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1,3-DITHIANES WITH ACID FUNCTIONALITIES: POTENT INHIBITORS AND CANDIDATE AFFINITY PROBES FOR THE GABA-GATED CHLORIDE CHANNEL

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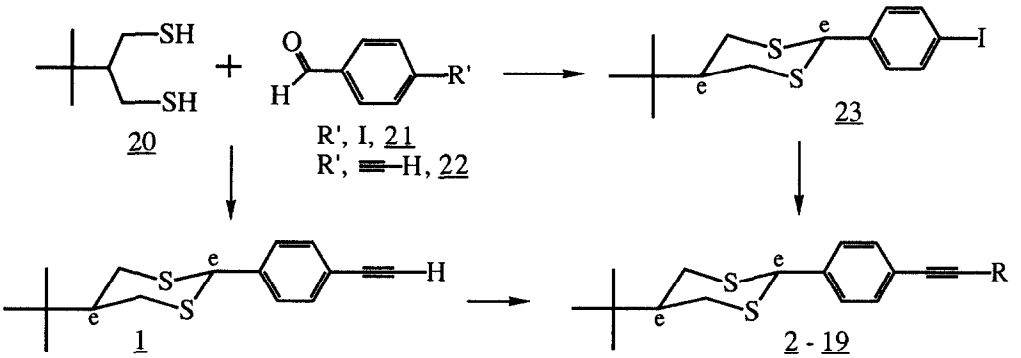
Abstract: 2e,5e-1,3-Dithianes of the type $(CH_3)_3C$-CH$(CH_2S)_2$CH-C_6H_4-4-C≡C-R (R = CO_2H, $CH_2CH_2CO_2H$ and $CH_2CH_2PO_3H_2$) are potent blockers of the GABA-gated chloride channel with 50% inhibition at 5-10 nM. Functionalization of the acid moieties provides candidate photoaffinity ligands [R = $C(O)CHN_2$ and $CH_2CH_2C(O)CHN_2$], affinity columns, and hapten-protein conjugates for antibody production.

The γ-aminobutyric acid (GABA)-gated chloride channel is one of the most sensitive and important targets for pharmaceuticals, convulsants and insecticides. Characterization of this channel has been facilitated by various radioligands, photoaffinity probes, and/or affinity columns specific for the GABA, benzodiazepine, or avermectin site.[1-3] The classical GABA antagonist, picrotoxinin, and a variety of insecticides act primarily at the noncompetitive blocker (NCB) site.[4] A preferred radioligand for the NCB site is 4'-ethynyl-4-[2,3-$^3H_2$]propylbicycloorthobenzoate ([^{3}H]EBOB), [$CH_3CH_2CH_2$-C$(CH_2O)_3$C-C_6H_4-4-C≡CH].[5,6] This radioligand, based on analogy with [^{35}S]tert-butylbicyclophosphorothionate [$(CH_3)_3$C-C$(CH_2O)_3$P=S],[7,8] measures GABA-stimulated chloride ion flux such that inhibition of radioligand binding is proportional to inhibition of chloride flux. 4-tert-Butylbicycloorthobenzoate [$(CH_3)_3$C-C$(CH_2O)_3$C-C_6H_5] has been modified at the 1-substituent to obtain candidate photoaffinity ligands [e.g. 1-diazocyclohexadienonyl[9] and 1-(4-azidophenyl)[10]] and a chemical affinity probe [1-(4-isothiocyanatophenyl)].[11] Although some of these orthocarboxylates are of relatively high affinity, their use is limited by acid lability and they are not practical at present.

Three recent discoveries provide an alternative approach to affinity probes for the NCB site. First, the bicycloorthocarboxylate moiety can be replaced with a dithiane with retention of much of the receptor affinity and greatly improved stability.[12,13] Second, the dithiane is further enhanced in potency by S-oxidation, particularly to the monosulfone.[13,14] Third, the optimal substituents with the 1,3-dithianes are 5e-tert-butyl and 2e-(4-ethynylphenyl),[12,13] the latter moiety providing a potential terminal site for functionalization. Previous additions to the ethynyl moiety in this series were not advantageous[12] but in contrast we find that this site can be extensively modified with high potency and substituents suitable for preparing affinity probes.

Synthesis of the candidate affinity probes and related compounds (1-19) is shown in the scheme. The intermediates were 2-tert-butylpropan-1,3-dithiol (20)[15] and aldehyde 21 (Karl Industries, Aurora, Ohio) which were quantitatively converted to 23 in formic acid.[12] 21 was also used to obtain 22 by palladium-catalyzed coupling with (trimethylsilyl)acetylene followed by removal of the trimethylsilyl protective group.[16]



Reaction conditions and yields for the substituted-ethynylphenyl derivatives (1-19, see Table for R substituents) were as follows:

1: 20, 22, HCO_2H; 100%

2-9, 16 and 17: 23, excess alkyne, $(Ph_3P)_2PdCl_2$, CuI/Et_3N; 90-100%

10 and 12: a, 14 or 16, KOH/H_2O/MeOH/CH_2Cl_2; b, H^+/H_2O; 100%

11: 15, HCl/THF; 31%

13: a, 17, Me_3SiBr; b, H_2O/acetone; 100%

14: a, 1, MeMgCl/THF, -20 °C; b, $ClCO_2Me$/THF, -20 °C; 60%

15: a, 1, n-BuLi /THF, -20 °C; b, BF_3·Et_2O, -20 °C; c, N_2CHCO_2Et, -20 °C; d, H_2O; 43%

