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SYNTHESIS OF DEUTERIUM LABELLED ANALOGUES OF S-OXIDATIVE METABOLITES OF THIORIDAZINE

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SUMMARY

The tetradeuterated analogues of the 2-methylsulfinyl (mesoridazine) and 2-methylsulfonyl (sulforidazine) metabolites of (±)-10-[2-(1-methyl-2-piperidinyl)ethyl]-2-methylthio-10H-phenothiazine (thioridazine) were obtained by treatment of the appropriate 2-substituted phenothiazine with the synthon, 2-(2-chloro[1,1,2,2-$^2H_4$]-ethyl)-1-methylpiperidine. Selective ring sulfur oxidation using nitrous acid of these deuterated products as well as the previously reported analogous isotopomer of thioridazine gave the tetradeuterated analogues of the 5-sulfoxide metabolites of thioridazine, mesoridazine and sulforidazine.

Key Words: Antipsychotic, thioridazine metabolites, S-oxidation, deuterium labelling

INTRODUCTION

The most commonly encountered piperidine type phenothiazine antipsychotic agents marketed as drugs are thioridazine (Scheme, 3a) and its sulfoxide and sulfone metabolites resulting from oxidation of the 2-methylthio group, namely mesoridazine (3c) and sulforidazine (3e), respectively. Deuterium labelled analogues of these drugs and their major metabolites were required for use in metabolic and pharmacokinetic studies and as true internal standards for GLC-MS assays (1). Previous papers described the syntheses of various deuterium labelled analogues of thioridazine (2,3), however, there are no such reports to any of its major metabolites. Apart from S-oxidation of the phenothiazine 2-substituent the other major metabolic routes of these drugs include S-oxidation of the 5-position of the phenothiazine ring (4). The

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present paper describes the synthesis of specifically labelled analogues of mesoridazine and sulforidazine and the 5-sulfoxide metabolites of thioridazine, mesoridazine and sulforidazine, each with four deuterium atoms in the ethyl group of the N-10 side chain.

