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Structural changes by sulfoxidation of phenothiazine drugs

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SUMMARY

The side-chain conformations of psychoactive phenothiazine drugs in crystals are different from those of biologically inactive ring sulfoxide metabolites. This study examines the potential energies, molecular conformations and electrostatic potentials in chlorpromazine, levomepromazine (methotrimeprazine), their sulfoxide metabolites and methoxypromazine. The purpose of the study was to examine the significance of the different crystal conformations of active and inactive phenothiazine derivatives, and to determine why phenothiazine drugs lose most of their biological activity by sulfoxidation. Quantum mechanics and molecular mechanics calculations demonstrated that conformations with the side chain folded over the ring structure had lowest potential energy in vacuo, both in the drugs and in the sulfoxide metabolites. In the sulfoxides, side chain conformations corresponding to the crystal structure of chlorpromazine sulfoxide were characterized by stronger negative electrostatic potentials around the ring system than in the parent drugs. This may weaken the electrostatic interaction of sulfoxide metabolites with negatively charged domains in dopamine receptors, and cause the sulfoxides to be virtually inactive in dopamine receptor binding and related pharmacological tests.

INTRODUCTION

Psychoactive phenothiazine derivatives have been used for nearly 30 years in the treatment of major psychiatric disorders. Their pharmacokinetics and metabolism have been extensively studied, and it has been demonstrated that like the structurally related thioxanthene drugs, the phenothiazines are converted in the body to several different metabolites [1]. It appears, however, that in general no more than 2–4 pharmacologically active drug metabolites are formed in significant amounts after usual therapeutic doses of phenothiazines [2].

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Sulfoxidation of the central thiazine ring is one of the major metabolic pathways of phenothiazine drugs. It has been demonstrated that after repeated oral doses of thioridazine [3], trifluoperazine [4], fluphenazine [5] and levomepromazine (methotrimeprazine) [6], patients usually have plasma concentrations of the ring sulfoxide which are well above the concentrations of the parent drug. This has raised questions concerning the possible contribution from sulfoxide metabolites to the side effects of treatment with phenothiazines, and it has been reported that the ring sulfoxide of thioridazine may contribute to cardiac side effects in patients [7,8].

The concentrations of chlorpromazine sulfoxide are often lower than those of chlorpromazine in plasma from psychiatric patients [9]. Chlorpromazine sulfoxide is virtually inactive in a variety of different biological systems [10], including assay of its binding affinity to dopamine D_2 receptors in the brain [11,12], antagonism of apomorphine-induced climbing in mice [13], and cardiodepressive effects on isolated rat atria [14]. Levomepromazine sulfoxide is virtually inactive in dopamine D_2 receptor binding [12], but has cardiodepressive effects on isolated rat atria, especially on electrophysiological parameters [14], and some potency in α_1 adrenergic receptor binding in rat cortex [12].

These findings led to a series of X-ray crystallographic studies on the 3-D molecular structures of phenothiazine drugs and metabolites, including levomepromazine [15] and its sulfoxide [16], chlorpromazine sulfoxide [17] and *N*-monodesmethyl chlorpromazine sulfoxide [18]. Chlorpromazine sulfoxide and *N*-monodesmethyl chlorpromazine sulfoxide, both of which are regarded as biologically inactive [10], had molecular conformations in crystals different from those in all known crystal structures of biologically active phenothiazine drugs [19]. The side chain in the inactive sulfoxide metabolites had extended 'down' conformations, as shown in Fig. 1 for chlorpromazine sulfoxide, while the side chains in biologically active phenothiazines had extended 'up' conformations, as illustrated in Fig. 1 for chlorpromazine, levomepromazine and levomepromazine sulfoxide. The only apparent exception to this rule was methoxypromazine maleate [20], which has a side chain conformation in crystals similar to that of chlorpromazine sulfoxide. This was, however, attributed to crystal packing forces involving the maleate anion, when it was found that the crystal structure of free methoxypromazine base [21] had a side chain conformation similar to those in chlorpromazine [22] and levomepromazine [15].

X-ray crystallographic studies of phenothiazine drugs have provided valuable structural insight, but have not necessarily shown all biologically significant conformations. The crystal structures do, however, provide a valuable basis for further examination of the different accessible conformations by computational methods. This study examines the potential energies, molecular conformations and electrostatic potentials in chlorpromazine, levomepromazine (methotrimeprazine), their sulfoxide metabolites and methoxypromazine, by quantum mechanics, molecular mechanics and other computational techniques. The principal goal of this study was to explain the structural features that are causing chlorpromazine but not levomepromazine to lose virtually all its biological activity by sulfoxidation.

METHODS

Molecular mechanical geometry optimization and molecular dynamics simulations in vacuo and in water were performed with the ensemble of AMBER programs [23], using the all atom force field [24]. A non-bonded cut-off radius of 99 Å was used in all calculations. A distance-dependent

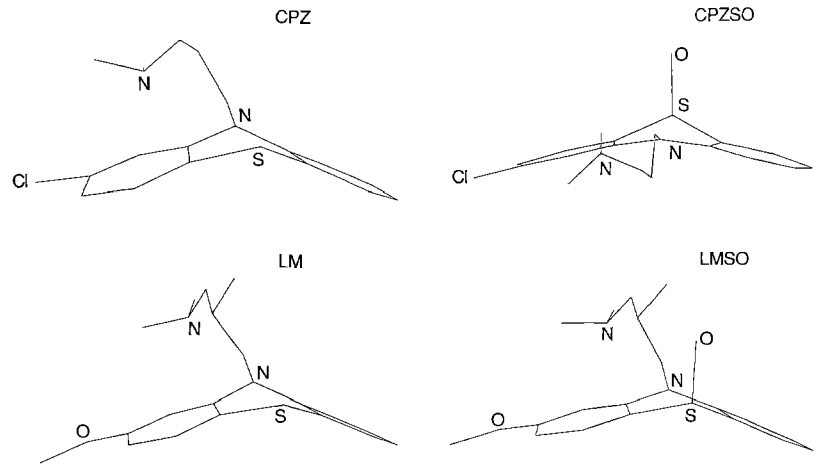

Fig. 1. Crystal structures of chlorpormazine (CPZ), chlorpromazine sulfoxide (CPZSO), levomepromazine (methotrimeprazine) (LM) and levomepromazine sulfoxide (LMSO). Hydrogen atoms are not shown. The side chain in CPZSO has an extended 'down' conformation with torsion angle T1 = 276.1°. The side chains in CPZ, LM and LMSO have extended 'up' conformations with T1 from 63.9° to 69.3° [19].

