Notes

Conformationally Restricted Phenothiazine Neuroleptics. 1. 3-(Dimethylamino)-1,2,3,4-tetrahydroazepino[3,2,1-*kl*]phenothiazine

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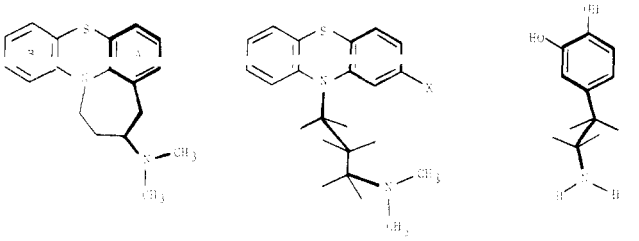
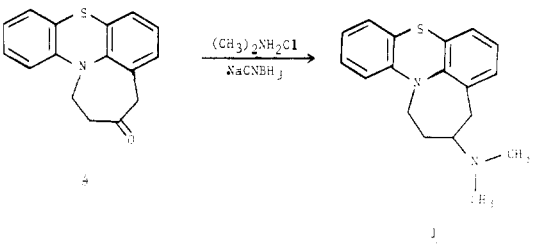
A rigid analogue of promazine, 3-(dimethylamino)-1,2,3,4-tetrahydroazepino[3,2,1-*kl*]phenothiazine (1), was prepared by reductive amination of the corresponding ketone 4. An X-ray crystallographic study revealed that the seven-membered ring of the hydrochloride salt of 1 exists as a half-chair-like form with the dimethylammonium group in an equatorial-like conformation. Compound 1 was approximately one-half as active as promazine as an inhibitor of [^{3}H]spiperone binding in rat corpus striatal homogenates. In homogenates obtained from calf caudate tissue, however, 1 was only about one-twentieth as active as promazine as an inhibitor of [^{3}H]spiperone binding. As a stimulator of homovanilic acid (HVA) synthesis in rat corpus striatum in vivo, it was about one-tenth as active as promazine.

The title compound 1, an analogue of promazine (**2a**),



was synthesized as part of a program directed toward the synthesis and pharmacological evaluation of conformationally restricted derivatives of the phenothiazine tranquilizers. The concept that the neuroleptics exert their antipsychotic actions through antagonism of dopamine receptors in the central nervous system is supported by an impressive body of evidence.[1-6] A molecular mechanism proposed to explain the dopaminergic receptor blocking action of chlorpromazine **2b** calls attention to the possible complementarity of the chlorpromazine and dopamine molecules.[3,7] Support for this suggestion has been found in the comparison of the observed solid-state trans (anti) conformation for dopamine hydrochloride[8] (**3**) with

Scheme I



an overlapping partially folded conformation found by X-ray analysis[9] of a variety of 2-substituted neuroleptic phenothiazines and thioxanthenes where the amino function is nearer the ring bearing the 2-substituent. Thus, it is proposed that the A ring of chlorpromazine, for example, is superimposable at the dopamine receptor with the aromatic ring of dopamine, wherein the sulfur atom of the phenothiazine ring system substitutes for the *m*-catechol oxygen atom of dopamine, and the protonated side-chain nitrogen atom folds over toward the A ring to correspond with a trans conformation for the protonated amino group of dopamine.

Compound 1 was selected as an early target of our efforts because its nearly rigid structure contains both the dimethylaminopropyl side chain of **2** and a dopamine (β-phenylethylamine) moiety. Furthermore, inspection of Dreiding molecular models of 1 suggested that in either of the most likely conformations of the tetrahydroazepine ring (i.e., half-chair-like or half-boat-like), the dimethylamino group would favor an equatorial orientation and would thus be trans to the A ring. Thus, if a *trans*-dopamine overlapping conformation at the receptor is assumed, the probability of an unfortuitous steric interaction

