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Synthesis, receptor binding and functional studies of mesoridazine stereoisomers

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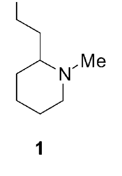
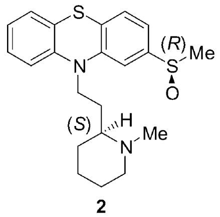
Abstract—The four stereoisomers of mesoridazine were synthesized and evaluated in D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, D_1, and D_3 receptor binding and functional assays. Two isomers demonstrated potent D_2 receptor binding ($K_i < 3$ nM) and functional antagonism ($IC_{50} \leqslant 10$ nM) activities. These two isomers also showed moderate affinity for the 5-HT_{2A} and D_3 receptors. A third isomer was devoid of significant D_2 receptor binding, but did have moderate affinity for the 5-HT_{2A} and D_3 receptors. The fourth isomer demonstrated poor affinity for all the receptors tested. Most significantly, the stereochemistry of the sulfoxide moiety played a dominant role in the observed structure–activity relationship (SAR).

Before the advent of efficient and economically viable synthetic methods for the preparation of enantiomerically pure pharmaceutical agents many drugs were introduced to patients in racemic form. However, with tremendous advances in asymmetric synthesis and with recognition that individual enantiomers could possess significantly different pharmacodynamic, pharmacokinetic and toxicological properties a move to introduce enantiomerically pure drugs began in earnest. Similarly, efforts were initiated to replace currently marketed racemic drugs with enantiomerically pure forms. These efforts continue today.[1] For example, the H^+K^+-ATPase inhibitor omeprazole, utilized for the treatment of gastroesophageal reflux disease (GERD), has been replaced with its sulfoxide (*S*)-enantiomer esomeprazole resulting in superior gastric-acid inhibition and clinical efficacy.[2]



1

Another compound whose enantiomers demonstrate differing biological activities is thioridazine, **1**.[3,4] It is a member of the phenothiazine class of neuroleptic agents used for the treatment of psychotic disorders, including schizophrenia.[5] The (*R*)-enantiomer has been shown to have 2.7 times greater affinity for the D_2 receptor compared to the (*S*)-enantiomer, whereas the (*S*)-enantiomer had ten times greater affinity for the D_1 receptor.[6]



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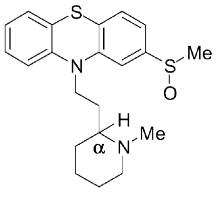

3 α-C: *R*; S(O): *S*
4 α-C: S; S(O): *S*
5 α-C: *R*; S(O): *R*

Mesoridazine (thioridazine-2-sulfoxide) is another member of the phenothiazine class of antipsychotics and a thioridazine analog.[7] In addition, it is a metabolite of thioridazine formed in several species, including humans.[8] The sulfoxide functionality in mesoridazine introduces an additional chiral center compared to thioridazine. Therefore, mesoridazine comprises a mixture of four stereoisomers (**2**–**5**). Herein we report the synthesis, receptor binding, and functional studies of these individual stereoisomers.

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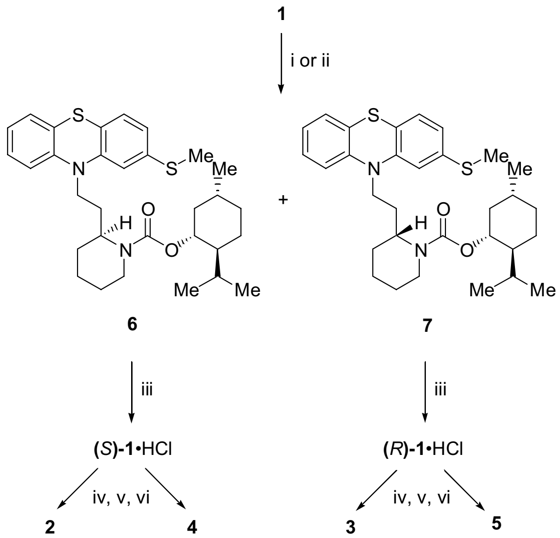

Scheme 1. Synthesis of **2**–**5**. Reagents and conditions: (i) (−)-menthyl chloroformate, *i*-Pr_2EtN, CH_2Cl_2, rt, 16h, 71%; (ii) 1-chloroethyl chloroformate, $ClCH_2CH_2Cl$, 0 °C to reflux, 2h then MeOH, reflux, 1h, then (−)-menthyl chloroformate, *i*-Pr_2EtN, CH_2Cl_2, rt, 16h, 60%; (iii) $LiAlH_4$, Et_2O, 0 °C to rt, 16h, then HCl, 54%; (iv) D- or L-dimethyl tartrate (4equiv), Ti(*i*-PrO)$_4$ (2equiv), H_2O (0.56equiv), CHP (1equiv), CH_2Cl_2, −20 °C, 18h, ~70%; (v) HPLC separation using chiral stationary-phase; (vi) oxalic acid (1equiv), Et_2O.