dielectric function, $\varepsilon = r_{ij}$, (r_{ij}: interatomic distance) was used in energy minimizations and molecular dynamics simulations in vacuo, in order to dampen long-range electrostatic interactions more than short-range interactions. A dielectric of 1.0 was used to calculate electrostatic interactions in simulations with explicit water molecules [25].

Quantum mechanical calculations of atomic charges

Net atomic point charges were calculated with the QUEST 1.0 program [26], on a VAX 11/780 computer connected with an FPS-264 array processor, using an STO-3G basis set. QUEST is an extended GAUSSIAN-80 program [27], which calculates electrostatic potentials over several layers of molecular surfaces by ab initio quantum mechanical methods, and projects the potentials into net atomic point charges by a least-squares optimization procedure. The molecular electrostatic potentials were calculated over 4 surface layers 0.2 Å apart, the inner-most surface corresponding to 1.4 times the van der Waals radii.

Molecular mechanics parameters

The AMBER force field did not contain parameters for bond lengths, angles and torsional barriers around the sulfur atom in the central thiazine ring. Rotational barriers were determined from molecular mechanics and quantum mechanics ab initio energy calculations of model compounds with the X–C–S–X torsion angle at different fixed positions, using the QUEST program and an STO-3G basis set. The rotational barrier for torsion angle X–C–S–X in phenothiazines, where X is either H or C, was determined from calculations on thiophenol. The X–C–S–O and X–C–S–C rotational barriers in phenothiazine sulfoxide were determined from calculations on *S*-methylthiophenol sulfoxide ($CH_3{\cdot}SO{\cdot}C_6H_6$) and thiophenol sulfoxide ($H{\cdot}SO{\cdot}C_6H_6$).

Rotational barriers about the –C–N– torsion angles in the central thiazine ring were determined

TABLE I
AMBER ALL ATOM FORCE FIELD PARAMETERS FOR THE CENTRAL THIAZINE RING IN PHENOTHIAZINE AND PHENOTHIAZINE RING SULFOXIDE[a]

Angle	k_θ (kcal mol^{-1} rad^{-2})	Θ_{eq} (°)	Source
C_A–S_P–C_A	62	98.9	Dimethylsulfide microwave [29]
C_A–N_P–C_A	50	113.0	Aniline [24]
C_A–N_P–C_T	50	113.0	Aniline [24]
C_A–S_O–C_A	62	96.6	Dimethylsulfoxide microwave [31]
C_A–S_O–O	62	106.7	Dimethylsulfoxide microwave [31]
C_A–N_O–C_A	50	121.4	Crystal structure of 14 different phenothiazine derivatives [19]
C_A–N_O–C_T	50	117.7	Crystal structure of 14 different phenothiazine derivatives [19]

Bond	K_r (kcal mol^{-1} Å^{-2})	r_{eq} (Å)	
S_P–C_A	300	1.75	Di-*p*-tolyl sulfide crystal structure [28]
S_O–C_A	300	1.76	Diphenylsulfoxide crystal structure [30]
S_O–O	300	1.50	Crystal structure of LMSO [16], CPZSO [17] and *N*-desmethyl CPZSO [18]
N_P–C_A	300	1.45	Crystal structure of 14 different phenothiazine derivatives [19]
N_O–C_A	300	1.45	Crystal structure of 14 different phenothiazine derivatives [19]

Torsion angle	V (kcal mol^{-1})	Phase shift angle	Periodicity
X–C_A–S_P–X	1.8	180	2.
X–C_A–N_P–X	3.0	180	2.
X–C_T–N_P–X	2.0	0	3.
X–C_A–S_O–O	0.0	180	2.
X–C_A–S_O–X	0.0	180	2.
X–C_A–N_O–X	6.0	180	2.
X–C_T–N_O–X	2.0	0	3.

[a] k_θ, bending force constant; Θ_{eq}, equilibrium bond angle; k_r, bond stretching force constant; r_{eq}, equilibrium bond length; V, rotational barrier. Atom types: S_P, sulfur in phenothiazine; S_O, sulfur in phenothiazine sulfoxide; N_P, nitrogen in phenothiazine; N_O, nitrogen in phenothiazine sulfoxide; C_A, aromatic carbon; C_T, alifatic carbon; X, any carbon or hydrogen.

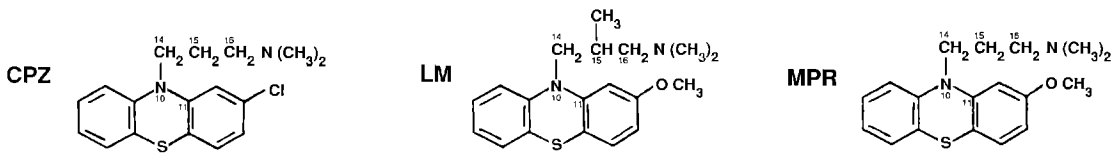

Fig. 2. Chemical structure of chlorpromazine (CPZ), levomepromazine (methotrimeprazine) (LM), and methoxypromazine (MPR). Torsion angles of the side chain; T1, C11–N10–C14–C15; T2, N10–C14–C15–C16.

by fitting AMBER optimized structures to the crystal structures of chlorpromazine and chlorpromazine sulfoxide, in order to obtain optimal resemblance. The parameters are given in Table 1.