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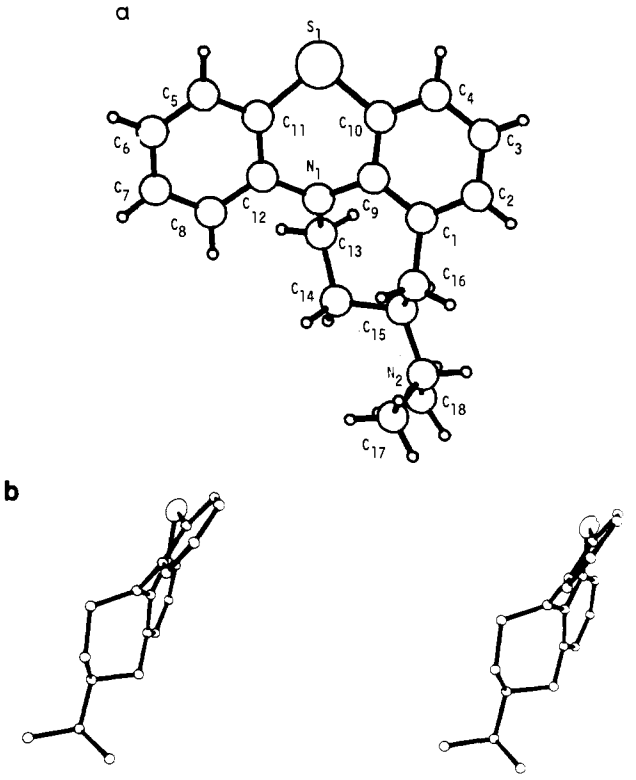

Figure 1. Solid-state structure of 3-(dimethylamino)-1,2,3,4-tetrahydroazepino[3,2,1-*kl*]phenothiazine hydrochloride: (a) top view with atom numbering; (b) stereoscopic pair (side view).

between 1 and the receptor, resulting from constraint of the side chain in a seven-membered ring, should be minimal.

Results and Discussion

Chemistry and X-ray Analysis. The rigid promazine analogue 1 was prepared by reductive amination of the previously reported[10] ketone 4 using dimethylamine hydrochloride and sodium cyanoborohydride (Scheme I).[11] The solid-state structure of the hydrochloride salt of 1 determined by X-ray crystallography[12] is shown in Figure 1. The seven-membered ring exists as a half-chair-like conformation with the β-phenylethylamine system in essentially a trans (anti) conformation. The C_1–C_{16}–C_{15}–N_2 torsion angle is 155°. As expected, the N_2–A ring distance (the distance from the side-chain nitrogen atom to the center of the A ring) of 5.17 Å is near the 5.14 Å distance found[8] for the trans conformation of dopamine hydrochloride in the solid state. The N_2–B ring distance is 7.03 Å. The comparable N_2–A ring and N_2–B ring distances for promazine hydrochloride in the solid state[13] are 6.09 and 7.39 Å, respectively. Thus, 1 resembles dopamine conformationally more closely than it does promazine and other neuroleptics. Somewhat suprisingly, N_2 is only 0.77 Å above the plane of the A ring despite the aryl-C_{16} torsion angles of −69° for C_9–C_1–C_{16}–C_{15} and 117° for C_2–C_1–C_{16}–C_{15}. Flattening of the β-phenylethylamine system of 1, brought about by constraints imposed by the fusion of the seven-membered ring onto the phenothiazine nucleus, apparently occurs. The N_2–B ring plane distance is 1.17 Å. Comparison of torsion angles for the dimethylaminopropyl side chains of 1 and **2a** reveals additional conformational differences between these molecules. The side chain for **2a** is more extended, while that for 1 is constrained in a folded conformation by the seven-membered ring fusion with the A ring.

Neurochemical Studies. Receptor-binding techniques provide a sensitive method for determining the in vitro receptor affinities of neuroleptic agents.[14] Several investigators[15-18] have studied the binding of [^{3}H]spiperone, a potent butyrophenone neuroleptic and dopamine antagonist in the caudate of rat, calf, and human brain. Inhibition studies[17,18] with dopamine agonists and antagonists indicate that [^{3}H]spiperone is a ligand of choice for the screening of neuroleptics in vitro. The rigid promazine analogue 1 was compared with promazine and chlorpromazine as an inhibitor of 100 pM [^{3}H]spiperone binding in membrane preparations obtained from both rat corpus striatum and calf caudate. The IC_{50} (the concentration required to inhibit specific binding by 50%) values in the rat were 2.15×10^{-6}, 1.04×10^{-6}, and 1.12×10^{-7} M for 1, promazine, and chlorpromazine, respectively. In the calf, the IC_{50} values for 1, promazine, and chlorpromazine were 2.4×10^{-6}, 1.3×10^{-7}, and 2.5×10^{-8} M. Thus, promazine was about 2 times and chlorpromazine 20 times the potency of 1 in the rat, while in the calf the relative potencies of promazine and chlorpromazine were nearly 20 times and 100 times that of 1.