The isomers were prepared according to the method outlined in Scheme 1. Racemic **1** was treated with (−)-menthyl chloroformate and diisopropylethylamine in dichloromethane (DCM) to give a mixture of diastereomers **6** and **7** that were readily separated by column chromatography.[9] Parenthetically, **1** could be demethylated in a two-step process utilizing 1-chloroethyl chloroformate and then the resulting amine converted to carbamates **6** and **7**.[10] However, the direct conversion of **1** to **6** and **7** was found to be quite efficient. Each carbamate was then reduced with lithium aluminum hydride (LAH) to give (*S*)-**1** and (*R*)-**1**, respectively.[11] Each enantiomer (as the corresponding hydrochloride salt) was subsequently oxidized with cumene hydrogen peroxide (CHP) in the presence of Ti(*i*-PrO)$_4$ and D- or L-dimethyl tartrate in DCM at −20 °C to yield mesoridazine isomers.[12] Typically the oxidation reactions proceeded with greater than 85% enantioselectivity as assessed by HPLC analysis. The D- and L-dimethyl tartrates gave the (*S*)- and (*R*)-sulfoxides, respectively.[13] Initially, diethyl tartrates were used, but the dimethyl esters were found to give fewer side-products. Two other oxidants, urea·H_2O_2 and (1*R*)-(10-camphorsulfonyl)oxaziridine, were also evaluated in place of CHP without success. Utilizing the hydrochloride salts of **1** also dramatically improved the selectivity of the reaction and the product yield compared to the free bases presumably by reducing the chelating ability of the amine. The diastereomeric purity of each isomer was further increased utilizing chiral stationary-phase HPLC purification giving **2**–**5** (free bases). The enantiomeric and chemical purity of each isomer was greater than 98%.[14–16] The sulfoxide stereochemical assignment was based on literature precedence for Ti(*i*-PrO)$_4$/H_2O/CHP or *t*-BuOOH oxidation of other aryl methyl sulfoxides.[13,17] Finally, each diasteriomer was converted to its corresponding oxalate salt for storage and biological testing.

The mesoridazine isomers **2**–**5** (oxalate salts) were evaluated in D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, D_1, and D_3 receptor binding assays.[18] Two isomers, **2** and **5**, had potent affinity for the D_2 receptor exhibiting K_i's at the high affinity binding site of 2.2 and 1.2nM, respectively (Table 1). Both compounds exhibited biphasic dose–response curves with Hill coefficients ~0.3 suggesting different binding affinities to various states of the D_2 receptor. Addition of guanosine 5′-[γ-thio]triphosphate (GTPγS; 1 μM) had no significant effect on the Hill coefficients of the dose–response curves, indicative of receptor antagonists.[19a] Although biphasic dose–response curves are unusual for D_2 antagonists, they are not unprecedented.[19b,c] The other two isomers, **3** and **4**, had significantly weaker affinity for the D_2 receptor ($K_i > 300$ nM). Compound **2** also had moderate affinity for the 5-HT_{2A} ($K_i = 55$ nM) and D_3 ($K_i = 23$ nM) receptors. Similarly, **5** had moderate affinity for these two receptors (5-HT_{2A} $K_i = 19$ nM; D_3 $K_i = 26$ nM). In addition, **5** had moderate affinity for the 5-HT_{1A} receptor ($K_i = 64$ nM). Isomer **3** only had modest affinity for the 5-HT_{2A} ($K_i = 112$ nM) and D_3 ($K_i = 95$ nM) receptors. Most interestingly, isomer **4** demonstrated relatively poor affinity ($K_i > 195$ nM) for all the receptors tested (Table 2).

Next, the mesoridazine isomers were evaluated in functional assays (Table 3).[20] D_1 agonism and antagonism was determined using a cyclic AMP functional assay in C_6D_1 low-density cells. D_2 and D_3 agonism was determined by stimulation of mitogenesis and antagonism was measured by inhibition of quinpirole-induced mitogenesis in CHOp cells. 5-HT_{2A} agonism and antagonism were determined using the rat aorta spiral (RAS) contraction assay.