Structure refinement

Molecular mechanics energy minimization of crystal structures was performed by the steepest-descent method for the initial 200 cycles, with an initial step length of 0.05, followed by conjugate gradient minimization until an energy gradient of 0.02 or lower was attained.

The reported crystal structures of chlorpromazine [22], chlorpromazine sulfoxide [17], levomepromazine [15], levomepromazine sulfoxide [16] and methoxypromazine [21] were used as starting coordinates in the calculations. The crystal structures were initially refined by energy minimization in vacuo without including electrostatic interactions. Atomic point charges were then calculated for the refined structures with the QUEST program, using an STO-3G basis set. A new set of structures was then generated by molecular mechanics energy minimization in vacuo, including electrostatic interactions.

Conformational analysis of all the molecules studied was carried out by the following procedure. Starting from the energy refined crystal structures, multiple conformations were generated as a function of torsion angle T1 and T2 of the side chain (Fig. 2). The torsion angles were varied at 15° intervals between 0° and 345°. Each of the 576 conformations generated this way was optimized in vacuo, by holding T1 and T2 fixed while the rest of the molecule was allowed to relax. Each conformation was refined by 10 cycles of steepest descent minimization followed by conjugate gradient minimization until convergence. The minimized energies were plotted as a function of T1 and T2, and energy contours drawn at 2 kcal/mol intervals (Fig. 3).

Structures representing minimum-energy conformations in the T1–T2 energy contour maps were further refined by energy minimization of the whole molecule. A hydrogen atom was added to the dimethylamino nitrogen atom in these refined structures, with a bond length of 0.96 Å and a bond angle of 109.5° with the two carbon atoms of the dimethylamino group. A new set of atomic charges were calculated for the protonated structures, which subsequently were energy minimized in vacuo as described above.

Molecular dynamics

Molecular dynamics simulations in vacuo and in water, without pressure monitoring and with conservation of total energy, were performed at 300 K with a step length of 0.001 ps, after 1 ps of initial heating and equilibrium dynamics starting from 0.1 K. Bonds involving hydrogen atoms were constrained during the simulations. The coordinates of the molecular system were saved at

0.1 ps or 0.5 ps intervals. The angle between the least-squares planes of the two phenyl rings and the distance between the nitrogen atom in the side chain and the center of the substituted phenyl ring were calculated for each of the saved coordinate sets.

A layer of water with thickness 9 Å, containing between 245 and 265 randomly distributed water molecules, was added around the protonated, energy refined structures, and the solute–water systems were refined by molecular mechanical energy minimization, including solute–solvent and electrostatic interactions. These refined structures were used as starting points for molecular dynamics simulations in solution.

Molecular graphics

The Molecular Interactive Display And Simulation (MIDAS) programs [32,33] were used for

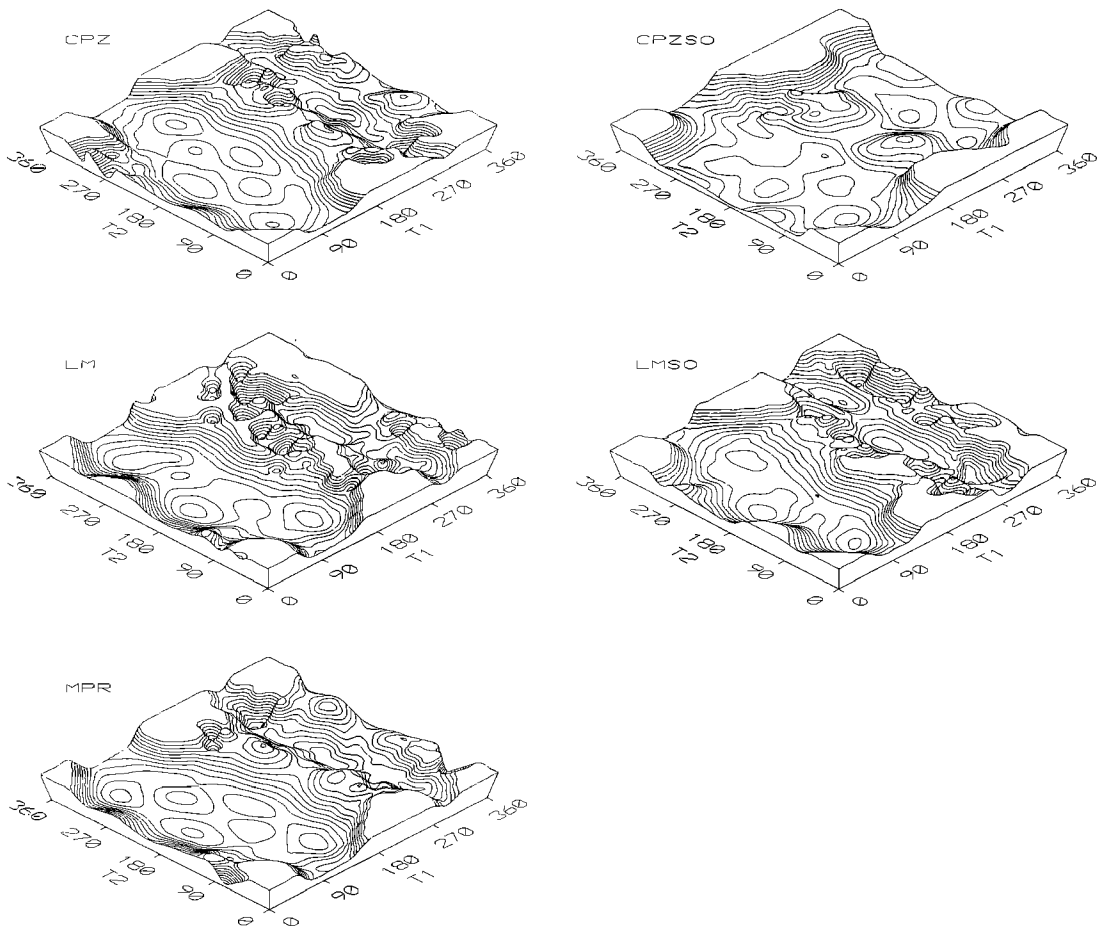

Fig. 3. Energy contour maps of chlorpromazine (CPZ), chlorpromazine sulfoxide (CPZSO), levomepromazine (LM), levomepromazine sulfoxide (LMSO) and methoxypromazine (MPR). T1 and T2: torsion angles of the side chain. The contours show relative potential energies at 2 kcal/mol intervals, starting at 1 kcal/mol. Energies above 24 kcal/mol are not indicated.

