(43) A. Bendich in "The Nucleic Acids", E. Chargaff and J. N. Davidson, Ed., Academic Press, New York, N.Y., 1955, p 81.
(44) L. H. Lister in "The Purines", Wiley, New York, N.Y., 1971, p 38.
(45) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **81**, 2515 (1959).
(46) A. Giner-Sorolla and J. T. Segarra in "Improved Procedures in Nucleic Acid Chemistry", L. Townsend, Ed., Wiley-Interscience, New York, N.Y., 1977, in press.
(47) J. A. Montgomery and L. B. Holum, *J. Am. Chem. Soc.*, **79**, 2185 (1957).
(48) Cf. H. Priewe and A. Poljack, *Chem. Ber.*, **88**, 1932 (1955).
(49) Cf. C. W. Noell and R. K. Robins, *J. Am. Chem. Soc.*, **81**, 5997 (1959).
(50) Wellcome Foundation Ltd., British Patent 767 216 (1957); *Chem. Abstr.*, **51**, 14796 (1957).
(51) A. G. Beaman and R. K. Robins, *J. Appl. Chem.*, **12**, 432 (1962).
(52) G. D. Hartman, M. Sletzinger, and L. M. Weinstock, *J. Heterocycl. Chem.*, **12**, 1081 (1975).
(53) Kindly supplied by Merck, Sharp & Dohme, Inc., Rahway, N.J.
(54) Supplied by courtesy of Stauffer Co., Westport, Conn.
(55) J. H. Burchenal, J. R. Burchenal, M. N. Kushida, S. F. Johnston, and B. S. Williams, *Cancer*, **2**, 113 (1949).
(56) R. L. Kassel, L. J. Old, E. A. Carswell, N. C. Fiore, and W. D. Hardy, Jr., *J. Exp. Med.*, **138**, 925 (1973).
(57) C. Lopez and A. Giner-Sorolla, *Ann. N.Y. Acad. Sci.*, **284**, 351 (1977).
(58) J. W. Hadden, E. Hadden, J. R. Sadlik, and R. G. Coffey, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1717 (1976).

5-Chloro-2-phenyl-1-benzo[*b*]thiophene-3-alkanamines, Potential Antipsychotic Agents

Michael J. Kukla,* Chi M. Woo,

Department of Chemistry

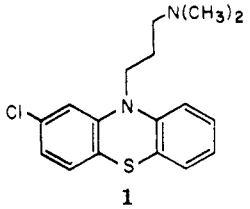
Janet R. Kehr, and Arni Miller

Department of Biology, G. D. Searle and Company, Chicago, Illinois 60680. *Received September 19, 1977*

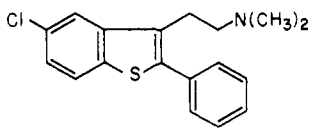
The title compounds (most notably **2a**) were synthesized on the basis of the N-methylation hypothesis of schizophrenia. They were evaluated in dopamine and haloperidol receptor assays. The binding characteristics were comparable in some cases to known neuroleptics.

The N-methylation hypothesis of psychosis is based on the premise that endogenous neurotransmitters are methylated by an *N*-methyltransferase to yield aberrant chemicals which are causative in abnormal behavior.[1] Isolation of the aforementioned enzyme[2] and demonstration of the hallucinogenic properties of *N,N*-dimethyltryptamine[3] have lent credence to these assertions.

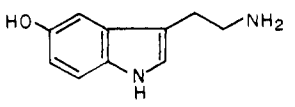
Smythies has reported[4] that chlorpromazine is an effective inhibitor of the transmethylation of tryptamine and implied that this property is related to the effectiveness of chlorpromazine as an antipsychotic agent. Consequently, the title benzothiophenes (most notably **2a**) were synthesized on the basis of their obvious structural similarity to both chlorpromazine (**1**) and serotonin (**3**), a tryptamine derivative which serves as a neurotransmitter. The expectation was that a molecule closer in structure to the natural substrate could be a more effective inhibitor of the methyltransferase and thus exhibit neuroleptic properties. The logic of this rationale was bolstered by Kier's study of the conformational similarities of **1** and **3** and the possible correlation of this conformation with biological activity of the former.[5]



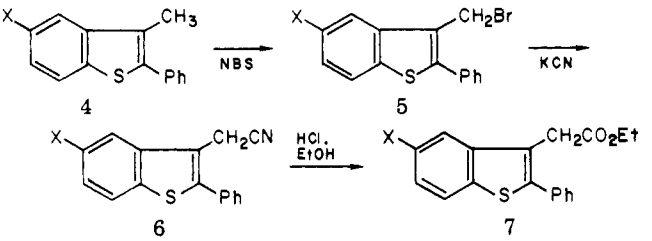
1



2a



Scheme I



4 5 6 7

a, X = Cl
b, X = H
c, X = F

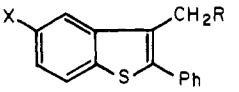
More recently, Snyder[6] has pointed out that chlorpromazine can attain a conformation that mimics the extended form of dopamine (3,4-dihydroxyphenethylamine) in the distance relationship between a phenyl ring and terminal amine. This was the basis for the contention that chlorpromazine acts as a dopamine antagonist. It seems entirely likely that **2a** might also assume a conformation common to these molecules and function in a similar manner.