Isomers **2** and **5** (oxalate salts) exhibited potent D_2 and 5-HT_{2A} antagonism (**2**: D_2 $IC_{50} = 10$ nM, 5-HT_{2A} $K_e = 7$ nM; **5**: D_2 $IC_{50} = 4$ nM, 5-HT_{2A} $K_e = 2.5$ nM) and moderate D_3 antagonism ($IC_{50} = 35$ and 16nM,

Table 1. D_2 receptor binding[18]

Cmpd	IC_{50} (nM)	K_i (nM)	Hill slope	K_i (nM) High affinity	%	K_i (nM) Low affinity	%
2	77	ND	0.34	2.2 ± 0.18	55.6	1545 ± 160	44.4
3	837	304 ± 87	ND	ND	ND	ND	ND
4	1173	426 ± 118	ND	ND	ND	ND	ND
5	63	ND	0.33	1.2 ± 0.22	56.1	1841 ± 301	43.9

Table 2. 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, D_1, and D_3 receptor binding[18]

Cmpd	5-HT_{1A} K_i (nM)	5-HT_{2A} K_i (nM)	5-HT_{2C} K_i (nM)	D_1 K_i (nM)	D_3 K_i (nM)
2	105 ± 14	55 ± 15	412 ± 21	103 ± 11	23 ± 7
3	349 ± 28	112 ± 17	704 ± 43	1963 ± 107	95 ± 24
4	395 ± 4	414 ± 44	410 ± 48	620 ± 8	197 ± 59
5	64 ± 1	19 ± 3	112 ± 5	317 ± 1	26 ± 9

Table 3. D_1, D_2, D_3, and 5-HT_{2A} functional antagonism assays[20]

Cmpd	D_1 IC_{50} (nM)	D_2 IC_{50} (nM)	D_3 IC_{50} (nM)	5-HT_{2A}		
				K_e (nM)	pA_2	*n*
2	137 ± 24	10 ± 2	35 ± 4	7 ± 0.8	8.2 ± 0.05	6
3	ND	161 ± 8	256 ± 9	28 ± 5	7.6 ± 0.07	5
4	1251 ± 172	232 ± 52	408 ± 55	ND	ND	—
5	460 ± 1	4 ± 0.08	16 ± 5	2.5 ± 0.2	8.6 ± 0.04	6

respectively). Furthermore, both compounds were inactive in the D_1, D_2, or D_3 agonism assays. Isomer **3** was also a 5-HT_{2A} antagonist ($K_e = 28$ nM), but isomer **4** was devoid of any functional activity below 200 nM for all the receptors tested in agreement with the receptor ligand binding studies.

Given the efficaciousness of mesoridazine, particularly in refractory schizophrenics,[21] while recognizing the desire to mitigate its potential cardiac side-effects[22] stemming from inhibition of human ether-a-go-go-related gene (HERG) potassium channels[23] and extrapyramidal adverse effects an investigation of the individual stereoisomers that comprise the currently available formulation was warranted. Based on the reported structure–activity relationship (SAR) for the enantiomers of thioridazine, **1**, the chiral center in the piperidine ring of mesoridazine was anticipated to significantly influence its SAR. However, most surprising was the finding that the chirality at the sulfoxide moiety of mesoridazine had a much more pronounced influence on the SAR of the individual isomers for D_2 receptor binding activity than the chiral center in the piperidine ring. These results raise several interesting possibilities related to mesorid- azine's therapeutic effects and observed toxicities. Further studies of isomers **2**–**5** could reveal if the observed in vivo effects reported for mesoridazine are due to a repertoire of various stereoisomers or primarily due to a single isomer. Such a deconvolution may also assist in a further understanding of the receptor binding profile of effective anti-psychotic agents.[24] Likewise, the associated toxicities of mesoridazine could be evaluated for each isomer in the hope that efficacy and adverse effects diverge among the various stereoisomers.

Mesoridazine is classified as a 'typical' anti-psychotic agent. More recently developed compounds, referred to as 'atypical' agents (reportedly associated with fewer extrapyramidal adverse effects) have been introduced. However, a recent study supports the effectiveness and pharmacoeconomic benefits of the 'typical' anti-psychotic agent haloperidol in combination with prophylactic benztropine compared with the 'atypical' agent olanzapine.[25] Such benefits may similarly be realized with a diastereo- and enantiomerically pure form of mesoridazine.

In conclusion, the four stereoisomers of mesoridazine were synthesized from racemic thioridazine. Each of the isomers was evaluated in D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, D_1, and D_3 receptor binding and functional assays. Two isomers, **2** and **5**, demonstrated potent D_2 receptor binding and functional antagonism activities. These two isomers also showed moderate affinity for the 5-HT_{2A} and D_3 receptors. A third isomer, **3**, was devoid of significant D_2 receptor binding, but did have moderate affinity for the 5-HT_{2A} and D_3 receptors. The fourth isomer, **4**, demonstrated poor affinity for all the receptors tested. Most significantly, the stereochemistry of the sulfoxide moiety played a dominant role in the observed SAR.

