hair growth cycle were used. All initiators were applied topically to the shaved region in 0.2 mL of peroxide-free THF, and control mice received solvent only. Mice were initiated with various doses of each test compound followed 2 weeks later by twice-weekly applications of 3.4 nmol of TPA in 0.2 mL of acetone. Development of skin papillomas was observed and recorded weekly. Papillomas were removed at random for histological verification. The data in this study are presented as the average number of papillomas per mouse and the percent of mice with papillomas. Statistical analyses of differences between mean papilloma responses were performed (where appropriate) with the Student's t test. The level of significance was set at $p \leq 0.05$.

Acknowledgment. Support of this research by grants from the National Cancer Institute, DHHS (CA 36097 and CA 14599), and the American Cancer Society (BC-132) is gratefully acknowledged.

Registry No. **2a**, 114326-33-9; **2a** diacetate, 114326-32-8; **2b**, 114326-36-2; **2b** diacetate, 114350-59-3; **3a**, 114326-34-0; **4a**, 66-99-9; **4b**, 3453-33-6; **6**, 114326-26-0; **7**, 114326-27-1; **8**, 114326-28-2; **9a**, 83136-28-1; **9b**, 83136-32-7; **10**, 76214-37-4; **12a**, 114326-29-3; **12b**, 114326-30-6; **13a**, 114326-31-7; **13b**, 114326-35-1; *N,N*-diethyl-6-methoxy-1-naphthamide, 114326-25-9; 2-bromo-6-methoxynaphthalene, 5111-65-9.

Heterocyclic Muscarinic Agonists. Synthesis and Biological Activity of Some Bicyclic Sulfonium Arecoline Bioisosteres

Per Sauerberg,† Erik Falch,† Eddi Meier,‡ Hanne L. Lembøl,‡ and Povl Krogsgaard-Larsen*†

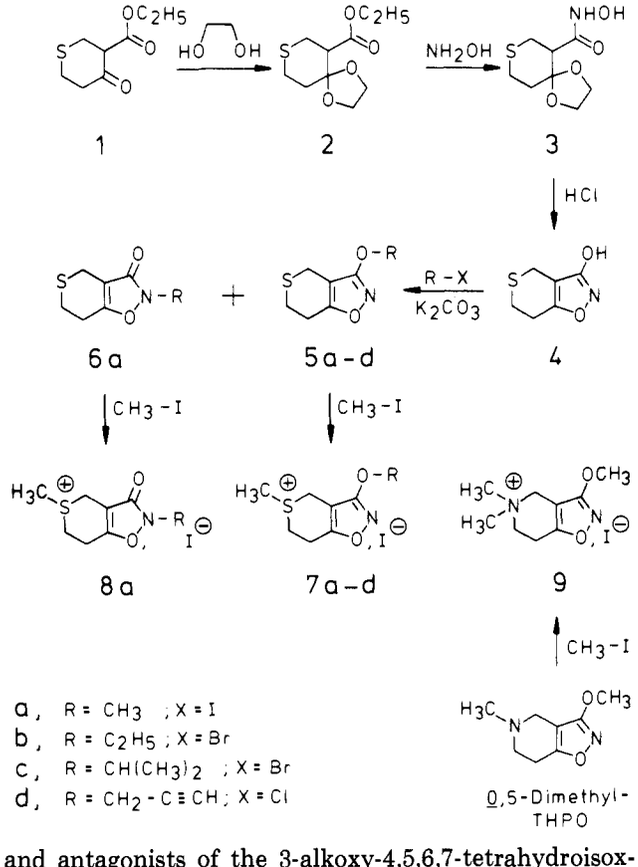
Department of Chemistry BC, The Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark, and Department of Pharmacology, H. Lundbeck A/S, DK-2500 Valby, Copenhagen, Denmark. *Received November 4, 1987*

A number of *S*-methylsulfonium analogues of the conformationally restricted muscarinic agonists of the 3-alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine (*O*-alkyl-THPO) type have been synthesized. The effects on muscarinic receptors of these 3-alkoxy-5-methyl-6,7-dihydro-4*H*-thiopyrano[3,4-*d*]isoxazol-5-ium (*O*-alkyl-*S*-methyl-DHTO) analogues (**7a–d**) were assessed in receptor-binding experiments with tritiated oxotremorine M, pirenzepine, and quinuclidinyl benzilate as ligands and were supported by studies on the isolated guinea pig ileum. The degree of muscarinic agonist activity of the compounds (M-agonist index) and their selectivity for M-1 or M-2 muscarinic receptor subtypes (M-2/M-1 index) were estimated on the basis of receptor-binding studies. The in vitro pharmacological profiles of the compounds were compared with those of arecoline and its sulfonium and 3-methoxyisoxazole isosteres, sulfoarecoline and *O*,5-dimethyl-THPO, respectively. While *O*-methyl-DHTO (**5a**) and *N*-methyl-DHTO (**6a**) were inactive, all of the sulfonium analogues **7a–d** were muscarinic agonists with the exception of *O*-ethyl-*S*-methyl-DHTO (**7b**), which showed a muscarinic antagonist profile.