The initial goal was to obtain **2a**. Subsequently, the desired variations were to assess (a) the effect of different amine functions; (b) whether slight changes to the aromatic substituent would alter biological activity; and (c) the alteration of chain lengths of **2** to one fewer and one more methylene.

Chemistry. Our synthetic efforts centered around a series of steps which have been used to synthesize ester **7a**[7] (Scheme I) and from which target structure **2a** and amine analogues **9**–**13** were accessible. Reduction of **7a** with lithium aluminum hydride ($LiAlH_4$), followed by derivatization with *p*-toluenesulfonyl chloride as shown in Scheme II, yielded the requisite tosylate **8** for amine nucleophilic displacements.

0022-2623/78/1821-0348$01.00/0

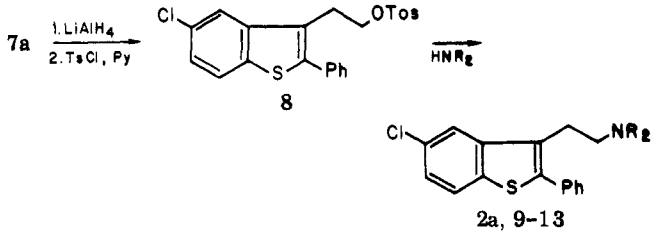
Table I. Displacement of [^{3}H]Dopamine (DA) and [^{3}H]Haloperidol (HALO)



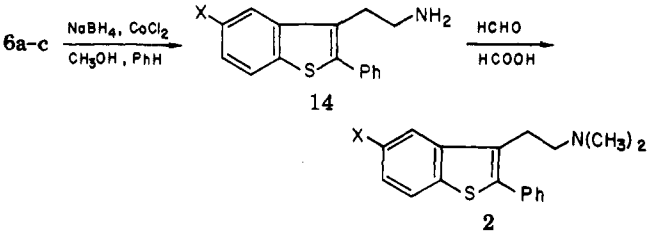
Compd	X	R	[^{3}H]-DA[a]	[^{3}H]-HALO[a]	Ratio[b]
21	Cl	$-N(CH_3)_2$	$>10^{-4}$	1.1×10^{-5}	
22	H	$-N(CH_3)_2$	4.3×10^{-5}	2.4×10^{-6}	17.9
14a	Cl	$-CH_2NH_2$	1.6×10^{-5}	3×10^{-6}	5.3
14b	H	$-CH_2NH_2$	1.6×10^{-5}	3.4×10^{-6}	4.7
14c	F	$-CH_2NH_2$	5.8×10^{-6}	3.5×10^{-6}	1.7
9	Cl	$-CH_2NHCH_3$	8.8×10^{-6}	1.9×10^{-6}	4.6
2a	Cl	$-CH_2N(CH_3)_2$	2×10^{-5}	6.5×10^{-7}	31
2c	F	$-CH_2N(CH_3)_2$	2×10^{-6}	8.5×10^{-7}	2.4
2b	H	$-CH_2N(CH_3)_2$	1.3×10^{-5}	6.2×10^{-7}	21
10	Cl	$-CH_2NEt_2$	6×10^{-6}	1.2×10^{-7}	50
11	Cl	$-CH_2N$-n-Bu_2	$>10^{-4}$	1.3×10^{-6}	
12	Cl	$-CH_2N(CH_2)_4$	4.8×10^{-5}	6.5×10^{-7}	74
19	Cl	$-(CH_2)_2N(CH_3)_2$	4.8×10^{-5}	3.5×10^{-7}	137
1	Chlorpromazine		2.5×10^{-6}	3.4×10^{-8}	74
	Clozapine		8×10^{-6}	1.8×10^{-7}	44
	Haloperidol		5×10^{-7}	1.8×10^{-9}	278

[a] These are molar drug concentrations which inhibit specific binding of 5 nM [^{3}H]dopamine or 1.6 nM [^{3}H]haloperidol by 50% (i.e., IC_{50}). Nonspecific binding is measured in the presence of 10^{-5} M (+)-butaclamol for [^{3}H]-DA and 10^{-4} M dopamine for [^{3}H]-HALO. IC_{50} values were determined from log probit plots using four to six concentrations of each compound assayed in triplicate. [b] IC_{50} [^{3}H]-DA/IC_{50}[^{3}H]-HALO.

