid ($GABA_A$)–Gated Chloride Channel

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Abstract: New photoaffinity ligand candidates were synthesized based on 5-*t*-butyl-2-(4-(substituted-ethynyl)phenyl)-1, 3-dithiane for the noncompetitive blocker site on the gamma-aminobutyric acid -gated chloride channel. Their half-maximal inhibition concentrations ranged from 4 to 32 nmol/L as measured by 4'-ethynyl-4-*n*-[2,3-$^3H_2$]-propylbicycloorthobenzoate (^3H EBOB) assay.

Keywords: Photoaffinity labeling, $GABA_A$ receptor, 1, 3-dithianes.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the nervous system of animals. The $GABA_A$-receptor in insects is the binding site for many insecticides such as picrotoxinin, bicyclophosphorus esters, 1,3-dithiane derivatives[1a-c], heptachlor, heptachlor epoxide, dieldrin, lindane, and fipronil[2]. A number of pharmacological differences have been detected between the GABA-gated chloride channels of insects and vertebrates. The potential exists for exploring such differences in the design of novel, safer insecticides. However, regional localization and characterization of insecticide-induced inhibition or activation of the $GABA_A$ receptor in mammalian brains remain unclear even when radioligand, fluorescent binding, or electrophysiological assays were used[3a-c].

5-*t*-Butyl-2-[4-(3-carboxymethoxy-1-propynyl)phenyl]-1,3-dithiane **1** derivatives bind at the noncompetitive blocker (NCB) site on the GABA-gated chloride channel as channel blockers, leading to inhibition of GABA-simulated chloride flux to induce hyperactivity, tremors[4]. Some photoaffinity probe candidates for the NCB site were reported based on **1**, for example compounds **2** and **3** showed 9 nmol/L of half-maximal inhibition concentrations (IC_{50}), but their use as photoaffinity probes is still limited by possible base lability and low radioactive specificity[5].

In this study, photoaffinity probe candidates were modified based on previous studies[5]. The new compounds contain an oxy-ester instead of a thio-ester for improvingits stability in binding buffer, and easy incorporation of radioactive iodine to increase radioactive specificity in the photoaffinity probes.

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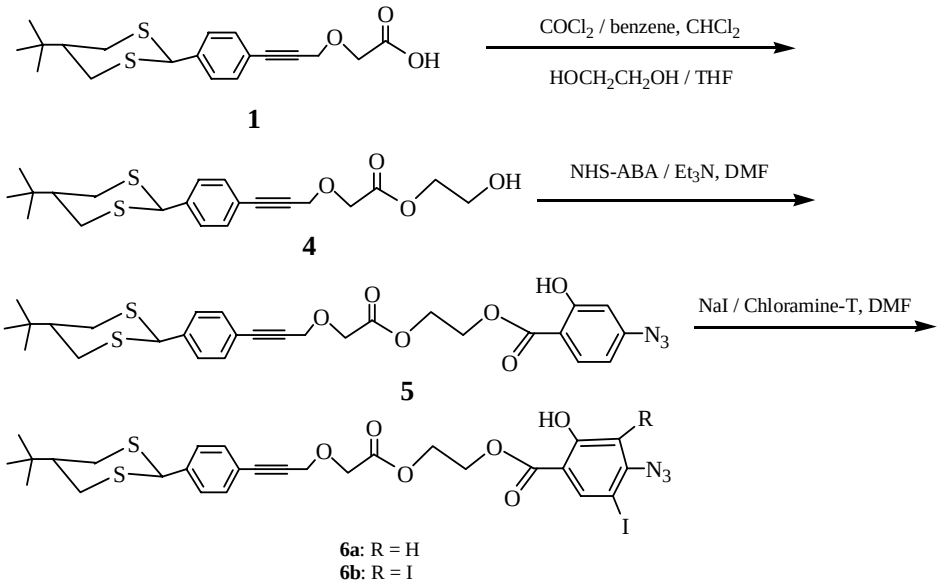
Scheme 1 Synthetic route of new photoaffinity probe candidates



The new photoaffinity ligand candidates (**6a**, **6b**) were synthesized *via* the route outlined in **Scheme 1**. 5-*tert*-Butyl-2-[4-(3-carboxymethoxy-1-propynyl)phenyl]-1,3-dithiane **1** was prepared from diethyl 2-*tert*-butylmalonate[6a-c]. N-Hydroxysuccinimidyl -4-azidobenzoic acid (NHS-ABA) was prepared from 4-aminosalicyclic acid[7]. **4** was obtained by reaction of **1** with oxalyl chloride in benzene and methylene chloride to generate the acyl chloride of **1** and subsequent reaction with ethylene glycol (yield 65%)[8]. The reaction of **4** with NHS-ABA in dry acetonitrile gave 5-*tert*-butyl-2-{4-[3-(2-(4-azido-2hydroxybenzoyloxy) ethoxyacetyloxy)-1-propynyl]phenyl}-1,3-dithiane **5** in 83% yield. Finally, treatment of **5** with sodium iodide and chloramine-T as catalysis in DMF at room temperature gave photoaffinity probe candidates **6a** and **6b**, which were separated by preparative TLC plate in 45% and 33% yield, respectively. The structures of all new compounds were confirmed by ^{1}H-NMR and MS[9].

