TRITIUM LABELLED (±)-10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-(methylmercapto)-[3-^{3}H(n)]phenothiazine (Thioridazine)

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SUMMARY

The antipsychotic drug, thioridazine, differs from more classical antipsychotic drugs with respect to both some atypical psychopharmacological characteristics and to the critical involvement of active metabolites in its pharmacology. Tritium labelled thioridazine has been prepared in our laboratory by palladium catalyzed reduction of an aryl brominated precursor using carrier free tritium gas in THF. The product is labelled in the 3-position of the phenothiazine ring system and a specific activity of 12 Ci/mmole was obtained.

Key Words: thioridazine, (±)-10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-(methylmercapto)phenothiazine, catalytic reduction, deuterium, tritium

INTRODUCTION

Like most other antipsychotic agents, the phenothiazine, thioridazine (Mellaril®; 10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-(methylmercapto)phenothiazine), probably exerts its clinical effects principally via blockade of dopamine receptors. However, several factors contribute to the rather interesting pharmacology of this drug. For one, thioridazine has long been known to produce rather atypical behavioral effects, in both animals and man, compared to some of the more

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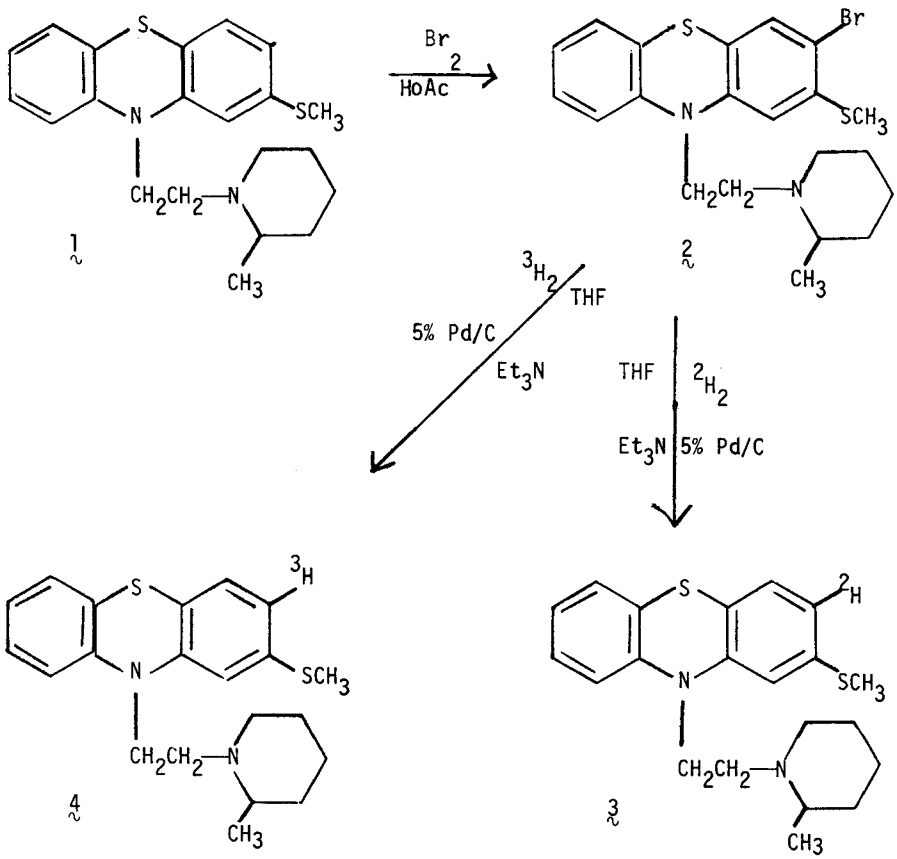


Figure 1

classical antidopaminergic antipsychotic agents such as haloperidol or chlorpromazine (1,2,3,4). The mechanisms behind these atypical behavioral effects have not been fully defined, although several hypotheses have been proposed (5,6).

Secondly, thioridazine, like most phenothiazines is extensively metabolized to both active and inactive compounds in animals and man. The side chain sulfoxide and sulfone metabolites in particular have been reported to be active as

antipsychotics when administered directly (7,8,9). These two sulfoxidized metabolites also have been reported to be more potent antidopaminergic compounds than thioridazine in radioligand binding studies (10,11,12,13). Furthermore, these two metabolites are more potent antidopaminergic agents in studies of dopamine and acetylcholine release from perfused striatal slices (14,15). The greater sensitivity of detection methods for radiolabelled compounds would allow us to explore these aspects of thioridazine pharmacology further. Therefore, tritium labelled thioridazine was prepared.

DISCUSSION

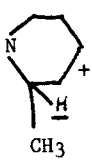
Aryl labelling of the 3-position of the phenothiazine ring system by bromination and subsequent reductive debromination with tritium gas appeared to be a reasonable approach for obtaining the labelled product. Therefore, racemic thioridazine free base (1) (Figure 1) was treated with one equivalent of bromine in glacial acetic acid at room temperature to afford the 3-bromo derivative 2 as evidenced by ^{1}H-NMR and mass spectral data. Upon treatment of 2 with 1.0 atm of deuterium gas in the presence of triethylamine, 5% Pd/C and THF, the substrate was rapidly debrominated to the extent of approximately 40-50%. Further exposure for up to 16 h effected no further progression probably due to catalyst poisoning by divalent sulfur in the substrate's structure. Mass spectral incorporation studies on the product (3) indicated d_0=52.54%, d_1=39.12% and d_2=8.34%. This extent of deuterium incorporation would predict a specific activity upon tritiation of ~ 16 Ci/mmole. Upon tritiation using 5.0 Ci of carrier free tritium gas under similar conditions, 296 mCi of pure tritiated product (4) was obtained with a specific activity of 12 Ci/mmole (32 mCi/mg).