Scheme II



Scheme III



Nitrile **6** served as a useful intermediate to obtain (Scheme III) a group of three primary amines (**14a–c**) and two additional dimethylamines (**2b,c**) in which the aromatic substituent has been varied slightly. It was resistant to reduction with $LiAlH_4$ under extended reflux or even in the presence of aluminum chloride. However, treatment with sodium borohydride and 2 equiv of cobaltous chloride[8] smoothly accomplished the desired transformation. Reductive alkylation of the primary amines under Eschweiler–Clarke conditions yielded dimethyl derivatives **2b,c**.

Chain shortening of **2** was a simple matter of displacing bromide **5** with dimethylamine. Homologation, on the other hand, required the multistep sequence shown in Scheme IV. Alkylation of the sodium salt of diethyl malonate with bromide **5a** led to diester **15**, as well as a small amount of dialkylated material (**16**). Carboxylic acid **17** was formed as a result of subsequent base hydrolysis of **15**, followed by spontaneous decarboxylation upon acid workup. Interestingly, a substantial amount of potassium salt **18** could be isolated when it crystallized out of the reaction mixture. Further treatment of **18** with aqueous acid gave a quantitative conversion to **17**. Generation of the acid chloride, followed by treatment with dimethylamine or *N*-methylpiperazine, gave **19** or **20**, respectively, after hydride reduction.

Results and Discussion

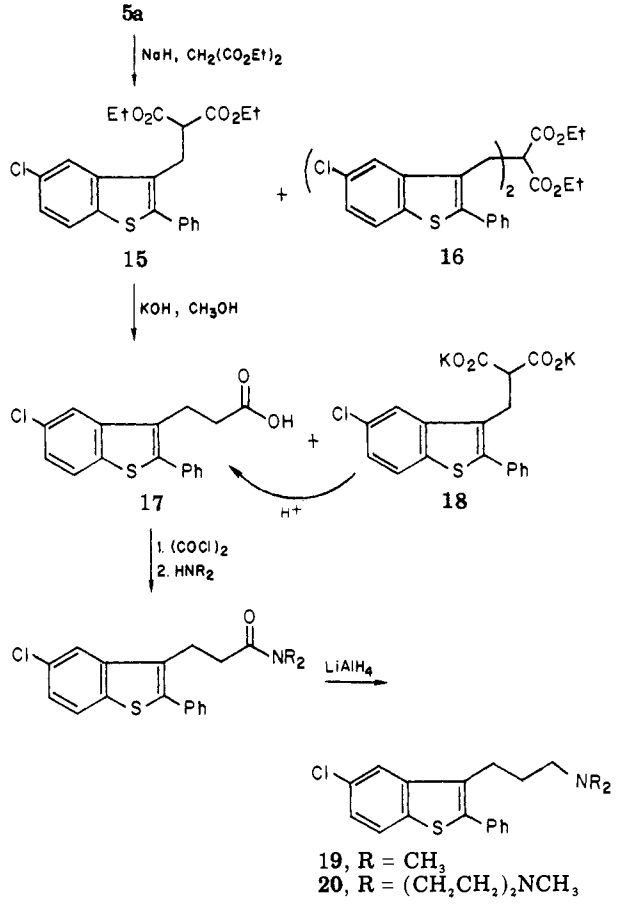
Our biological evaluation of the title compounds was based not so much on the original rationale for synthesis as on recent developments in the literature. Much has been written about a dopamine hypothesis of schizophrenia.[9] Of primary concern is the possible involvement of this neurotransmitter in the etiology of the disease state and, more importantly, the apparent similarity of all antipsychotic agents in the blockade of dopamine functions. However, verification has come only after two groups have isolated a dopamine receptor in membrane preparations from calf brain.[10–15] By use of two labeled ligands, [^{3}H]dopamine and [^{3}H]haloperidol, they have purportedly differentiated between an agonist and antagonist state of the receptor.[11] A large number of compounds including neuroleptics of widely variable structure were tested in the assay. Studies showed that the effectiveness (IC_{50}) of drug in displacing labeled haloperidol correlated well with ED_{50} doses for a number of in vivo animal tests. These included blockade of amphetamine or apomorphine stereotypic behavior in the rat, as well as apomorphine induced emesis in dogs.[14] Most important is the impressive correlation of the average clinical dose to binding affinity.[12]

Our results using this binding assay on the title benzothiophenes are given in Table I.

There are two indicators from the assay data as to whether a compound is a probable neuroleptic. Firstly, it must be a dopamine antagonist. This is determined by a higher affinity for [^{3}H]haloperidol than [^{3}H]dopamine sites. In other words, it must have a high [^{3}H]-DA/[^{3}H]-HALO IC_{50} ratio.[11] Note that the opposite is true for a dopamine agonist. Secondly, the absolute value for displacement of labeled haloperidol seems to correlate with the corresponding potency in vivo.