The increase in central dopamine metabolites following treatment with neuroleptics has been attributed to blockade of postsynaptic dopamine receptors. The increase in the major dopamine metabolite, homovanillic acid (HVA), in rat corpus striatal tissue following treatment with standard neuroleptics has been shown to be dose related and correlates reasonably well with neuroleptic-induced suppression of stereotypy and other animal tests for neuroleptic activity.[19] When compared with promazine at the ED_{50} dose of 200 μmol/kg[19] in the rat in vivo, the rigid promazine analogue 1 increased striatal HVA synthesis only by 24%, while promazine brought about a 250% increase. Since the ED_{50} for chlorpromazine in the HVA assay is around 5.8 μmol/kg,[19] it is evident that 1 shows little promise as a neuroleptic agent.

Structure–Activity Considerations. The very low order of neuroleptic potency of the rigid promazine analogue 1 provides evidence against a promazine (and possibly chlorpromazine) side-chain conformation at the dopamine receptor which truly overlaps the trans (anti) form of dopamine which is found in the solid state. It is probable that in the receptor bound conformation(s) of the neuroleptic phenothiazines and thioxanthenes the average distance between the center of the nearest aryl ring and the basic nitrogen atom is somewhat greater than the 5.14 Å distance observed in the solid state[8] for dopamine hydrochloride. With the exception of chlorpromazine base which has a 5.12 Å distance,[20] the distance between the

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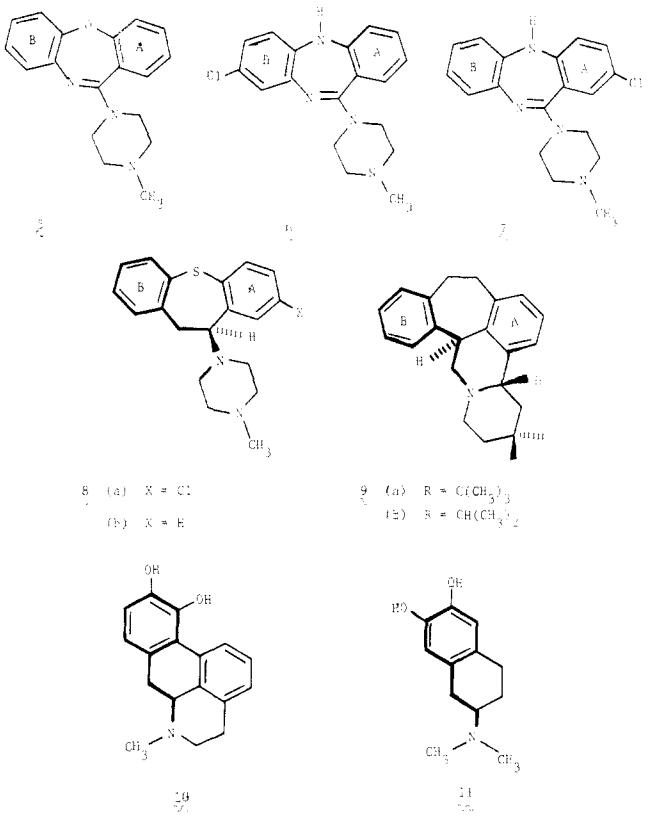
A ring centroid and the side-chain nitrogen for a large group of tricyclic neuroleptics ranges from 5.7 to 6.7 Å,[9,21] in the solid state. In chlorpromazine hydrochloride it is 6.70 Å.[22] Chlorpromazine and its hydrochloride are flexible molecules in which the side chain can probably exist in several solution (and gas phase) conformations differing only slightly in potential energy. It is, therefore, not surprising that their solid-state conformations differ, since the ionic and hydrogen bonding forces that are very important (if not dominant) in the crystal lattice of the salt are absent in the free base. X-ray crystallographic studies of several closely related semirigid tricyclic neuroleptics, namely loxapine (**5**), cloxapine (**6**) and its positional isomer HF-2046 (**7**),[23] and (+)-octaclothiepin (**8**),[24] however, point to the same conformational asymmetry of the side chain observed for the conformationally flexible neuroleptics in the solid state.[9]