There is strong evidence of major deficits in central cholinergic transmission in patients with the pathology characteristic of Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT).[1-5] On the basis of clinical and animal behavioral studies, this cholinergic deficit may be of particular relevance to disturbances in learning and memory in AD/SDAT patients.[1,3,4,6] Neurochemical examination of autopsy and biopsy brain material from Alzheimer patients have revealed loss of the presynaptic marker enzyme, choline acetyltransferase, and presynaptic muscarinic receptor sites of the M-2 subtype correlating with dementia score and severity of neurohistopathology. Postsynaptic muscarinic receptor sites, primarily of the M-1 subtype, do, however, seem to survive the loss of cholinergic nerve terminals.[1,2,7] There may actually be an up-regulation of postsynaptic M-1 receptors in Alzheimer patients.[8] Although the degree of functional integrity of muscarinic receptors in AD/SDAT brains is, as yet, unknown, much interest is focused on such receptors as therapeutic sites of attack. Whereas antagonists at presynaptic M-2 receptors might be useful drugs at the early stages of AD/SDAT,[9] agonists at postsynaptic M-1 receptors or, perhaps, compounds with mixed M-1 agonist/M-2 antagonist profiles appear to be of particular therapeutic interest.[8,10]

These aspects have accelerated the pharmacological characterization of M-1 and M-2 receptors.[11] As part of our attempts to elucidate the muscarinic pharmacophore(s) relevant to AD/SDAT, we have developed a series of potent and conformationally restricted muscarinic agonists and antagonists of the 3-alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine (*O*-alkyl-THPO) type.[12-15]

Scheme I



† The Royal Danish School of Pharmacy.
‡ H. Lundbeck A/S.

0022-2623/88/1831-1312$01.50/0

This paper describes the syntheses (Scheme I) of the *S*-methylsulfonium analogues (**7a–d**) of the respective *O*-alkyl-THPO muscarinic agonists and antagonists and studies of their effects on muscarinic cholinergic receptors in vitro. A number of related compounds, including *O*,5,5-trimethyl-THPO (**9**) and the isomer of **7a**, compound **8a**, were synthesized and tested.

Chemistry. The ketalized β-oxo ester **2** was converted into the corresponding hydroxamic acid **3**, which was deprotected and cyclized to give 6,7-dihydro-4*H*-thiopyrano[3,4-*d*]isoxazol-3-ol (DHTO, **4**) (Scheme I). The 3-isoxazolol anion of **4**, which is a nonsymmetric isostere of the carboxylate ion, was alkylated to give the expected O- and N-alkylated products **5** and **6**, respectively. Since the aim of this work was to study 3-alkoxyisoxazoles as bioisosteres of ester groups in muscarinic cholinergic agonists, we only isolated one of the N-alkylated isomers of **5a–d** in a pure form. This compound, **6a**, and **5a–d** were converted into the sulfonium derivatives **8a** and **7a–d**, respectively, by prolonged treatment with methyl iodide at room temperature. Treatment of *O*,5-dimethyl-THPO with methyl iodide at 40 °C for 18 h gave the quaternized analogue **9**. The structures of all of the new compounds **2–9** were established by ^{1}H NMR and IR spectroscopy, supported by elemental analyses. ^{1}H NMR spectroscopic data are only given for the final products **7a–d**, **8a**, and **9**.

Evaluation of Biological Effects. Receptor-binding assays were used to determine the affinity of the compounds under study for muscarinic receptor sites in the rat brain and heart. The ability of the compounds to displace radioactive pirenzepine (PZ), a selective antagonist for M-1 muscarinic cholinergic receptors,[16] was used to estimate their affinity for M-1 receptor sites.[17] In contrast to PZ, quinuclidinyl benzilate (QNB) is a nonselective antagonist for M-1 and M-2 muscarinic cholinergic receptors.[17] On the basis of the potency of the compounds as inhibitors of [^{3}H]QNB binding to membranes isolated from the heart, a tissue containing a predominance of M-2 receptors,[17-19] the affinity of the compounds for M-2 receptor sites was determined. Oxotremorine M (Oxo-M) is a potent agonist for muscarinic cholinergic receptors, and the potency of the compounds as inhibitors of [^{3}H]Oxo-M binding was interpreted in terms of affinity of the test compounds for the "agonist conformational state" of the muscarinic receptor sites. The isolated guinea pig ileum was used as a functional test system for the evaluation of the pharmacological profile of the compounds at muscarinic receptors.