Five of the present series, i.e., **2a,b**, **10**, **12**, and **19**, have antagonist ratios which are comparable to clozapine and chlorpromazine (see Table I). Interestingly, the primary amines **14a–c** and the single secondary amine **9** all have a ratio less than **10**. In comparing compounds **2a–c** one finds no dramatic effect by the slight changes in aromatic

Scheme IV



substituent (i.e., Cl → H → F). In the homologous series of **21**, **2a**, and **19**, similar binding properties for the two (**2a**) and three (**19**) carbon side chains were observed. The single methylene analogue **21** displayed a decreased binding affinity for both sites.

The troubling aspect is the relative lack of activity that the compounds displayed in in vivo testing. All have been evaluated in blockade of amphetamine lethality in mice and most in blockade of conditioned avoidance response in trained male rats (see the Experimental Section for a description of these procedures). Although a few members of the series do show minimal activity, only **2a,b** and **10** of the five compounds mentioned above protect mice from a lethal dose of *d*-amphetamine at the relatively high doses of 16, 64, and 64 mg/kg, respectively (ca. minimum effective doses). In comparison, we found that chlorpromazine and clozapine blocked amphetamine lethality at 4 mg/kg (MED).

Only **2b** and **10** were active in blockade of CAR at a dose of 40 mg/kg. At the lower dose of 10 mg/kg, each of these compounds was rated as inactive. In fact, although statistically significant in blockade of behavior at the higher dose, the decreases in response were only 8.5 and 3.9%, respectively. On the other hand, chlorpromazine disrupted the number of conditioned responses 21.6% at 2 mg/kg and clozapine 53.2% at 10 mg/kg.

This lack of activity could very well be a reflection of the concentrations which we found necessary for displacement of [^{3}H]haloperidol. Although each of the five antagonists have IC_{50} values similar to the antipsychotic drug clozapine, they are $^1/_{10}$–$^1/_{100}$ as potent as chlorpromazine and haloperidol. Alternatively, the problem could be one of bioavailability or metabolism prior to entry in the CNS. We are hopeful that further studies will shed light on this problem. In the future we also hope to evaluate the ability of this series to inhibit the *N*-methyltransferase enzyme system.

Table II. Physical Characteristics of 5-Substituted 2-Phenyl-1-benzo[*b*]thiophene-3-alkanamines

Compd	Formula	Analyses	Mp, °C
2a	$C_{18}H_{18}NSCl \cdot HCl$	C, H, N, Cl	231–232
2b	$C_{18}H_{19}NS \cdot HCl$	C, H, N, Cl	220 dec
2c	$C_{18}H_{18}NSF \cdot HCl$	C, H, N, S, Cl	245 dec
9	$C_{17}H_{16}NSCl \cdot HCl$	C, H, N, S, Cl	212 dec
10	$C_{20}H_{22}NSCl \cdot HCl \cdot 0.5H_2O$	C, H, N, S, Cl	178–179
11	$C_{24}H_{30}NSCl \cdot HCl$	C, H, N, S, Cl	176–177
12	$C_{20}H_{20}NSCl \cdot HCl$	C, H, S, Cl	170–175
13	$C_{21}H_{23}N_2SCl \cdot 2HCl$	C, H, Cl	>250 dec
14a	$C_{16}H_{14}NSCl \cdot HCl$	C, H, N	>250 dec
14b	$C_{16}H_{15}NS \cdot HCl$	C, H, N, Cl	200–205 dec
14c	$C_{16}H_{14}NSF \cdot HCl$	C, H, N, S, Cl	260 dec
19	$C_{19}H_{20}NSCl \cdot HCl$	C, H, N	252–253.5
20	$C_{22}H_{25}N_2SCl \cdot 2HCl$	C, H, N	239–240
21	$C_{17}H_{16}NSCl$	C, H, N, S	114–115
22	$C_{17}H_{17}NS \cdot HCl$	C, H, N, Cl	231–235

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. In the cases where more than one reaction was carried out by basically the same procedure, only a typical example is reported, even though all compounds are listed in Table II.

Haloperidol ([^{3}H]-G), specific activity 15.867 Ci/mmol, and dopamine ([*ethyl*-1-^{3}H]-N), specific activity 8.95 Ci/mmol, were purchased from New England Nuclear. Nonradioactive dopamine was purchased from Sigma Chemical Co. Thioridazine and (+)-butaclamol were gifts from Sandoz Pharmaceuticals and Ayerst Laboratories, respectively.