18 and 19: a, 10 or 12, excess $(COCl)_2/CH_2Cl_2$/benzene, 0 °C; b, CH_2N_2/Et_2O, 0 °C; 5% for 18, 36% for 19

1 was obtained by coupling 20 and 22 according to Elliott et al.[12] Compounds 2-9 and 16 were prepared by reacting 23 with an excess amount of the corresponding propargyl reagent (commercially available) in the presence of bis-triphenylphosphine palladium dichloride and a catalytic amount of copper(I) iodide in anhydrous triethylamine.[16] 12 was obtained from basic hydrolysis of its methyl ester (16). 10 was also prepared as the methyl ester (14) by a procedure similar to that of Earl and Townsend[17] followed by hydrolysis. 15, prepared by the general method of Hooz and Layton,[18] was subjected to acid hydrolysis to give 11. 13 was obtained via 5e-tert-butyl-2e-[4-(diethyl butyn-3-ylphosphono)phenyl]-1,3-dithiane (17) by reaction of 23 with diethyl butyn-3-ylphosphonate [synthesized by coupling 3-(trimethylsilyl)propargyl bromide with diethyl methylphosphonate[19]] followed by selective de-ethylation with bromotrimethylsilane.[20] 18 and 19 were prepared by treating the corresponding carboxylic acids (10 and 12) with excess oxalyl chloride followed by excess diazomethane.[21]

no	R	NMR, δ ppm[22] ^{13}C	NMR, δ ppm[22] ^{1}H	mp °C	IC_{50} nM
1	H	--	3.10	148-149	21
2	CH_3	4.4	2.03	161-162	35
3	CH_2CH_3	13.1, 13.9	1.22, 2.40	130-131	690
4	$CH_2CH_2CH_3$	13.5, 21.4, 22.2	1.03, 1.58, 2.37	133-134	6700
5	CH_2OH	51.5	4.48	148-149	76
6	CH_2CH_2OH	23.8, 61.1	2.67, 3.79	146-147	400
7	$CH_2CH_2CH_2OH$	15.9, 31.2, 61.5	1.82, 2.51, 3.78	147-148	410
8	$CH(OH)CH_3$	24.3, 58.8	1.54, 4.74	153-154	3100
9	$C(CH_3)_2OH$	31.4, 65.5	1.60	177-178	>10000
10	CO_2H	154.1	--	160 (dec)	7
11	CH_2CO_2H	26.5, 173.6	3.55	156-161	23
12	$CH_2CH_2CO_2H$	15.2, 33.2, 177.5	2.69, 2.73	221	5
13	$CH_2CH_2PO_3H_2$	14.4, 28.0	2.01, 2.68	185-186	10
14	CO_2CH_3	52.8, 154.3	3.83	181-182	9
15	$CH_2CO_2CH_2CH_3$	14.0, 26.6, 61.4, 167.8	1.30, 3.49, 4.22	119-126	460
16	$CH_2CH_2CO_2CH_3$	15.4, 33.5, 50.9, 172.3	2.62, 2.72, 3.71	133-135	17
17	$CH_2CH_2P(O)(OCH_2CH_3)_2$	13.4, 16.4, 25.3, 61.6	1.33, 2.07, 2.70, 4.12	102	3000
18	$C(O)CHN_2$	60.1, 189.5	5.59	147 (dec)	690
19	$CH_2CH_2C(O)CHN_2$	15.2, 39.5, 54.8, 192.6	2.61, 2.73, 5.35	134 (dec)	830

The new dithianes 3-19 were compared with known compounds 1 and 2 for potency as inhibitors (IC_{50}= concentration for 50% inhibition) of [^{3}H]EBOB binding using bovine brain membranes.[6] Addition to 1 of terminal alkane substituents results in progressively lower receptor potency with increasing chain length (2 - 4). In the n-alkanol series (5-7) potency drops off with chain length, while secondary and tertiary alcohols (8 and 9) have very low activity. The results with acids 10-13 are very surprising since there are no reports of $GABA_A$ receptor antagonists with acidic functionalities acting at the NCB site. The exceptional potencies of carboxylic acids 10 and 12[23] and phosphonic acid 13 are therefore quite remarkable. Interestingly, there are clusters of arginines and lysines at the channel mouth which provide positively-charged residues that are presumed to serve as anion-concentrating regions normally important for chloride ion flow[1]. These findings and earlier structure-activity studies[24,25] suggest that the tert-butyl moiety may enter the channel and approach the NCB site first with the acidic functionality possibly directed towards the mouth of the channel. Esterification of the carboxylic acid moiety reduces the receptor potency 1.3- to 20-

fold (10-12 vs. 14-16) whereas with the phosphonic acid (13) the diethyl phosphonate (17) is 300-fold less active. The high activity of the carboxylic esters implies that suitable linkage to agarose gels or proteins might yield affinity columns or hapten-protein conjugates as immunogens for antibody production. Diazoketones 18 and 19 combine moderate receptor potency with outstanding photoreactivity in pH 7.4 phosphate buffer on irradiation at 254 nM and are therefore candidate photoaffinity probes.

In conclusion, the dithianes with suitably-positioned acidic functional groups retain high receptor potency and form the basis for further derivatizations to obtain photoaffinity probes and affinity columns.

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