Inhibitory constants (K_i's) were estimated by using the formula

$$K_i = IC_{50}(\text{compound})/[1 + ([^3\text{H}]\text{ligand})/K_d]$$

where IC_{50} values were determined by conventional methods and K_d values were derived from Scatchard analyses following procedures analogous with those described earlier.[17]

In analogy with a published procedure for estimation of muscarinic agonist efficacy,[20,21] the ratio between the K_i values of the compounds determined in [^{3}H]QNB (brain) and [^{3}H]Oxo-M (brain) binding experiments was used as a muscarinic agonist index (M-agonist index) of the compounds. According to a scale introduced by Freedman et al.,[20,21] values of this index above 4000 indicate full agonism, whereas values of 100–300 and below 10 predict partial agonism and antagonism, respectively, of muscarinic agents:

$$\text{M-agonist index} = K_i(\text{QNB, brain})/K_i(\text{Oxo-M, brain}) = IC_{50}(\text{QNB, brain})/IC_{50}(\text{Oxo-M, brain}) \times 0.162$$

The ratio between the K_i values of the compounds determined in [^{3}H]QNB (heart) and [^{3}H]PZ (brain) binding experiments was used as an index of M-1 selectivity (M-2/M-1 index), higher values of this index indicating higher degrees of M-1 selectivity:

$$\text{M-2/M-1 index} = K_i(\text{QNB, heart})/K_i(\text{PZ, brain}) = IC_{50}(\text{QNB, heart})/IC_{50}(\text{PZ, brain}) \times 0.125$$

Structure–Activity Relationships and Discussion. In previous studies we have determined the relationship between structure and in vivo and in vitro biological activity of a series of bicyclic muscarinic agonists and antagonists of the *O*-alkyl-THPO type, of which *O*-methyl-THPO and *O*,5-dimethyl-THPO are conformationally restricted bioisosteres of norarecoline and arecoline, respectively.[12-15] In the present paper, we have extended these structure–activity studies to include analogues of such mono- and bicyclic muscarinic agonists containing quaternary ammonium and sulfonium groups. As model compounds for these studies we have synthesized *O*,5,5-

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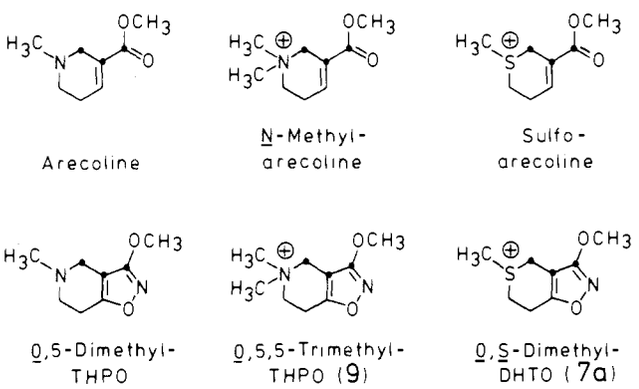
Table I. In Vitro Effects of Some Heterocyclic Muscarinic Cholinergic Agonists and Antagonists

compound	effects on receptor ligand binding: IC_{50}, μM						effects on ileum	
	[^{3}H]QNB brain	[^{3}H]QNB heart	[^{3}H]PZ brain	[^{3}H]Oxo-M brain	M-agonist index	M-2/M-1 index	agonism EC_{50}, μM	antagonism IC_{50}, μM
O-methyl-DHTO (**5a**)	>1000	>1000	>1000	>1000			>50	>50
N-methyl-DHTO (**6a**)	>1000	>1000	>1000	>1000			>50	>50
O,S-dimethyl-DHTO (**7a**)	36	1.6	4.1	0.019	307	0.05	0.84	>0.4
N,S-dimethyl-DHTO (**8a**)	110	10	30	0.22	81	0.04	9.3	>6
O-ethyl-*S*-methyl-DHTO (**7b**)	4.6	4.9	0.53	0.23	3.3	1.2	>50	15
O-isopropyl-*S*-methyl-DHTO (**7c**)	4.8	1.8	0.43	0.039	20	0.53	6.2	>3
O-propargyl-*S*-methyl-DHTO (**7d**)	2.6	0.44	0.25	0.0033	128	0.22	0.56	>0.2
O,5,5-trimethyl-THPO (**9**)	74	9.5	2.8	0.043	279	0.42	8.2	>3
O,5-dimethyl-THPO	38	16	6.4	0.028	220	0.31	0.83	>0.2
arecoline	11	0.59	1.1	0.0019	938	0.07	0.15	>1
sulfoarecoline	34	2.5	2.6	0.0014	3930	0.12	NT[a]	NT
sulfoarecaidine ethyl ester	22	3.9	0.4	0.0092	387	1.2	NT	NT