5-Chloro-2-phenyl-1-benzo[*b*]thiophene-3-ethanol. A solution of ester **7**[7] (25 g, 75.6 mmol) in 100 mL of ether was added dropwise over a 15-min period to a suspension of $LiAlH_4$ (11.5 g, 300 mmol) in refluxing ether (100 mL) under a nitrogen atmosphere. The resultant mixture was heated under reflux for 48 h before it was cooled to 0 °C and quenched with the cautious sequential addition of 11.5 mL of water, 11.5 mL of 15% NaOH solution (aqueous), and 34.5 mL of water. It was stirred an additional period before the granular aluminum salts were removed by vacuum filtration and washed liberally with ether. Concentration of the filtrate under vacuum yielded 20.8 g (95%) of white solid which was used without further purification: NMR ($CDCl_3$, Me_4Si) δ 1.9 (s, 1 H, –OH), 3.1 (t, *J* = 7 Hz, 2 H, methylene), 3.8 (t, *J* = 7 Hz, 2 H, $-CH_2OH$), 7.1–7.9 (m, 8 H, aromatics).

3-(2-Tosyloxyethyl)-5-chloro-2-phenyl-1-benzo[*b*]thiophene (8). Tosyl chloride (22.4 g, 117 mmol) was added in one portion to a solution of alcohol (16.9 g, 58.6 mmol) in 100 mL of pyridine at 0 °C under nitrogen in an Erlenmeyer flask. The mixture was stirred at 0 °C until all the reagent had dissolved. After storage in a refrigerator at 0 °C for 45 h, it was poured into 500 mL of ice water and extracted with three portions of ether. The combined organic extracts were washed with 6 N HCl (three times, total 500 mL) and brine, dried with a mixture of sodium sulfate–sodium carbonate, and concentrated under vacuum to yield 15.5 g (60%) of an off-white solid. A portion was recrystallized twice from ethanol: mp 123–125 °C. Anal. ($C_{23}H_{19}S_2O_3Cl$) C, H, Cl.

5-Chloro-2-phenyl-1-benzo[*b*]thiophene-3-ethanamines (2a and 9–13). Typical Reaction. Tosylate 8 (3.0 g, 6.7 mmol) and dimethylamine (21 mL, 33.5 mmol) were mutually dissolved in 50 mL of dry dimethylformamide and heated in a bomb (or under a reflux condenser in the case of higher boiling amines) at 68 °C for 26 h. The mixture was then poured into water and extracted with ether. The organic layer was separated, washed with water (three times) and brine, dried with $MgSO_4$, and concentrated on a rotary evaporator to yield 2.0 g (95%) of pale yellow oil: NMR ($CDCl_3$, Me_4Si) δ 2.3 (s, 6 H, $-CH_3$), 2.4–2.8 (m, 2 H, $-CH_2-$),

2.9–3.3 (m, 2 H, $-CH_2N$), 7.2–7.9 (m, 8 H, aromatic). The hydrochloride salt was formed by slow addition of 5 mL of 2-propanolic HCl (ca. 0.27 g/mL) to a stirred solution of the amine in 70 mL of isopropyl alcohol. A flocculent white solid separated from solution and after 30 min 70 mL of ether was added to further precipitate the salt. Filtration yielded, after washing with ether, 2.4 g of the analytically pure amine hydrochloride **2a**; see Table II.

5-Chloro-2-phenyl-1-benzo[*b*]thiophene-3-ethanamine (14). Nitrile **6** (6.9 g, 24.3 mmol) was suspended in a solution of cobaltous chloride hexahydrate (11.6 g, 48.6 mmol) in 300 mL of methanol–benzene (5:1). Sodium borohydride (9.2 g, 243 mmol) was added slowly at 0 °C. When the addition was complete the mixture was allowed to warm to room temperature and stirred for 19 h. The dark solution was then diluted with 100 mL of 3 N HCl, and stirring was continued for 2 h before it was partitioned between water and ether. The aqueous layer was separated, basified with concentrated ammonium hydroxide, and extracted three times with ether. These organic fractions were combined, dried, and concentrated to yield 3.55 g (51.5%) of pale yellow oil: NMR ($CDCl_3$, Me_4Si) δ 1.25 (s, 2 H, $-NH_2$), 3.0 (s, 4 H, methylenes), 7.1–7.8 (m, 8 H, aromatics). Purification was accomplished by hydrochloride salt formation as previously described. Anal. ($C_{16}H_{15}Cl_2NS$) C, H, N.

***N,N*-Dimethyl-5-chloro-2-phenyl-1-benzo[*b*]thiophene-3-methanamine (21).** Dimethylamine (ca. 5 mL) was condensed into a cooled (5 °C) suspension of anhydrous potassium carbonate (3 g) in 120 mL of methyl ethyl ketone. Immediately afterward bromide **5** (5 g, 15 mmol) in 40 mL of dimethylformamide was added. This mixture was stirred at 5 °C for 2 h and then at ambient temperature overnight. Workup gave a solid residue which was recrystallized from isopropyl alcohol to yield 3.4 g (75%) of **21**; see Table II.

Amine **22** was synthesized with the same procedure and purified as the hydrochloride.