In view of the fact that promazine is significantly less potent than chlorpromazine in animal tests for neuroleptic activity,[19,25] it is possible that the apparent pharmacological inertness of **1** can be attributed entirely to the lack of an electronegative substituent at an appropriate position in the A ring. The absolute essentiality of an electronegative aryl substituent is disputed, however, by the observed potent neuroleptic activity of unsubstituted members of the benzocycloheptapyridoisoquinoline and dibenzo[*b*,*f*][1,4]thiepin series, e.g., (+)-butaclamol[26] (**9a**) and perathiepin[27] (**8b**), respectively. In fact, single chlorine atom substitutions in either the A or B rings of the isopropyl analogue **9b** tend to reduce neuroleptic activity in animals.[28] The conformational similarities between the *trans*-β-phenyethylamine moieties of (+)-butaclamol and (+)-octaclothepin have been noted by Humber et al.[28] who,[26] along with Horn et al.,[9] suggested that (+)-butaclamol mimics dopamine conformationally at receptors in the CNS. Although the β-phenylethylamine moiety of the rigid promazine analogue **1** also exists in a conformation wherein the phenyl ring and nitrogen atom are trans, the torsion angles of −69 and 117° for the C_1–C_{16} bond differ considerably from the comparable aryl carbon–α carbon torsion angles in **8** and **9**, which are nearly 0°. Thus, the atoms forming the β-phenylethylamine systems in **8** and **9** are nearly coplanar, whereas in **1** the phenyl ring is approximately perpendicular to the plane made by the ethylamine system bonded to it. X-ray crystallographic studies on the dopamine agonists 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene[29] (A-6,7-DTN, **10**) and apomorphine[30] indicate that the β-phenylethylamine moieties of these potent dopaminergic agonists are also nearly coplanar. Perhaps a nearly coplanar arrangement of these atoms is essential for firm receptor binding of both agonists and antagonists of the β-phenylethylamine type. The apparent low affinity of **1** for dopamine receptors in the CNS may therefore be principally due to an unfavorable β-phenylethylamine conformation rather than to the absence of an electronegative A ring substituent.

Experimental Section

The melting point was determined on a Mel-Temp apparatus and is uncorrected. The proton magnetic resonance spectrum was obtained in deuteriochloroform with tetramethylsilane as an internal standard using a Varian Model EM360 60-MHz spectrophotometer. The elemental analysis was performed in the Microanalytical Laboratory of the Department of Chemistry, State University in Groningen, The Netherlands.

3-(Dimethylamino)-1,2,3,4-tetrahydroazepino[3,2,1-*kl*]-phenothiazine. To a solution of 920 mg (11.3 mmol) of dimethylamine hydrochloride in 40 mL of MeOH was added, with stirring, 250 mg (4.5 mmol) of KOH. When the KOH had completely dissolved, a solution of 630 mg (2.1 mmol) of 1,2,3,4-tetrahydroazepino[3,2,1-*kl*]phenothiazin-3-one[10] (**4**) in 10 mL of THF and 1 g of 3Å molecular sieves were added. The resulting yellow suspension was stirred at 25 °C for 15 min and a solution of 60 mg (0.95 mmol) of $NaCNBH_3$ was added. The resulting mixture was stirred at 25 °C for 12 h and then tested with pH paper. Since the system was slightly alkaline (pH 8–9), an additional 50 mg of dimethylamine hydrochloride and 20 mg of $NaCNBH_3$ were added, and the resulting mixture was allowed to stir for an additional 36 h. The reaction mixture was filtered and the filtrate concentrated in vacuo to give a viscous orange oil, which was dissolved in 30 mL of MeOH and treated with 6 N aqueous HCl. After most of the MeOH was removed in vacuo, the largely aqueous solution was extracted with 50 mL of C_6H_6, separated, basified with 5% NaOH solution, and finally extracted with two 50-mL portions of $CHCl_3$. The $CHCl_3$ extracts were combined, washed with distilled H_2O (50 mL), and dried over anhydrous $MgSO_4$. Evaporation of the solvent in vacuo afforded 590 mg (84%) of the amine as a viscous orange oil, a sample of which chromatographed as a single spot on a silica gel G TLC plate (4:1 $CHCl_3$/MeOH, R_f 0.65): NMR ($CDCl_3$) δ 2.05 (m, 2 H, aliphatic C-CH_2-C), 2.15 [s, 6 H, $N(CH_3)_2$], 2.7–2.8 (2 m, 3 H,

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benzylic CH_2 and equatorial N_1 CH), 3.1-4.1 (2 m, 2 H, axial N_1 CH and axial N_2 CH), 6.7-7.2 (m, 7 H, aryl H). The oil was dissolved in 20 mL of dry Et_2O and a solution of 20 mL of Et_2O saturated with HCl gas was added dropwise to precipitate the hydrochloride salt as a yellow powder. The powder was recrystallized from MeOH to give 580 mg (67%) of rhombic crystals, mp 121 °C dec. Anal. ($C_{18}H_{20}N_2S$·HCl) C, H, N, S.