TABLE 2
MINIMUM-ENERGY CONFORMATIONS AND RELATIVE ENERGIES IN VACUO OF THE FREE BASES OF CHLORPROMAZINE (CPZ), CHLORPROMAZINE SULFOXIDE (CPZSO), LEVOMEPROMAZINE (LM), LEVOMEPROMAZINE SULFOXIDE (LMSO) AND METHOXYPROMAZINE (MPR)[a]

Compound	Conformation type[b]	Torsion angle		Plane angle A(°)	Rel. energy (kcal/mol)	
		T1(°)	T2(°)		E_T	E_T-E_E
CPZ	UF	84.3	295.5	153.0	0.0	0.0
	UE	59.6	177.5	136.0	2.8	2.5
	DE	278.9	172.1	152.5	4.8	4.3
CPZSO	UF	60.5	56.3	149.8	0.0	0.0
	UE	72.4	178.4	147.5	1.2	0.6
	DE	275.1	171.9	153.0	2.8	1.4
LM	UF	77.7	62.5	148.4	0.0	0.0
	UE	57.0	178.1	138.2	1.4	1.5
LMSO	UF	81.8	60.5	146.7	0.0	0.0
	UE	56.6	182.4	143.9	0.3	0.4
	DE	264.1	147.2	153.1	4.8	4.2
	DF	285.9	303.0	155.7	3.9	2.8
MPR	UF	86.9	295.9	152.7	0.0	0.0
	UE	58.5	175.0	135.7	2.5	2.5
	DE	279.9	185.0	152.0	4.3	4.3

[a] T1 and T2, torsion angles of the side chain; A, angle between the least-squares planes of the phenyl rings; E_T, relative total potential energy; E_T-E_E, Relative total − electrostatic energy.
[b] U, 'up'; D, 'down'; F, folded; E, extended.

molecular graphics on an Evans and Sutherland PS390 system with a DEC VAX 780/VMS system as the host machine. Solvent-accessible molecular surfaces [34,35] and electrostatic potentials 1.4 Å outside the surfaces were calculated with the MIDAS programs, and the potentials were illustrated by color coding of the surfaces. The electrostatic potentials were calculated with a distance dependent dielectric function ($\varepsilon = r_{ij}$) and a nonbonded cutoff radius of 10.0 Å.

RESULTS

Figure 1 illustrates the difference between the 'up' conformations of the side chains in the crystal structures of chlorpromazine, levomepromazine and levomepromazine sulfoxide, and the 'down' conformation in the crystal structure of chlorpromazine sulfoxide. 'Up' conformations are defined here as those with torsion angle T1 (Fig. 2) between 0° and 180°, and 'down' conformations as those with T1 between 180° and 360°, which places the side chain below the folded ring structure in the projection shown in Fig. 1.

Accessible conformations and energies

The potential energies of the drugs and sulfoxide metabolites are shown in Fig. 3 as a function of torsion angle T1 and T2, which were rotated in 15° intervals. In some cases, with T1 constrained near 0° or 180°, this placed the side chain in an energetically unfavorable position close to one of the phenyl rings. During energy minimization of the rest of the molecule, the ring system and the side chain were then forced away from each other, sometimes resulting in inversion of the central thiazine ring such that the structure was transformed to an energetically more stable 'up' conformation. This appeared as a circular indent in the potential energy surface. However this phenomenon, which only was observed with T1 constrained near 0° or 180°, probably has little relevance for the conformational behavior of these molecules in vacuo or in solution.

As illustrated in Fig. 3, the energy barrier between 'up' and 'down' conformations was significantly higher in chlorpromazine (13 kcal/mol) than in chlorpromazine sulfoxide (6 kcal/mol). Levomepromazine, which has a methyl group on the side chain and a more bulky 2-substituent than in chlorpromazine, also had a higher energy barrier between 'up' and 'down' conformations (> 20 kcal/mol). Levomepromazine sulfoxide had an energy barrier of 14 kcal/mol between 'up' and

TABLE 3

MINIMUM-ENERGY CONFORMATIONS AND RELATIVE ENERGIES IN VACUO OF PROTONATED CHLORPROMAZINE (CPZ), CHLORPROMAZINE SULFOXIDE (CPZSO), LEVOMEPROMAZINE (LM), LEVOMEPROMAZINE SULFOXIDE (LMSO) AND METHOXYPROMAZINE (MPR)[a]

Compound	Conformation type[b]	Torsion angle		Plane angle A(°)	Rel. energy (kcal/mol)	
		T1(°)	T2(°)		E_T	E_T–E_E
CPZ	UF	84.7	295.9	154.7	0.0	0.0
	UE	56.8	174.3	139.9	2.2	0.7
	DE	275.2	152.8	152.2	5.5	2.4
CPZSO	UF	55.1	58.7	145.3	0.0	0.0
	UE	79.0	174.2	147.7	3.4	2.6
	DE	273.5	189.7	150.9	8.4	6.5
LM	UF	67.7	66.6	146.7	1.1	1.7
	UE	64.9	197.2	152.2	0.0	0.0
LMSO	UF	72.7	291.6	144.1	0.6	0.8
	UE	78.5	201.9	146.0	0.0	0.2
	DE	263.6	147.4	128.7	4.7	0.9
	DF	283.4	304.1	152.5	2.0	0.0
MPR	UF	86.7	286.9	153.1	0.0	0.6
	UE	57.4	172.2	137.7	0.3	0.0
	DE	281.5	167.9	150.7	1.7	1.7

[a] T1 and T2, torsion angles of the side chain (Fig. 2); A, angle between the least-squares planes of the phenyl rings; E_T, relative total potential energy; $E_T - E_E$, relative total − electrostatic energy.