***N,N*-Dimethyl-5-chloro-2-phenyl-1-benzo[*b*]thiophene-3-ethanamine (2a). Eschweiler–Clarke Procedure.** Amine **14** (0.9 g, 3.1 mmol) was dissolved in 7 mL of 90% aqueous formic acid before the addition of 0.7 mL of 37% aqueous formaldehyde. The resulting mixture was heated at 70 °C in a nitrogen atmosphere for 15 h and 8 h at 100 °C. It was partitioned between 10% HCl (aqueous) and ether. The aqueous layer was separated, basified with 50% NaOH (aqueous) solution with cooling, and extracted with benzene (three times). The combined benzene extracts were dried and concentrated to yield 0.6 g of yellow oil whose NMR was identical with that of the material obtained previously.

3-[2,2-Bis(ethoxycarbonyl)ethyl]-5-chloro-2-phenyl-1-benzo[*b*]thiophene (15). Diethyl malonate (26.1 g, 0.16 mol) in 50 mL of THF was added over a 30-min period to a cooled (0 °C) and stirred suspension of sodium hydride (3.93 g, 0.16 mol) in 50 mL of THF. To the resultant solution was added bromide **5** (50 g, 0.15 mol) in THF (150 mL) over a 5-min period. The mixture was then stirred at ambient temperature for 18 h before the precipitated sodium bromide was removed by vacuum filtration. Concentration of the filtrate yielded 62 g of white solid of which 57 g was recrystallized twice from Skelly B. The isolated solid (38.5 g) analyzed ($C_{22}H_{21}O_4SCl$) correctly (C, H, Cl) for structure **15**: mp 97.5–98.5 °C; NMR ($CDCl_3$, Me_4Si) δ 1.1 (t, J = 7 Hz, 6 H, $-CH_3$), 3.5 (s, 3 H, methylenes and methine), 4.0 (q, J = 7 Hz, 4 H, $-CH_2CH_3$), 7.2–7.8 (m, 5 H, aromatics). The mother liquors were concentrated and chromatographed on 100 g of silica gel with Skelly B–benzene as the elution system. Thus an additional 5 g of **15** was obtained as well as 4.2 g of **16** which was identified by virtue of its NMR, IR, and molecular ion (m/e 672).

5-Chloro-2-phenyl-1-benzo[*b*]thiophene-3-propionic Acid (17). Potassium hydroxide (28 g) was added to a suspension of diester **15** (35 g) in methanol. The resultant mixture was stirred at ambient temperature for 24 h. A white solid which had precipitated and was isolated by vacuum filtration was determined to be the dipotassium salt **18** on the basis of its subsequent conversion to **17** below. The methanol solution was acidified with 250 mL of 50% HCl (aqueous). Most of the methanol was removed on a rotary evaporator and the remaining aqueous mixture was heated on a steam bath for ca. 30 min. An oil separated which, after cooling, was extracted into ether. The organic layer was dried with brine and $MgSO_4$ and concentrated to yield 24.5 g of **17** as a viscous oil: NMR ($CDCl_3$, Me_4Si) δ 3.6 (s, 4 H, methylenes), 7.2–7.8 (m, 8 H, aromatics), 9.5 (br s, 1 H, $-CO_2H$). An additional 6.8 g of product was obtained by treatment of **18** from above with 10% HCl (aqueous) on a steam bath for 2 h.

***N,N*-Dimethyl-5-chloro-2-phenyl-1-benzo[*b*]thiophene-3-propionamide.** Oxalyl chloride (25 g) was added to a suspension of carboxylic acid **17** (25 g) in 500 mL of benzene, and the resultant mixture was heated under reflux for 21 h. The solvent and excess oxalyl chloride were distilled at atmospheric pressure until a volume of 200 mL remained. It was then concentrated on a rotary evaporator. Two 100-mL portions of benzene were added and subsequently concentrated to a viscous yellow oil (26.1 g) which was used without further purification.

Dimethylamine was bubbled into a THF solution of the acid chloride (13 g), cooled to 0 °C with an ice bath. The mixture was allowed to warm to room temperature and stirred an additional 19 h. It was then partitioned between water and ether. The organic layer was separated, washed with water (three times) and brine, dried with $MgSO_4$, and concentrated in vacuo to yield 10.3 g of yellow oil. Crystallization from isopropyl alcohol yielded 4.9 g of the amide: mp 129–131 °C; NMR ($CDCl_3$, Me_4Si) δ 2.6 (s, 3 H, methyl), 2.8 (s, 3 H, methyl), 3.5 (s, 2 H, H_2O), 3.5–4.0 (m, 4 H, methylenes), 7.2–7.9 (m, 8 H, aromatics). Anal. ($C_{19}H_{18}NOSCl\cdot H_2O$) C, H, N.

N-Methylpiperazine could be substituted for dimethylamine to yield the respective amide.