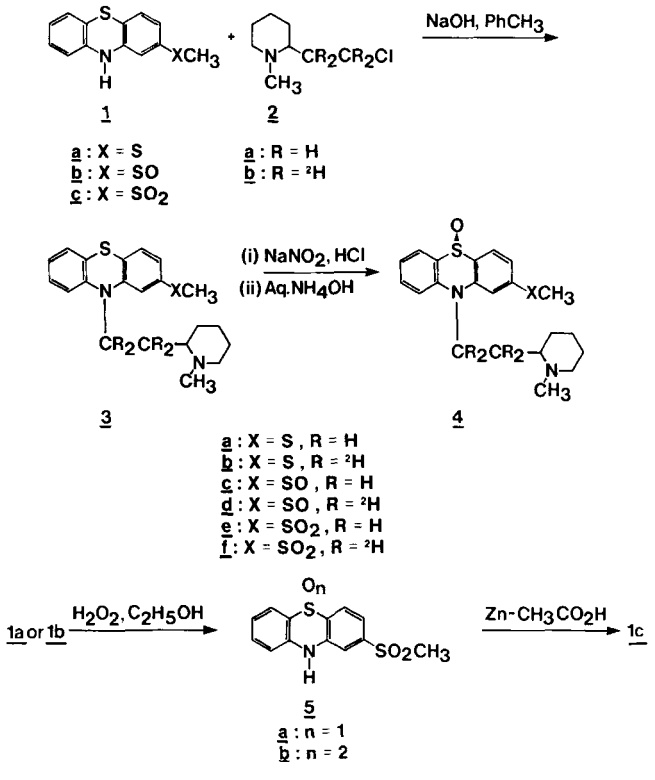
DISCUSSION

The choice to label the metabolites of thioridazine in the ethyl group of the N-10 side chain with four deuterium atoms was made since no known routes of metabolism involve this part of the molecule and with a mass difference of four between labelled and nonlabelled mass spectral fragments (retaining the entire label) consideration of the natural abundance of isotopes in the measurement of monitored MS-SIM peaks is not necessary. The reported tetra-deuterated synthon 2b (2), previously used in the synthesis of 1,1,2,2-$^2H_4$ labelled thioridazine (3b) was also utilised in the present work to allow four deuterium atoms to be introduced in each of the metabolite structures 3c and 3e. Thus N-10 alkylation of 2-methylsulfinyl-(1b) and 2-methylsulfonyl-10H-phenothiazine (1c) with 2b using sodium hydroxide as base in refluxing anhydrous toluene, respectively gave 1,1,2,2-$^2H_4$ labelled mesoridazine (3d) and sulforidazine (3f) in good yields. Whereas the former phenothiazine starting material 1b was available as a gift it was necessary to synthesize 1c. The only report to the synthesis of this sulfone 1c from its corresponding sulfide, 2-methylthio-10H-phenothiazine (1a), involved four steps (5). In the present work it was found that the desired sulfone could be readily obtained in only two steps from either the corresponding sulfide or sulfoxide. Thus 1a or 1b was treated with 30% hydrogen peroxide to provide a mixture of the phenothiazine ring S-oxidation products 5a and 5b of the desired sulfone, which were not separated. The oxidised ring sulfur was in each case subsequently selectively reduced with zinc-acetic acid to give the desired 1c.

The isotopic purity of the labelled mesoridazine (3d) and sulforidazine (3f) was determined by a single ion monitoring technique using electron impact mass spectrometry. The ratios for the molecular ions $^2H_0/^2H_4$ of 3d and 3f were found to be 0.92 and 0.77%, respectively. Correction of these ratios for the $M^{+\cdot}-4$ ions originating from the respective nondeuterated compound indicated that the isotopic purity of each labelled compound was greater than 99%. This

isotopic purity of each of these isotopomers of mesoridazine and sulforidazine is sufficient for their use in metabolic and pharmacokinetic studies, as well as true internal standards in GLC-MS assays. These samples of 3d and 3f, as well as the previously reported similarly labelled thioridazine (3b) of isotopic purity also >99% were each oxidized to obtain the labelled 5-sulfoxide metabolites 4d, 4f and 4b, respectively.

Scheme : Synthesis of S-oxidative metabolites of thioridazine



The procedure used to obtain the 5-sulfoxide metabolites of the piperidine type phenothiazine antipsychotic agents was that previously reported for the synthesis of thioridazine-5-sulfoxide (6). Thus treatment of thioridazine, mesoridazine and sulforidazine or their deuterated analogues with nitrous acid gave in each case the corresponding sulfoxide (3 → 4) in good yield. It is noteworthy that this nonstereoselective chemical oxidation introduces an asymmetric centre in molecules with at least one further such centre and therefore, the products were obtained as diastereoisomeric mixtures. Such a mixture has

been separated in large quantities into diastereoisomeric pairs in the case of thioridazine 5-sulfoxide (6), but no such attempt was made in the present work. The initial evidence indicates that in man, dog or rat there is little stereoselectivity in the formation or elimination of these metabolites (7–9).

EXPERIMENTAL

Melting points (mp) were determined on a Gallenkamp mp apparatus and are uncorrected. Literature mp refers to the nondeuterated compounds. TLC was performed on pre-coated fluorescent plates of 0.2 mm thickness (Kieselgel 60 F_{254}; E. Merck) and spots were visualized under short wave UV light and/or iodine vapours. Column chromatography was carried out using Baker silica gel 60–200 mesh. IR spectra (KBr disks) were recorded on a Beckman Acculab 4 spectrophotometer. Unless otherwise specified ^{1}H NMR spectra were measured on a Varian T-60 (60 MHz) spectrometer in deuteriochloroform. Chemical shift values are expressed in δ units relative to internal tetramethylsilane at δ 0.00 ppm. In situations where multiplets could not be measured easily, the center of gravity was taken as the chemical shift. Low resolution electron impact (EIMS) and chemical ionization (amm) (CIMS) mass spectra of probe samples were recorded on a Vg Micromass 7070HE instrument at 70 eV coupled to a Vg 2035 data system; relative intensity is noted in parentheses after each fragment. All organic extracts were dried over anhydrous sodium sulfate. The removal of solvent from crude reaction mixtures was carried out on a Büchi Rotavapor-R connected to a water aspirator. Unless otherwise specified all chemicals were procured from Aldrich Chemical Co., Milwaukee, WI.