EXPERIMENTAL PROCEDURES

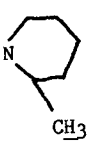
All chemicals were used as obtained from the manufacturer. Melting points

were obtained on a MEL-TEMP melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a JEOL FX-60 60 MHz FT spectrometer using $CDCl_3$ (TMS) as solvent. Radiopurity was determined using a Packard Radioscanner Model 7201. Tritium was counted using a Packard Tricarb Minaxi Liquid Scintillation Counter Model 4000 (external standard) with Scintiverse® (Fisher) counting solution. Silica gel plates (UV) were used for TLC analysis. Elemental composition of novel compounds was determined by high resolution mass spectrometry using an AEI MS-902 mass spectrometer.

(±)-10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-(methylmercapto)-3-bromophenothiazine (2). Bromine (428 mg, 2.68 mmol) in 1.0 ml of glacial acetic acid was added dropwise to a solution of 900 mg (2.43 mmol) of racemic thioridazine (1) base in 8.0 ml of glacial acetic acid with stirring at room temperature. A dark blue color appeared immediately as well as a precipitate. After 15 min. the volatiles were removed in vacuo and the dark blue residue was shaken between CH_2Cl_2 and saturated $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated in vacuo to afford an amber colored solid. Recrystallization from acetone afforded 700 mg (64%) of product as amber colored crystals; mp = 133-136° m/e = 448.0643 ($C_{21}H_{25}BrN_2S_2$ requires 448.0644). ^{1}H-NMR ($CDCl_3$, TMS) δ 7.41-6.75 (m, 5H, ArH_5) 6.68 [s, 1H, ArH(1)], 3.90 (t, 2H,-CH_2 -N), 2.75 (m,3H,



+ -CH_2 CH_2 -N), 2.48 (s, 3H, SCH_3), 2.22 (s, 3H,



), (m, 8H, piperidinyl H's).

(±)-10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-(methylmercapto)-[3-^{2}H(n)] phenothiazine (3). A solution of 80.3 mg (0.179) mmol) of 2 and 250 µl of triethylamine in 3.0 ml of dry THF was stirred in the presence of 50 mg of 5% Pd/C under 1.0 atm of deuterium gas for 4 h at room temperature. TLC indicated about 40 -50% conversion to the higher R_f deuterated product. Longer reaction times did not better the extent of reaction. The catalyst was filtered off through a Celite pipet column and the filtrate evaporated in vacuo. The residue was shaken between CH_2Cl_2 and saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated in vacuo to afford 29.8 mg of a gum. Purification on two 20 x 20 cm x 0.25 mm silica gel plates (EtoAc-Hex-EtOH-NH_4OH 60:30:10:1) afforded 14.7 mg (22%) of pure product which was identical to authentic thioridazine with regard to R_f value and ^{1}H-NMR spectrum (except for presence of deuterium). Mass spectral data indicated d_0=52.54%; d_1=39.12% and d_2=8.34%. ^{1}H-NMR ($CDCL_3$, TMS) δ 7.40 - 6.72 (m, 5H, Ar<u>H</u>$_5$), 3.90 (t, 2H, -C<u>H</u>$_2$CH$_2$N), 2.76 (m, 3H, -CH$_2$C<u>H</u>$_2$N + ), 2.12 - 1.20 (m, 8H, piperidinyl <u>H</u>'s).

(±)-10-[2-(1-Methyl-2-piperindinyl)ethyl]-2-(methylmercapto)-[3-^{3}H(n)]phenothiazine (4). A solution of 34 mg (0.075 mmol) of 2 and 100 µl of triethylamine in 1.0 ml of dry THF was stirred at room temperature for 4 h in the presence of 30 mg of 5% Pd/C under 5.0 Ci (0.086 mmol) of carrier free tritium gas. The catalyst was filtered off through a Celite/Na_2SO_4 pipet column and the filtrate evaporated in vacuo. The residue was shaken between CH_2Cl_2 and saturated $NaHCO_3$, the organic layer dried (Na_2SO_4) and counted to afford 1,080 mCi of crude product. The solvent was removed in vacuo and the residue chromatographed on two 20 x 20 cm x 0.25 mm silica gel plates (EtoAC-Hex-EtOH-NH_4OH 60:30:10:1) using thioridazine (1) and 3-bromothioridazine (2) as reference standards. Removal of the appropriate band,

elution with CH_2Cl_2 - MEOH (4:1) and evaporation of the solvents in vacuo afforded 296 mCi (33% chemical yield) of product which was dissolved and stored in 500 ml of absolute ethanol. TLC-radioscan indicated > 99% radiochemical purity. A 3.0 ml aliquot of the ethanol solution was evaporated and the residue dissolved in 10 ml of methanol and examined on a Cary 15 UV spectrometer scanning from 320 nm to 250 nm. This procedure previously performed using authentic unlabelled thioridazine indicated λ_{max} =264 nm and $\varepsilon=2.96 \times 10^4$. The UV spectrum of the labelled product was identical to that of the authentic thioridazine and indicated a yield of 9.22 mg and a specific activity of 12 Ci/mmol (32 mCi/mg). The ethanol stock solution was stored at 4°C.

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