***N,N*-Dimethyl-5-chloro-2-phenyl-1-benzo[*b*]thiophene-3-propanamine (19).** The amide from above (4.0 g) was dissolved in 25 mL of ether and added dropwise to a suspension of $LiAlH_4$ (1.0 g) in 25 mL of refluxing ether. The mixture was heated under reflux an additional 20 h before it was cooled to 0 °C and quenched with the cautious sequential addition of water (1 mL), 15% NaOH (aqueous) (1 mL), and water (3 mL). It was stirred for 30 min before the granular aluminum salts were removed by vacuum filtration and washed liberally with ether. The filtrate was concentrated to yield 3.2 g of a colorless oil. Purification was accomplished by hydrochloride salt formation as previously described. Amine **20** was obtained by the same procedure. See Table II.

Blockade of *d*-Amphetamine Lethality in Mice. Groups of ten male HAM/ICR mice supplied by Charles River and weighing between 20 and 30 g were used in this procedure. Thirty minutes following intraperitoneal administration of saline (control) or test compound, mice were given intraperitoneal injections of *d*-amphetamine (115 mg/kg) and placed in individual observation cages. Thirty minutes after *d*-amphetamine administration, the mice were observed for lethal effects. This dose of *d*-amphetamine caused approximately 90% lethality in saline-treated control mice. A dose of test compound was rated active if lethality was blocked in a significant number of the treated groups as compared with a concurrent control (Fisher exact probability test, $p \leq 0.05$).

Conditioned Avoidance Response in Trained Rat. The apparatus consisted of a shuttle box divided into two compartments and enclosed in a sound attenuating chamber. The floor of the shuttle box was an electrifiable grid. Following intraperitoneal injection of the vehicle or the drug, the rat was placed into the shuttle cage and was allowed to acclimate for approximately 1 min. A 5-s conditioned stimulus, consisting of a tone and a light, preceded a 0.2-mA footshock delivered to the grid floor of the cage. The shock was automatically terminated after 30 s if the rat failed to respond. A shuttle response during the conditioned stimulus period terminated the conditioned stimulus, prevented the onset of the shock, and was scored as an avoidance response. A shuttle response during the shock period terminated the conditioned stimulus and the shock and was scored as an escape response. If no escape response was made, an escape failure was scored. Each conditioned stimulus presentation was separated by a 15-s interval, and a response during this time resulted in the onset of the shock and the conditioned stimulus until the rat returned to the other side. Each rat was presented with 100 trials (i.e., 100 conditioned stimulus presentations) which took approximately 30 min. Each rat was trained to an average criterion of ≥85 avoidance responses per 100 trial session when

administered vehicle control solution. Four rats per dose were tested and each rat's performance under the drug treatment was compared to his previous performance under vehicle control treatment. Comparisons were made by means of a paired t test ($p \leq 0.05$, two tailed). Usually three doses of a compound were evaluated. Most drugs were evaluated 30 min postinjection but intervals of 1–90 min could be used.

Receptor Binding Studies. Calf caudate nuclei were dissected from freshly obtained brains and stored frozen at −76 °C. As needed, caudate tissue was homogenized and prepared following procedures outlined by Creese and co-workers.[11]

Receptor binding studies were performed as reported in the literature[11,13,15] with slight modifications. A typical sample contained 2 mL of caudate membrane homogenate (10 mg of original tissue/mL) in a final ligand concentration of either 5 nM [^{3}H]dopamine or 1.6 nM [^{3}H]haloperidol. Test compounds were added as 20-μL aliquots from stock solutions prepared in absolute ethanol or 0.1% ascorbic acid. Samples were incubated in triplicate at 37 °C for 15 min when [^{3}H]dopamine was used and for 10 min when [^{3}H]haloperidol was present.

Immediately following all incubations, proteins were recovered on Whatman GF/B glass fiber filters under reduced pressure. Trapped membranes were solubilized off the filters using 1 mL of NCS tissue solubilizer (Amersham/Searle Corp.) at 50 °C for 1 h. Then the pH was adjusted by adding 0.1 mL of glacial acetic acid, 10 mL of PCS (Amersham/Searle Corp.) was added, and the samples were analyzed for membrane-bound radioactivity using a Mark II liquid scintillation counter (Searle Analytical, Inc.).

Nonspecific binding was measured in the presence of 10^{-5} M (+)-butaclamol for the [^{3}H]dopamine studies and 10^{-4} M nonradiolabeled dopamine for the [^{3}H]haloperidol studies. IC_{50} values were determined from log probit using four to six concentrations of each compound.

Acknowledgment. Appreciation is extended to Mr. J. Bloss for in vivo biological assays, Mr. E. Zielinski for microanalytical results, Mr. R. Ray for chemical assistance, Dr. F. Hershenson for useful discussions, and Ms. D. Sheller and Ms. J. Vassilatos for preparation of the manuscript.