10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-methylsulfinyl-10H-phenothiazine (Mesoridazine) (3c): A stirred yellow suspension of 2-methylsulfinyl-10H-phenothiazine (1b, 0.522 g, 2 mmol) in dry toluene (10 ml) (dried over molecular sieves type 5A) containing finely powdered NaOH (0.32 g, 8 mmol) was heated at reflux under nitrogen in the absence of direct intense light. Upon refluxing the suspension dissolved and after 2 h a red coloured solution was produced. The hydrochloride of 2a (2) (0.435 g, 2.2 mmol) was added slowly to the solution at reflux in small portions over a period of 1 h. The refluxing was continued for an additional 4 h whereupon a light green coloured solution was formed. The reaction mixture was cooled and the organic phase was washed with water and then extracted with 15% tartaric acid. The combined tartaric acid

extracts were washed with benzene and then basified with NaOH. The liberated base was extracted into methylene chloride. The combined organic extracts were washed with water, dried and evaporated on a rotavapor. The resultant oily residue was purified by column chromatography using 5% methanol in chloroform as eluent to obtain mesoridazine (3c, 0.595 g, 77%), which was found to be identical with the free base of an authentic commercial sample by comparison of TLC and co-TLC in a number of solvent systems (benzene:95% ethanol::1:1; chloroform:95% ethanol::4:1; 12 N ammonium hydroxide:chloroform:95% ethanol:: 0.05:4:1; acetic acid:ethyl acetate:water::2:5:2) and spectral correlation; ^{1}H NMR: 1.17-2.13(m,10H,C_3-H_2,C_4-H_2,C_5-H_2,C_6-H_2,$Ar_2NCH_2\underline{CH_2}$), 2.20(s,3H,$NC\underline{H_3}$), 2.53-3.03(m containing $S(O)CH_3$ spike at 2.67,4H,$S(O)\underline{CH_3}$,C_2-H), 3.97(m,2H,$Ar_2N\underline{CH_2}$), 6.80-7.43(m,7H,Ar$\underline{H}$); EIMS:m/z 386(12, $M^{+\cdot}$), 98(100). The oil (0.580 g, 1.5 mmol) was dissolved in absolute ethanol (1.2 ml) and treated with benzenesulfonic acid (Eastman Kodak Co., Rochester, NY) (0.240 g, 1.5 mmol) in absolute ethanol (1.2 ml) at 0°C. The resultant solution was refrigerated for 48 h when 3c besylate crystallised as a white solid; mp 174-175°C [lit. (10) mp 181-183°C] and admixture with the authentic commercial sample (mp 174-175°C) did not show any depression.

10-[2-(1-Methyl-2-piperidinyl)[1,1,2,2-$^2H_4$]ethyl]-2-methylsulfinyl-10H-phenothiazine (3d): This was prepared from 1b and the chloro compound 2b (2) by the method described for 3c; TLC, co-TLC and mp and admixture mp of the besylate salt as for 3c; ^{1}H NMR(free base): 1.10-2.13(m,8H,C_3-H_2,C_4-H_2, C_5-H_2,C_6-H_2), 2.20(s,3H,$NC\underline{H_3}$), 2.53-3.03(m containing $S(O)CH_3$ spike at 2.67, 4H,$S(O)\underline{CH_3}$,C_2-H), 6.77-7.43(m,7H,Ar$\underline{H}$); EIMS:m/z 390(31, $M^{+\cdot}$), 98(100).

2-Methylsulfonyl-10H-phenothiazine (1c): A stirred mixture of 1a or 1b (25 mmol) in 95% C_2H_5OH (20 ml) and 30% H_2O_2 (14 ml) was refluxed gently for 24 h. After cooling the reaction mixture to room temperature and subsequent refrigeration the solid was filtered and washed with ethanol to give 5 as a cream coloured solid (5.92 g), mp 269-270°C (dec.) [lit. (5) mp of 5a 305-310°C]. The high resolution ^{1}H NMR spectrum (Bruker AM-300, 300.13 MHz) in $(C^2H_3)_2SO$ exhibited two singlets due to $S(O_2)CH_3$ protons at δ 3.33 and 3.40 in a respective ratio of 1:4. Also, the EIMS showed the highest m/z value at 309.

A stirred mixture of 5 in DMF (25 ml) and acetic acid (10 ml) containing Zn dust (3.50 g) was heated at 95-100°C for 10 h. While hot, the reaction mixture

was filtered and the filtrate diluted with water. The precipitated solid was filtered, washed with water and dried. Three crystallizations from benzene provided pure 1c (3.40 g, 49%), mp 158-159°C [lit. (5) mp 162°C]; IR: 3360(N-H), 1460, 1320(O=S=O), 1290, 1145(O=S=O), 1095, 775, 760 cm^{-1}; ^1H NMR: 3.07(s,3H,S(O_2)C$\underline{H}_3$), 6.50-7.50(m,7H,Ar$\underline{H}$); EIMS:m/z 277(100, $M^{+\cdot}$). Anal. Calcd. for $C_{13}H_{11}NO_2S_2$: C,56.30; H,4.00; N,5.05. Found: C,56.43; H,4.20; N,4.97.