[a] NT = not tested.

trimethyl-THPO (**9**), the quaternized analogue of *O*,5-dimethyl-THPO, and the compounds **7a–d**, in which the secondary amino groups of the respective *O*-alkyl-THPO analogues have been replaced by *S*-methylsulfonium groups. The described structure–activity studies include the earlier described muscarinic agonists sulfoarecoline and the corresponding ethyl ester, sulfoarecaidine ethyl ester.[22,23]



It is generally accepted that muscarinic agonists containing secondary or tertiary amino groups bind to and activate the receptors in the protonated forms. Accordingly, the complete inactivity of *O*-methyl-DHTO (**5a**) and *N*-methyl-DHTO (**6a**) (Table I) is not surprising. The sulfonium analogues of **5a** and **6a**, *O,S*-dimethyl-DHTO (**7a**) and *N,S*-dimethyl-DHTO (**8a**), respectively, do, however, show muscarinic agonist activity, the former compound, which contains the 3-methoxyisoxazole moiety as a bioisostere of the methyl ester group in arecoline, being the most agonistic and potent compound (Table I).

Among the compounds **7a–d**, *O*-propargyl-*S*-methyl-DHTO (**7d**) is the most potent muscarinic agonist in agreement with the findings for *O*-propargyl-THPO.[14] Like *O*,5-dimethyl-THPO, **7d** has an M-agonist index indicating partial agonist activity, and these compounds show a degree of M-1 selectivity similar to that of arecoline but significantly higher than those of **7a** or **8a**.

Whereas the M-agonist index values for **7a** and **7d** reflect partial agonist character, the in vitro and in vivo pharmacological profiles of **8a**, *O*-isopropyl-*S*-methyl-DHTO (**7c**), and *O*-ethyl-*S*-methyl-DHTO (**7b**) become increasingly antagonistic with simultaneous increase in M-1 selectivity (Table I). This structure–activity relationship is quite surprising in light of the partial agonist character of *O*-ethyl-THPO and antagonist profile of *O*-isopropyl-THPO at muscarinic receptors.[14,15]

The quaternized arecoline analogue, *N*-methylarecoline, is substantially less active than the parent compound as a muscarinic agonist.[22] A similar loss of efficacy at ileal muscarinic receptors is observed after quaternization of *O*,5-dimethyl-THPO to *O*,5,5-trimethyl-THPO (**9**), although the in vitro pharmacological profiles of these two compounds are similar (Table I). Like *O*,5-dimethyl-THPO, its sulfonium analogue *O,S*-dimethyl-DHTO (**7a**) has the characteristics of a partial agonist, and these two compounds show comparable potencies at ileal muscarinic receptors. Nevertheless, **7a** shows a considerably higher selectivity for M-2 receptor sites than *O*,5-dimethyl-THPO as revealed by its effects on [^{3}H]QNB binding to heart membranes and its relatively low M-2/M-1 index. Similarly, the sulfonium analogue of arecoline, sulfoarecoline, shows a preferential affinity for M-1 receptor sites as compared with the parent compound. The exceptionally high M-agonist index of sulfoarecoline, indicative of high muscarinic efficacy of this compound, is not shown by the corresponding ethyl ester, sulfoarecaidine ethyl ester, the in vitro pharmacological profile of which is consistent with a predominantly M-1 agonist or partial agonist character as supported by its effects on [^{3}H]PZ binding and its relatively high M-2/M-1 and *M*-agonist indexes. It is interesting to note that, like sulfoarecaidine ethyl ester, the structurally related bicyclic compound, *O*-ethyl-*S*-methyl-DHTO (**7b**), shows a high degree of M-1 selectivity, but whereas the former compound, like *O*-ethyl-THPO,[15] is a partial agonist, **7b** is an antagonist.

These structure–activity studies indicate that, within the class of reverse ester bioisosteres of acetylcholine, a number of structural parameters affect the muscarinic pharmacological profile. The structural parameters of major importance appear to be (1) the degree of conformational flexibility, (2) the structure of the cationic head, and (3) the structure of the ester alkyl group. These structural parameters cannot be analyzed separately, reflecting that alteration of one of these parameters of a compound more or less profoundly affects all of the structural parameters characterizing the compound. Further studies on these complex structure–activity relationships for muscarinic agonists are in progress.