[b] U, 'up'; D, 'down'; F, folded; E, extended.

'down' conformations. In methoxypromazine, which has an *O*-methoxy ring substituent like levomepromazine, and a side chain similar to that in chlorpromazine, the energy barrier between 'up' and 'down' conformations was also similar to that in chlorpromazine (13 kcal/mol).

The potential energy maps in Fig. 3 show several distinct minima among the 'up' conformations (T1 0–180°), corresponding to various *anti* and *gauche* conformations of torsion angle T2. As might have been expected, the contour maps have a much narrower 'valley' of energy minima for the 'down' conformations (T1 180–360°), which have the side chain below the folded ring system in the projection shown in Fig. 1. The energy minima for 'up' conformations were also lower than the minima for 'down' conformations, and this difference was more pronounced in chlorpromazine and levomepromazine than in the corresponding sulfoxides (Fig. 3).

The potential energy map for levomepromazine sulfoxide has two distinct energy minima among the 'down' conformations, corresponding to a folded *gauche* (T2) conformation and an extended *anti* (T2) conformation. The energy maps for the other four compounds showed only one distinct minimum, corresponding to an extended *anti* (T2) conformation, for T1 between 180° and 360° (Fig. 3).

Energy minimization of the whole molecule, starting from the various energy minima in the contour maps shown in Fig. 3, resulted in several different extended and folded 'up' and 'down' conformations of the side chain. Energy minimization of a T1 $-$*gauche*, T2 *anti* ('down') conformation of levomepromazine did not converge in a 'down' conformation, but resulted in ring inversion which placed the side chain in an 'up' conformation. This shows that the structure ob-

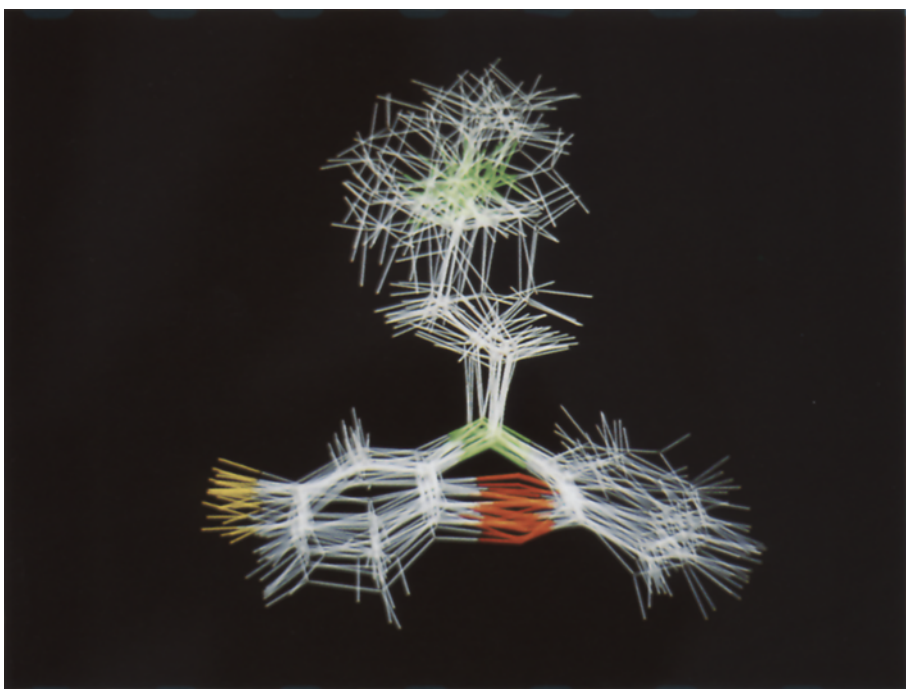

Fig. 4. Molecular structures of protonated chlorpromazine observed during 25 ps of molecular dynamics in vacuo. The 50 superimposed structures were observed at 0.5 ps intervals. Color coding of atoms: nitrogen, green; chlorine, yellow; sulphur, red; hydrogen and carbon, white.

TABLE 4
STARTING CONFORMATION FOR EQUILIBRIUM PHASE DYNAMICS, AND CONFORMATIONS OBSERVED DURING SUBSEQUENT MOLECULAR DYNAMICS SIMULATIONS OF PROTONATED CHLORPROMAZINE (CPZ) AND CHLORPROMAZINE SULFOXIDE (CPZSO), 25 ps IN VACUO AND 10 ps IN WATER[a]

Compond	Phase[b]	Conformation type[c]		Distance d(Å)	Plane angle α(°)
		Start equil.	During simulation		
CPZ	vac	UE	UF	5.1–7.0 (6.1)	109–169 (143)
CPZ	vac	DE	UF	3.5–7.0 (5.3)	101–173 (142)
CPZ	wat	UE	UF	4.7–6.4 (5.9)	141–178 (157)
CPZ	wat	DE	UF	4.3–5.7 (5.0)	92–165 (132)
CPZSO	vac	DE	DE,UF	4.8–7.0 (5.9)	107–167 (141)
CPZSO	vac		UF	4.4–7.1 (5.7)	105–178 (141)
CPZSO	vac	UE	UF	3.7–5.8 (5.1)	107–166 (140)
CPZSO	wat	DE	DE	6.0–7.3 (7.0)	101–176 (151)

[a] α, Angle between least-squares planes of the two phenyl rings; d, distance between the center of the substituted phenyl ring and the nitrogen atom of the dimethylamino group in the side chain. Observed range with mean value in parentheses.
[b] vac, in vacuo; wat, in water.
[c] U, 'up'; D, 'down'; F, folded; E, extended.

tained by minimization of the rest of the molecule with fixed T1 = *−gauche* and T2 = *anti* conformations, did not correspond to a true energy minimum for the whole LM molecule. Compared to CPZ, LM both has a more bulky ring substituent and an additional methyl substituent on the side chain. Steric hindrance may therefore have made the 'down' conformations less stable for LM than for CPZ, as also reflected in the energy contour maps (Fig. 3).