10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-methylsulfonyl-10H-phenothiazine (sulforidazine) (3e): This was prepared from 1c and the chloro compound 2a using a method similar to that described for 3c, however, the work up was different. The green coloured solution obtained after completion of the reaction was washed with water and dried. The solvent was evaporated on a rotavapor and the crude residual oil was purified by column chromatography using 5% methanol in chloroform as eluent to afford sulforidazine (3e, 0.318 g, 79%) as a pale yellow solid, mp 115-116°C [lit. (10) mp 121-123°C] and admixture with the authentic commercial sample (mp 117-119°C) did not show any depression; ^1H NMR: 1.07-2.13(m,10H,C_3-H_2,C_4-H_2,C_5-H_2,C_6-H_2,$Ar_2NCH_2C\underline{H}_2$), 2.20(s,3H,NC$\underline{H}_3$), 2.80(m,1H,$C_2$-H), 3.00(s,3H,S($O_2$)C$\underline{H}_3$), 3.90(m,2H,$Ar_2NC\underline{H}_2$), 6.80-7.50(m,7H,Ar$\underline{H}$); EIMS:m/z 402(7, $M^{+\cdot}$), 98(100).

10-[2-(1-Methyl-2-piperidinyl)[1,1,2,2-$^2H_4$]ethyl]-2-methylsulfonyl-10H-phenothiazine (3f): This was prepared from 1c and the chloro compound 2b by the method described for 3e; TLC, co-TLC and mp and admixture mp as for 3e; ^1H NMR: 1.03-2.17(m,8H,C_3-H_2,C_4-H_2,C_5-H_2,C_6-H_2), 2.23(s,3H,NC$\underline{H}_3$), 2.83(m,1H, C_2-H), 3.03(s,3H,S(O_2)C$\underline{H}_3$), 6.80-7.60(m,7H,Ar$\underline{H}$); EIMS:m/z 406(55,$M^{+\cdot}$), 98(100).

10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-methylthio-10H-phenothiazine-5-sulfoxide (4a): A solution of $NaNO_2$ (0.5 g) in distilled water (5 ml) was added dropwise to a stirred solution of thioridazine hydrochloride (3a, 0.760 g, 19 mmol) in distilled water (50 ml) containing conc. HCl (5 drops). The colour of the solution changed from blue to yellow-brown and finally became orange after the complete addition of $NaNO_2$ solution. The reaction mixture was further stirred for 10 min, made alkaline with aqueous NH_4OH and extracted into methylene chloride. The combined organic extracts were washed with water and dried. The solvent was removed on a rotavapor and the crude product was purified by column chromatography using 5% methanol in chloroform as eluent to give the 5-sulfoxide 4a (0.570 g, 79%). The product recrystallized from hexane

as a light pink solid, mp 110-113°C [lit. (6) mp 113.5-128.5°C]; IR: 1580, 1465, 1425, 1250, 1040(S=O), 800, 770 cm^{-1}; ^1H NMR: 1.17-2.27(m,10H,C_3-H_2, C_4-H_2,C_5-H_2,C_6-H_2,$Ar_2NCH_2\underline{CH_2}$), 2.33(s,3H,$NC\underline{H_3}$), 2.53(s,3H,$SC\underline{H_3}$), 2.90(m,1H,$C_2-H$), 4.23(m,2H,$Ar_2NC\underline{H_2}$), 6.93-8.03(m,7H,Ar$\underline{H}$); CIMS:m/z 387$(M+H)^+$.

10-[2-(1-Methyl-2-piperidinyl)[1,1,2,2-$^2H_4$]ethyl]-2-methylthio-10H-phenothiazine-5-sulfoxide (4b): This was prepared from 3b (2) by the method described for 4a; TLC, co-TLC and mp and admixture mp as for 4a; IR: 1580, 1460, 1445, 1410, 1340, 1040(S=O), 760 cm^{-1}; ^1H NMR: 1.07-2.20(m,8H,C_3-H_2, C_4-H_2,C_5-H_2,C_6-H_2), 2.33(s,3H,$NC\underline{H_3}$), 2.53(s,3H,$SC\underline{H_3}$), 2.90(m,1H,C_2-H), 6.90-8.03(m,7H,Ar$\underline{H}$); CIMS:m/z 391$(M+H)^+$.