In contrast to some of the *O*-alkyl-THPO compounds, which have very favorable pharmacokinetic properties,[14,15] the sulfonium analogues described in this paper do not appear to be suitable for behavioral pharmacological studies, and preliminary pharmacological experiments in different animal models indicate that these compounds do not easily penetrate the blood–brain barrier.[15] The

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structure–activity relationships described are, however, of major importance for our attempts to design and develop muscarinic cholinergic agonists of therapeutic interest.

Experimental Section

Chemistry. Melting points were determined in capillary tubes and are uncorrected. Column chromatography (CC) was performed on silica gel 60 (70–230 mesh, ASTM, Merck) and thin-layer chromatography (TLC) was performed with silica gel F_{254} plates (Merck). ^1H NMR spectra were recorded on a Varian 360-L spectrometer. Analyses, indicated by elemental symbols, were within ±0.4% of the theoretical values and were carried out by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

3-(Ethoxycarbonyl)-2,3,5,6-tetrahydro-4-oxo-4*H*-thiopyran Ethylene Ketal (2). A mixture of 1[24] (30.5 g, 160 mmol), 4-toluenesulfonic acid (3.04 g, 16 mmol), ethylene glycol (45 mL), and toluene (350 mL) was refluxed for 24 h with a Dean–Stark water separator. Toluene (150 mL) was added, and the mixture was washed with sodium hydrogen carbonate (150 mL, 5%) and saturated sodium chloride (150 mL). The dried (K_2CO_3) toluene phase was evaporated in vacuo. The residue was subjected to CC with toluene–ethyl acetate (9:1) as an eluent to give **2** (28.9 g, 77%) as an oil. Anal. ($C_{10}H_{16}O_4S$) C, H, S.

2,3,5,6-Tetrahydro-4-oxo-4*H*-thiopyran-3-carbohydroxamic Acid Ethylene Ketal (3). To a stirred solution of hydroxylamine hydrochloride (12.1 g, 174 mmol) in methanol (100 mL) maintained at 40 °C was added dropwise a solution of sodium methoxide prepared from sodium (174 mmol) and methanol (50 mL). The reaction mixture was cooled to room temperature, and a solution of **2** (27 g, 116 mmol) in methanol (50 mL) was added followed by a solution of sodium methoxide, prepared from sodium (174 mmol) and methanol (50 mL). Stirring was continued for 2 days. The mixture was cooled to 0 °C, and a solution of hydrochloric acid in methanol was added to pH 4. After the mixture was stirred at 0 °C for 2 h, the precipitate was collected and extracted with three 300-mL portions of warm $CHCl_3$. The combined extracts were evaporated in vacuo, and the residue was crystallized from ethyl acetate to give **3** (21.6 g, 85%), mp 160–163 °C. Anal. ($C_8H_{13}NO_4S$) C, H, N, S.

6,7-Dihydro-4*H*-thiopyrano[3,4-*d*]isoxazol-3-ol (DHTO, 4). To a solution of **3** (7.1 g, 32.4 mmol) in methanol (100 mL) kept at 80 °C was added concentrated hydrochloric acid (16 mL). The mixture was stirred at 80 °C for 10 min and evaporated in vacuo. The residue was extracted with $CHCl_3$ (3 × 150 mL). The extracts were evaporated in vacuo, and the residue was crystallized from toluene–petroleum ether to give **4** (3.14 g, 62%), mp 161–163 °C. Anal. ($C_6H_7NO_2S$) C, H, N, S.

3-Methoxy-6,7-dihydro-4*H*-thiopyrano[3,4-*d*]isoxazole (*O*-Methyl-DHTO, 5a) and 2-Methyl-3-oxo-2,3,6,7-tetrahydro-4*H*-thiopyrano[3,4-*d*]isoxazole (*N*-Methyl-DHTO, 6a). A mixture of **4** (2.72 g, 17.3 mmol) and K_2CO_3 (5.93 g, 43 mmol) in DMF (130 mL) was stirred at 40 °C for 1 h. Methyl iodide (1.08 mL, 17.3 mmol) was added dropwise, and the mixture was stirred at 40 °C for 20 h. The reaction mixture was evaporated in vacuo, and water (50 mL) was added to the residue. The aqueous mixture was extracted with $CHCl_3$ (3 × 100 mL), and the combined and dried extracts were evaporated in vacuo. The resulting oil contained **5a** as well as **6a**. The two components were separated by CC with toluene–ethyl acetate (1:1) as an eluent. The first fractions contained **5a**. TLC: R_f 0.77 [eluent, toluene–ethyl acetate (1:1)]. Compound **5a** was recrystallized from petroleum ether to give 0.94 g (33%), mp 36.5–37.5 °C. Anal. ($C_7H_9NO_2S$) C, H, N, S. The later fractions contained **6a**. TLC: R_f = 0.25 [eluent, toluene–ethyl acetate (1:1)]. Compound **6a** was recrystallized from ether to give 0.94 g (34%), mp 102–103 °C. Anal. ($C_7H_9NO_2S$) C, H, N, S.