The inhibitory activity of all new compounds was measured through ^{3}H EBOB assay. The new compounds with (**5, 6a, 6b**) or without azidophenyl group **4** displayed strong inhibition to the binding site of ^{3}H EBOB on bovine brain membrane. The half-maximal inhibition concentrations (IC_{50}s) of compound **4**, **5, 6a, 6b** were 12, 4, 11 and 32 nmol/L, respectively. The results IC_{50} showed that the potential was reduced slightly after incorporation of iodine onto the aromatic ring. In summary, new compounds with aryl azide are expected to be good photoaffinity probes for the $GABA_A$ receptor, if radioisotope iodine (^{125}I) is incorporated.

Acknowledgment

This work was supported by a grant from Hawaii Heptachlor Education and Research Foundation, and USDA-ARS award number 58-5320-8-147.

References and Notes

1. a) P. Perret, X. Sarda, M. Wolff, T. Wu, D. Bushey, *J.Biol. Chem.*, **1999**, *274*, 22350. b) Y. Ozoe, M. Akamatzu, *Pest Management Science*, **2001**, *57*, 923. c) M. Kamijima, J. E. Casida, *Toxicol. Appl. Pharmacol.*, **2000**, *163*, 188.
2. D. Hainzl, L. M. Cole, J. E. Casida, *Chem. Res. Toxicol.*, **1998**, *11*, 1529.
3. a) L. M. Cole, J. E. Casida, *Pestic.Biochem.Physiol.*, **1992**, *44*, 1. b) J. R. Bloomquist, *Rev. Toxicol.*, **1998**, *2*, 333. c) S. J. Farrar, P. J. Whiting, T. P. Bonnert, R. M. McKernan, *J. Biol.Chem.*, **1999**, *274*, 10100.
4. A. Krantis, K. Nichols, A. Blas, W. Staines, *Neuroscience Lett.*, **1994**, *176*, 32.
5. Q. X. Li, J. E. Casida, *Bioorg. Med. Chem.*, **1995**, *3*, 1675.
6. a) Li, Q. X.; Casida, J. E. *Bioorg. Med. Chem.*, **1994**, *2*, 1423. b) W. R. Purdum, K. D. Berlin, *Organic Preparation and Procedures Int.*, **1975**, *7*, 283. c) M. Elliott, D. A. Pulman, J. P. Larkin, J. E. Casida, *J. Agric. Food Chem.*, **1992**, *40*, 147.
7. S. N. Husain, B. Gentile, R. R. Sauers, A. Eichholz, *Carbohydr. Res.*, **1983**, *118*, 57.
8. Compound **2** showed a better stability through HPLC monitoring, no hydrolysis occurs in the binding buffer (pH 8.0) 90 min at 37°C.
9. Data of new compounds: **4.** ^{1}H NMR ($CDCl_3$, δ ppm) 7.417 (s, 4H, Ar-H), 5.114 (s, 1H, CH), 4.535 (s, 2H, CH_2), 4.332-4.308 (t, 2H, J=6.1, CH_2), 4.308 (s, 2H, CH_2), 3.854-3.830 (t, 2H, J=6.1, CH_2), 2.991-2.789 (m, 4H, $2CH_2$), 1.820-1.755 (m, 1H, CH), 0.959 (s, 9H, $3CH_3$). HRMS (FAB): calcd. for $C_{21}H_{28}O_4S_2$ 408.1429, found 408.1410. m.p. 81-83°C. **5.** ^{1}H NMR ($CDCl_3$, δ ppm) 7.817-7.789 (d, 1H, J=8.6, Ar-H), 7.424-7.362 (m, 4H, Ar-H), 6.625-6.618 (d, 1H, J=2, Ar-H), 6.545-6.509 (dd, 1H, J=8.6, 2, Ar-CH), 5.109 (s, 1H, CH), 4.549-4.535 (m, 4H, $2CH_2$), 4.527 (s, 2H, CH_2), 4.298 (s, 2H, CH_2), 2.998-2.782 (m, 4H, $2CH_2$), 1.823-1.765 (m, 1H, CH), 0.959 (s, 9H, $3CH_3$), LCMS $C_{28}H_{30}N_3IO_6S_2$ 569. m.p. 89-91.5°C. **6a.** ^{1}H NMR ($CDCl_3$, δ ppm) 8.251 (s, 1H, Ar-H), 7.390 (s, 4H, Ar-H), 6.748 (s, 1H, Ar-H), 5.105 (s, 1H, CH), 4.555-4.512 (m, 4H, $2CH_2$), 4.542 (s, 2H, CH_2), 4.312 (s, 2H, CH_2), 2.989-2.782 (m, 4H, $2CH_2$), 1.821-1.765 (m, 1H, CH), 0.956 (s, 9H, $3CH_3$), LCMS $C_{28}H_{30}N_3IO_6S_2H^+$ 696.0, m.p. 45-47°C. **6b** ^{1}H NMR ($CDCl_3$, δ ppm) 8.256 (s, 1H, Ar-H), 7.399 (s, 4H, Ar-H), 5.108 (s, 1H, CH), 4.645-4.508 (m, 4H, $2CH_2$), 4.544 (s, 2H, CH_2), 4.308 (s, 2H, CH_2), 3.105-2.780 (m, 4H, $2CH_2$), 1.820-1.765 (m, 1H, CH), 0.960 (s, 9H, $3CH_3$), LCMS $C_{28}H_{29}N_3I_2O_6S_2$ 820.8, m.p. 62-63°C.

Received 20 June, 2003