The potential energies and torsion angles T1 and T2 for the lowest-energy conformations are given in Table 2 for the free bases and in Table 3 for the protonated compounds. As shown in Table 2, 'up' conformations of the free bases which have the side chain folded over the ring structure, had lowest energy in vacuo, while the 'down' conformations had highest energies. For the protonated structures folded 'up' conformations had lowest energies for chlorpromazine, chlorpromazine sulfoxide and methoxypromazine, while the extended 'up' conformations had slightly lower energies than the folded 'up' conformations in protonated levomepromazine and levomepromazine sulfoxide (Table 3).

Molecular dynamics

In order to further examine the difference between chlorpromazine and chlorpromazine sulfoxide regarding the energy barrier between their 'up' and 'down' conformations, molecular dynamics simulations of the protonated compounds were performed in vacuo and in water. Table 4 shows the type of conformations, the observed distance between the dimethylamino nitrogen atom and the center of the substituted phenyl ring, and the angle between the least-squares planes of the two phenyl rings during the simulations.

Figure 4 shows structures observed during a 25 ps molecular dynamics simulation of proto-

nated chlorpromazine in vacuo, after 1 ps of equilibrium dynamics starting from an extended 'up' conformation. The side chain stayed folded over the ring system, and both the side chain and the tricyclic ring system showed substantial flexibility during the simulation. Similar folded 'up' conformations were observed during 10 ps molecular dynamics of protonated chlorpromazine in solution, after equilibrium dynamics starting from an extended 'up' conformation.

Subsequent simulations with protonated chlorpromazine were performed in vacuo and in solution, starting the equilibrium phase from an extended 'down' conformation. Both in vacuo and in solution the side chain switched from a 'down' conformation to an 'up' conformation during the 1 ps equilibrium phase, and stayed in folded 'up' conformations during the following 25 ps simulation in vacuo and during the 10 ps simulation in solution.

In simulations with protonated chlorpromazine sulfoxide in solution, with the equilibrium simulation starting from an extended 'down' conformation, the side chain stayed in extended 'down' conformations both during the initial equilibrium phase and during the following 10 ps simulation, as shown in Fig. 5a. In a simulation of protonated chlorpromazine sulfoxide in vacuo the side chain stayed in extended 'down' conformations during the intitial equilibrium phase, and switched from a 'down' to an 'up' conformation during the following 25 ps dynamics simulation, as shown in Fig. 5b. The simulation in vacuo was then continued for another 25 ps, during which the side chain stayed in folded 'up' conformations as shown in Fig. 5c.

Molecular electrostatic potentials

The molecular electrostatic potentials 1.4 Å outside the water-accessible surfaces ranged from −2 kcal/mol to 34 kcal/mol, and were similar for protonated chlorpromazine, levomepromazine and methoxypromazine. Similar ranges of molecular electrostatic potentials (−3 kcal/mol to 32 kcal/mol) were also found for the folded and extended 'up' conformations of chlorpromazine sulfoxide and levomepromazine sulfoxide. However, extended and folded 'down' conformations of the side chain in the sulfoxide metabolites exposed an area of strong negative electrostatic potentials around the sulfoxy group, as shown in Fig. 6 for chlorpromazine sulfoxide. The molecular electrostatic potentials around the 'down' conformations of chlorpromazine sulfoxide and levomepromazine sulfoxide ranged from −11 kcal/mol to 33 kcal/mol.

DISCUSSION

Differences between the phenothiazines and their sulfoxide metabolites in the electronic properties of the central thiazine ring, which resulted in different molecular mechanics parameters, produced lower energy barriers between 'up' and 'down' conformations in sulfoxides than in the parent compounds. Regardless of the molecular mechanics parameters used in the present calculations, the energy barrier was substantially higher in levomepromazine sulfoxide than in chlorpromazine sulfoxide (Fig. 3). This was probably due to the methyl group at the side chain and the more bulky ring substituent in levomepromazine. It has previously been suggested, from the crystal structures of phenothiazine drugs and their sulfoxide metabolites, that an extended 'down' conformation may diminish the biological activity of phenothiazines [19]. The higher energy barrier between 'up' and 'down' conformations in levomepromazine sulfoxide than in chlorpromazine sulfoxide might be one reason for levomepromazine sulfoxide being slightly more biologically active than chlorpromazine sulfoxide.