Compounds **5b–d** were prepared in a similar manner, but no attempts were made to isolate the isomeric N-alkylated compounds.

3-Ethoxy-6,7-dihydro-4*H*-thiopyrano[3,4-*d*]isoxazole (*O*-ethyl-DHTO, 5b) from **4** (0.50 g, 3.18 mmol) and ethyl bromide (0.27 mL, 3.5 mmol): yield of **5b** 328 mg (56%); mp 32–33 °C (petroleum ether). Anal. ($C_8H_{11}NO_2S$) C, H, N, S.

3-Isopropoxy-6,7-dihydro-4*H*-thiopyrano[3,4-*d*]isoxazole (*O*-isopropyl-DHTO, 5c) from **4** (0.70 g, 4.45 mmol) and isopropyl bromide (0.46 mL, 4.9 mmol): yield of **5c** 379 mg (43%); mp 44–45 °C (ether–petroleum ether). Anal. ($C_9H_{13}NO_2S$) C, H, N, S.

3-(Propargyloxy)-6,7-dihydro-4*H*-thiopyrano[3,4-*d*]isoxazole (*O*-propargyl-DHTO, 5d) from **4** (0.73 g, 4.6 mmol) and propargyl chloride (0.36 mL, 5.1 mmol): yield of crude **5d** 400 mg (45%). An analytical sample was recrystallized from ether–petroleum ether, mp 44–46 °C. Anal. ($C_9H_9NO_2S$) C, H, N, S.

General Procedure for the Syntheses of 3-Alkoxy-5-methyl-6,7-dihydro-4*H*-thiopyrano[3,4-*d*]isoxazol-5-ium Iodides (7a–d) and 2,5-Dimethyl-3-oxo-2,3,6,7-tetrahydro-4*H*-thiopyrano[3,4-*d*]isoxazol-5-ium Iodide (8a). Methyl iodide (20 mmol) was added to a solution of **5** or **6a** (2 mmol) in methanol (5 mL). The mixture was left at room temperature in the dark for 4 days. In the case of **7a** and **8a**, the compounds precipitated. Otherwise, ether was added. All of the compounds were recrystallized from methanol–ether.

***O*,*S*-Dimethyl-DHTO (7a):** yield 42%; mp 142–143 °C; ^1H NMR (60 MHz, Me_2SO-d_6) δ 4.25 (2 H, pert d), 3.88 (3 H, s), 3.70 (2 H, m), 3.20 (2 H, m), 2.80 (3 H, s). Anal. ($C_8H_{12}INO_2S$) C, H, I, N, S.

***O*-Ethyl-*S*-methyl-DHTO (7b):** yield 29%; mp 126–127 °C; ^1H NMR (60 MHz, D_2O) δ 4.30 (2 H, q), 4.25 (2 H, pert d), 3.75 (2 H, m), 3.25 (2 H, m), 2.80 (3 H, s), 1.35 (3 H, t). Anal. ($C_9H_{14}INO_2S$) C, H, I, N, S.

***O*-Isopropyl-*S*-methyl-DHTO (7c):** yield: 41%; mp 117–119 °C; ^1H NMR (60 MHz, D_2O) δ 4.85 (1 H, m), 4.25 (2 H, pert d), 3.75 (2 H, m), 3.30 (2 H, m), 2.85 (3 H, s), 1.35 (6 H, d). Anal. ($C_{10}H_{16}INO_2S$) C, H, I, N, S.

***O*-Propargyl-*S*-methyl-DHTO (7d):** yield 36%; mp 128–130 °C; ^1H NMR (60 MHz, Me_2SO-d_6) δ 5.00 (2 H, d), 4.35 (2 H, pert d), 3.75 (2 H, m), 3.70 (1 H, d), 3.30 (2 H, m), 2.80 (3 H, s). Anal. ($C_{10}H_{12}INO_2S$) C, H, I, N, S.

***N*,*S*-Dimethyl-DHTO (8a):** yield 34%; mp 134–136 °C; ^1H NMR (60 MHz, Me_2SO-d_6) δ 4.15 (2 H, pert d), 3.70 (2 H, t), 3.35 (3 H, s), 3.10 (2 H, m), 2.80 (3 H, s). Anal. ($C_8H_{12}INO_2S$) C, H, I, N, S.