References and Notes

(1) R. J. Wyatt and J. C. Gillin, *Psychiatry Ann.*, **6**, 33 (1976), and references cited therein.
(2) (a) J. Axelrod, "Transmethylation and Methionine Biosynthesis", S. K. Shapiro and F. Schlenk, Ed., University of Chicago Press, Chicago, Ill., 1965, p 71; (b) A. J. Mandell, B. Buckingham, and D. Segal, "Advances in Behavioral Biology", Vol. 1, B. T. Ho and W. M. McIsaac, Ed., Plenum Press, New York and London, 1971, p 37.
(3) H. Tanimukai, R. Ginther, J. Spaide, J. R. Bueno, and N. E. Himrich, *Br. J. Psychiatry*, **117**, 421 (1970).
(4) F. Antun, D. Eccleston, and J. R. Smythies in ref 2b, p 61.
(5) L. B. Kier, *J. Theor. Biol.*, **40**, 211 (1973).
(6) A. P. Feinberg and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1899 (1975); A. S. Horn and S. H. Snyder, *ibid.*, **68**, 2325 (1971).
(7) M. Descamps, E. Van Durme, M. Colot, and R. Charlier, *Eur. J. Med. Chem.-Chim. Ther.*, **8**, 536 (1973).
(8) T. Satoh, S. Suziki, Y. Suziki, Y. Meyaji, and Z. Imai, *Tetrahedron Lett.*, 4555 (1969).
(9) S. H. Snyder, *Am. J. Psychiatry*, **133**, 197 (1976).
(10) S. H. Snyder, I. Creese, and D. R. Burt, *Psychopharmacol. Commun.*, **1**, 663 (1975).
(11) I. Creese, D. R. Burt, and S. H. Snyder, *Life Sci.*, **17**, 993 (1975).
(12) P. Seeman, M. Chau-Wong, J. Tedesco, and K. Wong, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4376 (1975).
(13) D. R. Burt, S. J. Enna, I. Creese, and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4665 (1975).
(14) I. Creese, D. R. Burt, and S. H. Snyder, *Science*, **192**, 481 (1976).
(15) D. R. Burt, I. Creese, and S. H. Snyder, *Mol. Pharmacol.*, **12**, 800 (1976).

Synthesis of O-Alkylated Lysine-vasopressins, Inhibitors of the Antidiuretic Response to Lysine-vasopressin

Lars-Eric Larsson,* Gunnar Lindeberg,

Institute of Biochemistry, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden

Per Melin,

Ferring AB, Fack, S-200 60 Malmö, Sweden

and Vladimir Pliška

Institute of Molecular Biology and Biophysics, ETH-Hönggerberg, CH-8093 Zürich, Switzerland. *Received November 4, 1977*

[Mpa^1,Tyr(Et)^2]-LVP (1-deamino-2-*O*-ethyltyrosine-8-lysine-vasopressin), [Mpa^1,Tyr(*n*-Pr)^2]-LVP, [Tyr(*n*-Bu)^2]-LVP, [Mpa^1,Tyr(*n*-Bu)^2]-LVP, and [Mpa^1,Tyr(*n*-hexyl)^2]-LVP were synthesized in solution by the *p*-nitrophenyl ester method. The previously prepared [Tyr(Et)^2]-LVP was resynthesized. All compounds possessed weak agonistic properties in both antidiuretic (0.5–2.0 IU/μmol) and pressor (0.5–3.0 IU/μmol) assays. In the rat none of the analogues inhibited the antidiuretic action of LVP when the two substances were given together in a single injection. However, when administered in low subthreshold doses, most of the deamino compounds suppressed the antidiuresis induced by a continuous infusion of LVP. Complete inhibition was obtained with [Mpa^1,Tyr(Et)^2]-LVP. The antagonistic potency seemed to decrease with increasing size of the alkyl substituent and [Mpa^1,Tyr(*n*-hexyl)^2]-LVP showed no antagonism. The molar inhibitor–LVP ratio for maximal inhibition was well below 100. Neither of the two amino analogues showed a clear-cut antagonism in the antidiuretic assay. Furthermore, none of the reported compounds was antagonistic to LVP in the rat pressor assay.

In the search for antagonists of the neurohypophyseal hormones, most work has been focused on oxytocins, and the antagonistic properties have been evaluated mainly in terms of oxytocin-like activities. The most potent inhibitors have proven to be analogues modified in position 1 alone or in combination with changes in positions 2 and 4 of the oxytocin molecule.[1,2]

Besides antagonizing the uterotonic action of oxytocin, some of these compounds also antagonize vasopressor responses to vasopressins. Thus, N-substituted [Tyr(Me)^2]oxytocins inhibit the vasopressor action of lysine-vasopressin (LVP) at inhibitor–LVP ratios[3] of about

0022-2623/78/1821-0352$01.00/0