Acknowledgements

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12. A solution of Ti(O-*i* -Pr)$_4$ (143 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (1 mL) was added to a solution of dimethyl tartrate (178 mg, 1 mmol) in anhydrous CH_2Cl_2 (2 mL). Next, water (2.5 μL, 0.14 mmol) was introduced via a microsyringe at room temperature. Then thioridazine hydrochloride (*R* or *S*, 100 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (5 mL) was added. The mixture was stirred at room temperature for 30 min and then cooled to −20 °C. After 30 min, cumene hydroperoxide (40 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was stirred at −20 °C for 18 h. A solution of saturated NH_4Cl was added and the reaction mixture was poured into ethyl acetate (100 mL), washed sequentially with saturated $NaHCO_3$ (3×50 mL) and saturated NaCl (3×50 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residual oil was purified first by column chromatography (silica gel, 5% MeOH in CH_2Cl_2) to give a colorless oil. This material was further purified by chiral stationary-phase HPLC (see Ref. 14).
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14. (a) HPLC instrument: Waters Alliance 2795; Column: Chiralpak AS (Daicel Chemical Industries, Ltd); elution solvents: hexane/EtOH/Et_2NH (81:19:0.2); elution rate: 0.8–1.0 mL/min; injection volume: 40–50 μL; injection sample concentration: 0.2 mg/μL; elution times (min) for **2**–**5**: 16.4–19.5, 12.0–12.7, 10.5–10.7, 18.9–23.4; (b) Eap, C. B.; Koeb, L.; Powell, K.; Baumann, P. *J. Chromatography* **1995**, *B669*, 271–279.
15. The ^{1}H NMR spectra of **2**–**5** (oxalate salts) were indistinguishable. ^{1}H NMR (CD_3OD, 500 MHz): δ 1.48–1.72 (3H, m), 1.81–1.87 (3H, m), 2.08–2.11 (1H, m), 2.16–2.20 (1H, m), 2.64–2.69 (3H, m), 2.82 (3H, s), 2.96–3.05 (1H, m), 3.10–3.24 (1H, m), 3.47–3.58 (1H, m), 4.08–4.13 (1H, m), 4.18–4.23 (1H, m), 7.04 (1H, t, J=8.5), 7.11 (1H, d, J=8.5), 7.21 (1H, d, J=7.0), 7.25–7.30 (2H, m), 7.35–7.38 (2H, m). **2** and **3** (oxalate salts) ^{13}C NMR (CD_3OD, 100.5 MHz): δ 163.39, 162.52, 147.66, 145.68, 145.42, 131.60, 129.33, 129.25, 128.81, 126.29, 124.95, 119.49, 117.61, 111.57, 65.35, 57.60, 44.42, 43.61, 41.60, 29.99, 29.09, 24.32, 22.97. **4** and **5** (oxalate salts) ^{13}C NMR (CD_3OD, 100.5 MHz): δ 163.37, 162.52, 147.73, 145.67, 145.35, 131.59, 129.32, 129.24, 128.83, 126.29, 124.96, 119.52, 117.59, 111.50, 65.40, 57.58, 44.43, 43.62, 41.57, 30.05, 29.11, 24.35, 22.95. Optical rotations (λ= 589 nm) of **2**–**5** (oxalate salts) were $[\alpha]_D^{24}$ +20.0 (*c* 0.29, MeOH), $[\alpha]_D^{24}$ −18.2 (*c* 0.275, MeOH), $[\alpha]_D^{24}$ −25.3 (*c* 1.69, MeOH), $[\alpha]_D^{24}$ +23.2 (*c* 1.14, MeOH), respectively.
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18. ND: not determined. Values ± SEM. Unless otherwise noted the Hill slopes were ~1 (range 0.88–1.41). (a) D_2 receptor: species: human; competitive ligand: [^{3}H]YM-09151-2 (0.21 nM); nonspecific ligand: chlorpromazine (1 μM); (b) 5-HT_{1A} receptor: species: human; competitive ligand: [^{3}H]8-OH-DPAT (0.5 nM); nonspecific ligand: dihydroergotamine (1 μM); (c) 5-HT_{2A} receptor: species: rat; competitive ligand: [^{3}H]ketanserin (0.4 nM); nonspecific ligand: ketanserin (1 μM); (d) 5-HT_{2C} receptor: species: rat; competitive ligand: [^{3}H]mesulergine (0.4 nM); nonspecific ligand: mesulergine (10 μM); (e) D_1 receptor: species: human; competitive ligand: [^{3}H]SCH-23390 (0.18 nM); nonspecific ligand: SCH-23390 (1 μM); (f) D_3 receptor: species: human; competitive ligand: [^{3}H]YM-09151-2 (0.21 nM); nonspecific ligand: chlorpromazine (1 μM).
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