3-Methoxy-5,5-dimethyl-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridinium Iodide (*O*,5,5-Trimethyl-THPO, 9). To a solution of 3-methoxy-5-methyl-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridinium chloride (*O*,5-dimethyl-THPO, HCl)[13] (0.65 g, 3 mmol) in water (10 mL) was added K_2CO_3 (0.69 g, 5 mmol). The mixture was extracted with three 15-mL portions of CH_2Cl_2. The combined and dried extracts were evaporated, and the residue was dissolved in methanol (15 mL). Methyl iodide (1.86 mL, 10 mmol) was added, and the mixture stirred at 40 °C for 18 h. Ether (10 mL) was added, and the precipitate was collected and recrystallized from methanol–ether to give **9** (0.58 g, 62%): mp 203–205 °C; ^1H NMR (60 MHz, D_2O) δ 4.20 (2 H, s), 3.90 (3 H, S), 3.65 (2 H, pert t), 3.20 (6 H, s), 3.10 (2 H, m). Anal. ($C_9H_{15}IN_2O_2$) C, H, I, N.

Muscarinic Cholinergic Agonism and Antagonism in Guinea Pig Ileum. A segment (30 mm long) of guinea pig ileum was placed isotonically in Tyrode solution at 37 °C in a 10-mL organ bath.[25] The agonistic activity of a test drug was estimated by measuring the muscle contraction induced by the drug in three to five different concentrations. Atropine was used to verify the muscarinic nature of the drug-induced contraction. In antagonist studies muscle contractions were induced by acetylcholine (0.22 μM). Comparisons were made between the acetylcholine-induced contractions before and 3 min after addition of the test drug in three to five different concentrations. EC_{50} (agonism) and IC_{50} (antagonism) values were estimated from dose–response curves.

Inhibition of Muscarinic Receptor Ligand Binding. [^3H]QNB binding to muscarinic receptor sites on membrane fractions prepared from rat brains was performed essentially as described by Watson et al.[17] Briefly, rat brains were homogenized in 100 vol (w/v) 10 mM sodium potassium phosphate buffer (pH 7.4) and diluted 1:10 with the same buffer. Aliquots (0.5 mg of

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tissue) were incubated with 0.12 nM [^{3}H]QNB (46 Ci/mmol, Amersham) alone or in the presence of test compound in a total volume of 5 mL for 30 min at 37 °C. The reaction was stopped by adding 5 mL of ice-cold buffer and rapid filtration through Whatman GF/B filters soaked previously in 0.1% polyethylenimine (Sigma) for a minimum of 30min. The filters were washed twice with the same volume of buffer and bound radioactivity estimated by liquid scintillation counting methods. Each compound was tested in five different concentrations, and nonspecific binding estimated at 20 μM atropine. All estimations were made in triplicate, and each displacement experiment was repeated at least twice. The dissociation constant (K_d) for the binding of [^{3}H]QNB to rat brain membranes was determined to 13.7 ± 0.9 pM based on Scatchard analysis following a previously described procedure.[17]

The procedure for determinations of inhibition of [^{3}H]QNB binding to rat heart tissue was the same as that described above with the exceptions that the tissue was homogenized in an ultraturrax homogenizer and that 4 mg of tissue was used per assay. A Scatchard analysis of [^{3}H]QNB binding to rat heart tissue gave a K_d value of 9.1 ± 0.7 pM.

The procedure for the determinations of inhibition of [^{3}H]PZ binding to rat brain membranes was analogous to that described above for [^{3}H]QNB binding to such membrane fractions: 3 mg of tissue were incubated with 1.0 nM [^{3}H]PZ (85 Ci/mmol, New England Nuclear) at 25 °C for 60 min in a total volume of 1.5 mL of buffer. The reaction was stopped by filtration under reduced pressure followed by three washes with 4 mL of ice-cold buffer. Nonspecific binding was estimated at 10 μM atropine. A K_d value of 1.8 ± 1.0 nM for the binding of [^{3}H]PZ to rat brain membranes was derived from a Scatchard analysis.

The procedure for determination of inhibition of [^{3}H]Oxo-M binding to rat brain membranes was analogous to that described above for [^{3}H]QNB binding to such membrane fractions: 5 mg of tissue were incubated with 0.2 nM [^{3}H]Oxo-M (84.4 Ci/mmol, New England Nuclear) at 30 °C for 40 min in a total volume of 1.5 mL of buffer. The reaction was stopped by adding 5 mL of ice-cold buffer, rapid filtration, and one wash with the same volume of buffer. A K_d value of 0.48 ± 0.03 nM for the binding of [^{3}H]Oxo-M to rat brain membranes was derived from a Scatchard analysis.