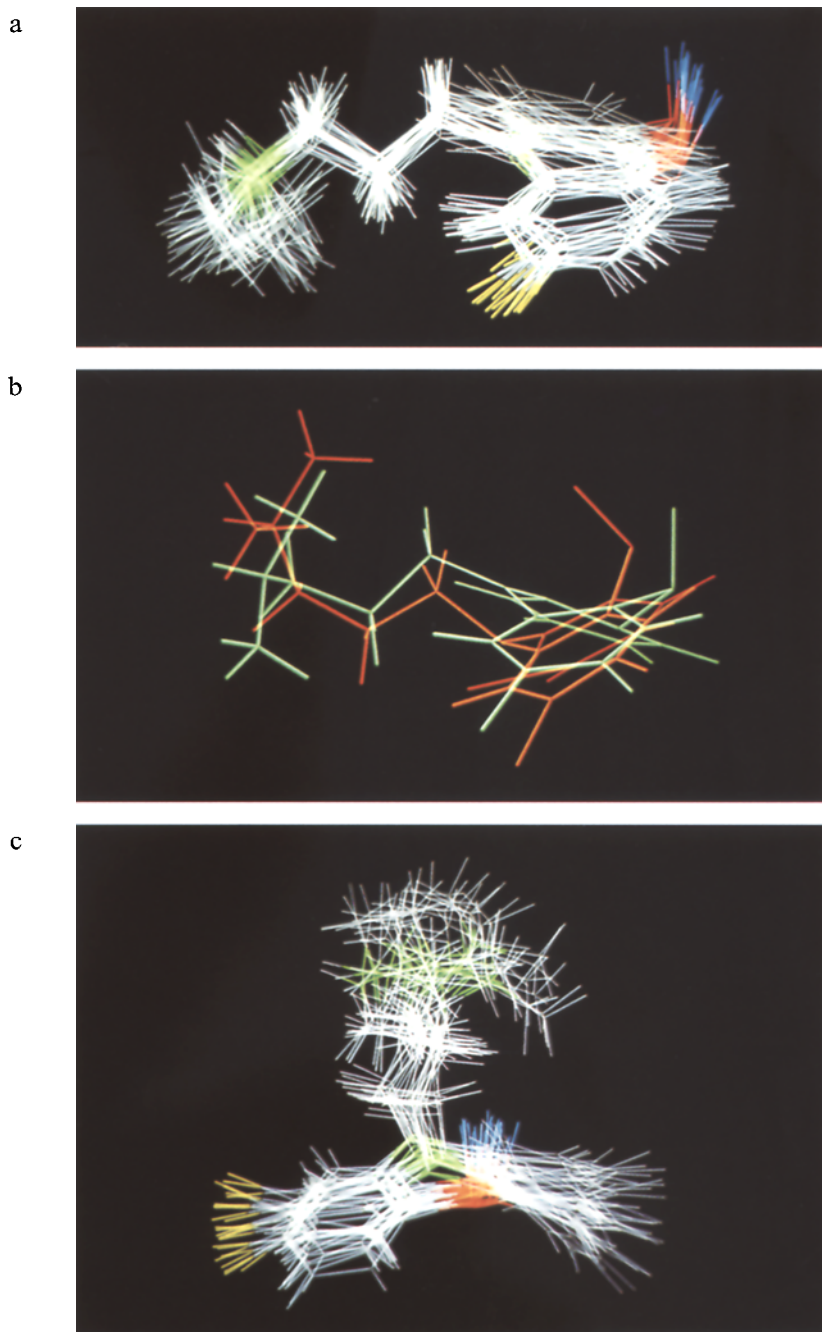

Fig. 5. Molecular dynamics of protonated chlorpromazine sulfoxide. (a) Extended 'down' conformations observed at 0.1 ps intervals during the initial 5 ps of a 10 ps simulation in water. (b) Transition from an extended 'down' conformation (green) to a folded 'up' conformation (red) during molecular dynamics in vacuo. The two conformations were observed at a 0.5 ps interval. (c) Folded 'up' conformations observed at 0.5 ps intervals during a 25 ps molecular dynamics simulation in vacuo. Color coding of atoms: nitrogen, green; chlorine, yellow; sulphur, red; oxygen, blue; hydrogen and carbon, white.