[^{3}H]Spiperone Binding. Binding experiments were carried out in twice washed 5% homogenates obtained from rat corpus striatum (or frozen calf caudate). Male, Sprague–Dawley rats (150–300 g) were decapitated, and their brains were immediately removed and dissected. The brain tissue was weighed and then homogenized in ice-cold 0.05 M sodium–potassium phosphate buffer (pH 7.4). After centrifugation at 48000g for 20 min (Sorvall RC2-B), the supernatant was discarded, the pellet was resuspended in distilled water, and the process was repeated. The final pellet was resuspended in 50 volumes of ice-cold 0.05 M Tris buffer (pH 7.4). Aliquots of brain tissue (final concentration 1.25 mg/mL), [^{3}H]spiperone (100 pM), and drugs (1, promazine hydrochloride and chlorpromazine hydrochloride) were incubated in buffer (final assay volume 2 mL) for 30 min at 37 °C. The binding reaction was terminated by filtration in vacuo over Whatman GF/B filters and rinsing with 3 × 5 mL of ice-cold buffer. Tissue radioactivity was extracted overnight in 6 mL of scintillation fluid [1 L of toluene (Baker), 1 L of Triton X-100 (NEN), 16 g of Omnifluor (NEN)] and measured in a Searle Mark II liquid scintillation counter (45% efficiency). Specific [^{3}H]-spiperone binding was defined as the difference between binding in the absence and in the presence of 1 μM (+)-butaclamol. The negative logarithm of the concentration of drug producing a 50% inhibition of specific binding (pIC_{50}) values were estimated graphically from logarithmic Hill plots composed of at least five points on the decline of the curve and converted to IC_{50} values.

Homovanillic Acid (HVA) Increase in Rat Corpus Striatum. The hydrochloride salt of 1 was dissolved in a 10% PEG 400 solution in distilled water. Promazine hydrochloride was dissolved in distilled water. Female Wistar rats (140–180 g) were injected ip with the drug solution or saline (controls). The rats were decapitated 2 h after the injections, and the corpus striatum was dissected on a cold plate, immediately frozen in liquid nitrogen, weighed, and homogenized in 1 mL of 0.4 M perchloric acid. After addition of KOH/potassium formate solution and centrifugation (15 min, 5000g, 2 °C), the supernatant was assayed for HVA by its isolation on small Sephadex G columns and its conversion to a fluorophor by oxidative dimerization with $K_3Fe(CN)_6$, followed by automated fluorimetric analysis.[31]

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Crystal and Molecular Structure of Nafoxidine and Stereochemical Features of Anticancer Antiestrogens

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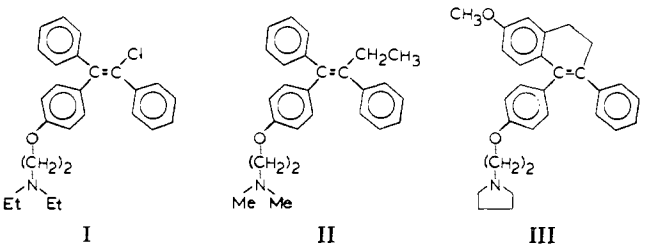
We have determined the molecular structure of the anticancer antiestrogen nafoxidine and compared its three-dimensional structure with two other clinically useful antiestrogens in order to delineate stereochemical parameters in these compounds. Crystals of nafoxidine hydrochloride–ethanol are monoclinic with cell dimensions a = 17.040, b = 7.967, c = 25.260 Å, β = 123.7°, and space group $P2_1/c$ with four formula units per cell. The structure was solved by direct phasing methods and refined to a discrepancy index of 0.068. The methoxyphenyl and phenyl rings are trans to each other relative to the ethylene bond, and the substituted amine–aryl ether chain has an extended conformation. Stereoscopic superposition drawings and tabular data are given to show structural similarities and differences in nafoxidine, clomiphene, and tamoxifen, the three antiestrogens with demonstrated clinical efficacy in the management of metastatic mammary carcinoma.

Breast cancer is the most common malignancy among North American and European women. Therapeutic management is an important problem, as about two-thirds of all patients eventually require treatment for disseminated disease. Hormonal manipulation has conventionally been the mainstay of therapy in this phase; however, only about one-third of patients show clinical response, and endocrine control is often temporary. In recent years the prospects for management of metastatic breast cancer have improved through (1) the identification of estrogen receptors (ER) in 50–65% of breast tumors and the predictive use of this test for potential response to hormonal therapy and (2) the development of nonsteroidal antiestrogens for use with patients with ER-positive tumors.[1]

Three antiestrogens with consistent activity in human breast cancer have been identified: Clomiphene (I) was



I II III

the first antiestrogen to show clinical usefulness,[2] but it has not been widely tested and is not currently approved for this use. Tamoxifen[3] (II) has recently been marketed after extensive trials; clinical results show it to be at least as effective as hormone treatment, with much reduced side effects. Nafoxidine (III) was originally synthesized[4] as an

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