10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-methylsulfinyl-10H-phenothiazine-5-sulfoxide (4c): This was prepared in 82% yield from the besylate salt of 3c by adopting the procedure described for 4a; mp: shrinks at ≈ 75°C and melts through 90-110°C; IR: 1580, 1455, 1420, 1055(S=O), 1035(S=O), 760 cm^{-1}; ^1H NMR: 1.10-2.50(m containing NCH_3 spike at 2.30,13H,C_3-H_2,C_4-H_2,C_5-H_2, C_6-H_2,$Ar_2NCH_2\underline{CH_2}$,$NC\underline{H_3}$), 2.63-3.07(m containing distorted s of $S(O)CH_3$ at 2.73,4H,C_2-H,$S(O)C\underline{H_3}$), 4.33(m,2H,$Ar_2NC\underline{H_2}$), 7.10-8.20(m,7H,Ar$\underline{H}$); CIMS:m/z 403$(M+H)^+$. Anal. Calcd. for $C_{21}H_{26}N_2O_2S_2$: C,62.65; H,6.51; N,6.96. Found: C,62.73; H,6.46; N,6.96.

10-[2-(1-Methyl-2-piperidinyl)[1,1,2,2-$^2H_4$]ethyl]-2-methylsulfinyl-10H-phenothiazine-5-sulfoxide (4d): This was prepared from 3d by the procedure adopted for 4c; TLC, co-TLC and mp and admixture mp as for 4c; IR: 1580, 1455, 1410, 1340, 1040(S=O), 760 cm^{-1}; ^1H NMR: 1.10-2.50(m containing NCH_3 spike at 2.33,11H,C_3-H_2,C_4-H_2,C_5-H_2,C_6-H_2,$NC\underline{H_3}$), 2.63-3.10(m containing distorted s of $S(O)CH_3$ at 2.75,4H,C_2-H,$S(O)C\underline{H_3}$), 7.10-8.20(m,7H,Ar$\underline{H}$); CIMS:m/z 407$(M+H)^+$.

10-[2-(1-Methyl-2-piperidinyl)ethyl]-2-methylsulfonyl-10H-phenothiazine-5-sulfoxide (4e): This was prepared in 57% yield from 3e by adopting the procedure described for 4a, mp 127-137°C (acetone); IR: 1585, 1455, 1425, 1320(O=S=O), 1160(O=S=O), 1055(S=O), 1035(S=O), 760 cm^{-1}; ^1H NMR: 1.10-2.50 (m containing NCH_3 spike at 2.33,13H,C_3-H_2,C_4-H_2,C_5-H_2,C_6-H_2,$Ar_2NCH_2\underline{CH_2}$,$NC\underline{H_3}$), 2.63-3.23(m containing $S(O_2)CH_3$ spike at 3.10,4H,C_2-H,$S(O_2)C\underline{H_3}$), 4.38(m,2H, $Ar_2NC\underline{H_2}$), 7.27-8.30(m,7H,Ar$\underline{H}$); CIMS:m/z 419$(M+H)^+$. Anal. Calcd. for $C_{21}H_{26}N_2O_3S_2$: C,60.26; H,6.26; N,6.69. Found: C,60.18; H,6.13; N,6.73.

10-[2-(1-Methyl-2-piperidinyl)[1,1,2,2-$^{2}H_{4}$]ethyl]-2-methylsulfonyl-10H-phenothiazine-5-sulfoxide (4f): This was prepared from 3f by the procedure adopted for 4e; TLC, co-TLC and mp and admixture mp as for 4e; IR: 1590, 1460, 1425, 1325(O=S=O), 1160(O=S=O), 1045(S=O), 760 cm^{-1}; ^{1}H NMR: 1.10-2.50 (m containing NCH_3 spike at 2.33,11H,C_3-H_2,C_4-H_2,C_5-H_2,C_6-H_2,NCH_3), 2.67-3.30(m containing $S(O_2)CH_3$ spike at 3.13,4H,C_2-H,$S(O_2)CH_3$), 7.17-8.30 (m,7H,ArH); CIMS:m/z 423$(M+H)^{+}$.

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