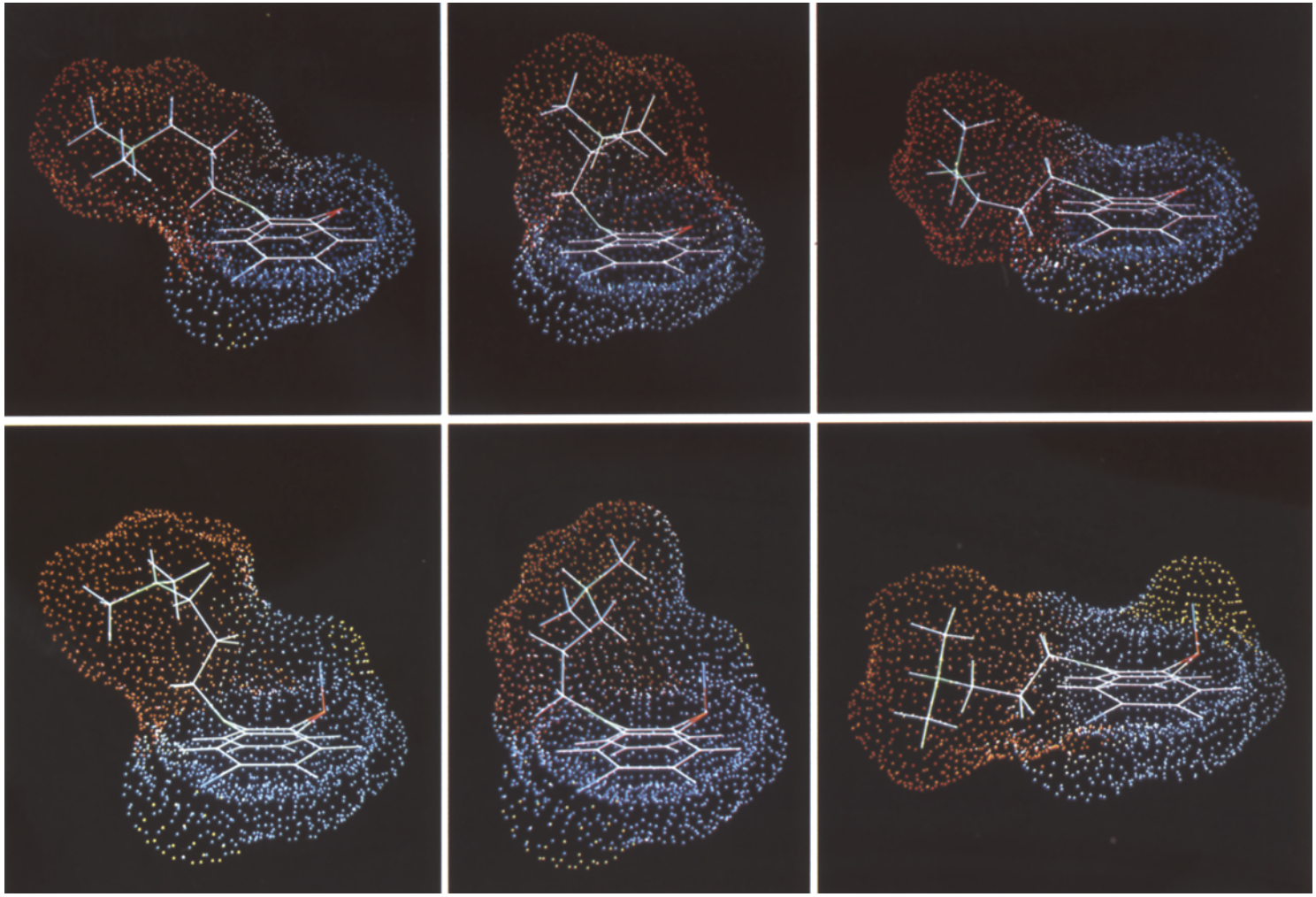

Fig. 6. Minimum energy conformations of chlorpromazine (upper row) and chlorpromazine sulfoxide (lower row) in vacuo. Left, extended 'up' conformations; middle, folded 'up: conformations; right, extended 'down' conformations. The dots show water accessible surfaces, colored according to molecular electrostatic potentials (e, kcal/mol) 1.4 Å outside the surface. Red, $e > 15$; white, $12 \leqslant e \leqslant 15$; blue, $0 \leqslant e < 12$; yellow, $e < 0$.

However, in our calculations both the phenothiazine drugs and the sulfoxides generally had lowest potential energy in conformations with the side chain folded over the ring system. This indicates that the substantial difference in biological activity between the phenothiazines and the sulfoxide metabolites is not mainly due to different side chain conformations, but to direct receptor interaction of the sulfoxy group.

It has been postulated that the primary interaction of protonated neuroleptic drug molecules with dopamine receptors is electrostatic, with negatively charged domains at the receptor surface [36–38]. It seems likely that the strong negative electrostatic field around the sulfoxy group in the 'down' conformations of chlorpromazine sulfoxide and levomepromazine sulfoxide may weaken such electrostatic interactions with dopamine receptors, and thereby cause the sulfoxides to be virtually inactive in dopamine receptor binding and related tests. However, the solvent may have a profound influence on conformational equilibria of flexible solutes in solution [39]. It is possible, therefore, that differences in solvation effects between phenothiazine drugs and the sulfoxide metabolites also may contribute to the differences in their dopamine receptor binding affinities.

The psychoactive thioxanthene derivatives have chemical structures and pharmacological effects similar to those of the phenothiazine drugs, but with a carbon atom instead of a nitrogen atom at the central ring, and an exocyclic C–C double bond. The thioxanthenes may therefore exist as *cis*(Z)- and *anti*(E)-isomers, and only the *cis*(Z)-isomers are considered pharmacologically active [40]. The molecular electrostatic potentials are significantly more negative around the 2-substituent in the inactive *anti*(E)-isomers than in the pharmacologically active *cis*(Z)-thioxanthenes, and it has been suggested that this may weaken the electrostatic interactions between *anti*(E)-thioxanthenes and dopamine receptors [41,42]. This offers a similar explanation for the lack of anti-dopaminergic potency of *anti*(E)-thioxanthenes and phenothiazine ring sulfoxides, and suggests that other tricyclic compounds with equally strong negative electrostatic potentials also may be devoid of anti-dopaminergic potency.

The tricyclic ring systems of chlorpromazine and its sulfoxide showed unexpectedly high flexibility in the molecular dynamics simulations, with variations between 92° and 178° in the angle between the planes of the two phenyl rings. Also, the motions from a 'down' to an 'up' conformation of the side chain in chlorpromazine and in chlorpromazine sulfoxide took place in a rather unexpected way. Instead of involving a rotation around the N10–C14 single bond in the side chain (Fig. 2) as might have been expected, the conformational change involved collective movements of all the atoms in the molecule, as illustrated for chlorpromazine sulfoxide in Fig. 5b.

It has previously been postulated from thermodynamic considerations, that flexible ligands may bind to a macromolecule in several successive steps while changing their conformation, instead of by a 'lock and key' mechanism by which the ligand stays in a specific conformation [43]. The rate and magnitude of the internal molecular movements in chlorpromazine during the molecular dynamics simulations support such a 'zipper' mechanism for its interaction with dopamine receptors.

The potential energy maps shown in Fig. 3 indicate that in addition to producing negative electrostatic potentials around part of the phenothiazine ring system, ring sulfoxidation also shifts the equilibrium between conformers and stabilizes the 'down' conformations. The calculations presented here indicate that both these structural effects contribute to the loss of anti-dopaminergic activity by ring sulfoxidation of phenothiazine drugs.

CONCLUSIONS

The 3-D structure, molecular electrostatic potentials and dynamics of chlorpromazine, levomepromazine and their sulfoxide metabolites were examined by molecular mechanics and quantum mechanics calculations, molecular dynamics simulations and computer graphics. 'Down' conformations of the side chains had higher potential energies than 'up' conformations with the side chain above the folded ring system. The energy differences between 'up' and 'down' conformations were higher in the phenothiazine drugs than in the sulfoxides, and the energy barriers between 'up' and 'down' conformations were smallest in the sulfoxides. 'Down' conformations of the side chain exposed strong negative electrostatic potentials around the sulfoxy group in the sulfoxides. It is suggested that this may weaken their electrostatic interactions with dopamine receptors, and thereby cause the sulfoxides to be virtually inactive in dopamine receptor binding and related tests.

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