Acknowledgment. This work was supported by grants from the Danish Medical and Technical Research Councils. The secretarial assistance of B. Hare and the technical assistance of T. Lindgreen and S. Stilling are gratefully acknowledged. The gifts of sulfoarecoline and sulfoarecaidine ethyl ester from Dr. U. Moser and Professor G. Lambrecht, Johann Wolfgang Goethe-Universität, Frankfurt/Main, West Germany, are cordially acknowledged.

Registry No. 1, 1198-44-3; 2, 113748-37-1; 3, 113748-38-2; 4, 113748-39-3; 5a, 113748-40-6; 5b, 113748-45-1; 5c, 113748-46-2; 5d, 113748-47-3; 6a, 113748-41-7; 7a, 113748-42-8; 7b, 113748-48-4; 7c, 113748-49-5; 7d, 113748-50-8; 8a, 113748-43-9; 9, 113748-44-0; 0,5-dimethyl-THPO·HCl, 95597-35-6; C_2H_5Br, 74-96-4; $(CH_3)_2CHBr$, 75-26-3; $HC{\equiv}CCH_2Br$, 624-65-7.

2,3-Diarylindenes and 2,3-Diarylindenones: Synthesis, Molecular Structure, Photochemistry, Estrogen Receptor Binding Affinity, and Comparisons with Related Triarylethylenes

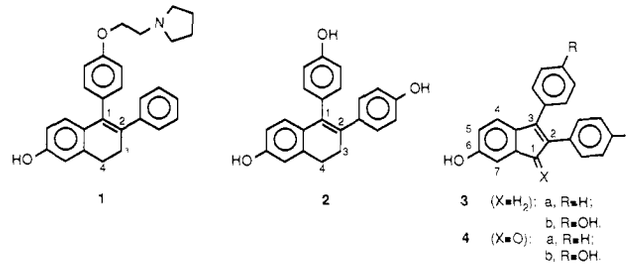
Gregory M. Anstead, Robert J. Altenbach, Scott R. Wilson, and John A. Katzenellenbogen*

Department of Chemistry, University of Illinois, Urbana, Illinois 61801. *Received December 7, 1987*

Two 2,3-diphenylindene and -indenone systems, with potential fluorescent and photofluorogenic properties, were prepared and studied as ligands for the estrogen receptor. The indene systems were prepared by Friedel–Crafts cyclization of appropriate α-benzyl desoxybenzoin systems, and the indenones either by oxidation of the indenes, by cyclization of α-benzoyl desoxybenzoins, or by acylium ion attack on tolan. Crystallographic analysis of the 2,3-diphenylindene and -indenone systems shows the phenyl substituents twisted out of the plane of the indene/indenone systems, with both torsional angles greater in the indenone than indene system; the phenyl attachment to the five-membered ring allows these systems to be considerably more planar than the related 1,2-diphenyl-3,4-dihydronaphthalene and the triarylethylene nonsteroidal estrogens. In contrast to the diphenyldihydronaphthalenes, the diarylindene and -indenone systems undergo photocyclization to phenanthrenes inefficiently. The estrogen receptor binding affinity of these systems is reasonably high (9–59% relative to estradiol), with the indenone systems having higher affinity than the indenes; additional hydroxyl substitution raises the affinity of the indenes but lowers that of the indenones. These trends can be rationalized by considering differences in molecular volumes or surface areas (related to torsional angles) and specific polar interactions.

Two nafoxidine[1] analogues of high estrogen receptor (ER) binding affinity, 1 and 2, have been described as photofluorogenic estrogens.[2,3] In these compounds, the *cis*-stilbene unit of the diaryldihydronaphthalene is photochemically converted to a fluorescent phenanthrenoid.[4] Such ER-targetted fluorophores have been proposed to quantitate the ER in individual cells, thereby providing a clinically useful prognostic technique in the management of breast cancer.[5]

The 2,3-diarylindenes (3)[6] were originally envisaged as another class of photofluorogenic estrogens. However, deletion of one methylene unit from the dihydronaphthalene caused dramatic changes in the ER binding affinity and in the photochemical and fluorescence properties. In addition, 2,3-diarylindenones (4) result from



1 2 3 (X=H_2): a, R=H; b, R=OH. 4 (X=O): a, R=H; b, R=OH.

* Address correspondence to John A. Katzenellenbogen, 461 Roger Adams Laboratory, Box 37, 1209 W. California St., University of Illinois, Urbana, IL 61801.

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