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Molecular Modelling Analysis of the Metabolism of Tolterodine

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Abstract: Tolterodine is a new antimuscarinic drug used for the treatment of patients with overactive bladder presenting urinary frequency, urgency and urge incontinence. *In vitro*, TTD has high affinity and specificity for muscarinic receptors and shows selectivity for the urinary bladder over salivary glands *in vivo*. It is a weak base that is rapidly absorbed in humans and eliminated mainly by metabolism. Two oxidative metabolic pathways of TTD involve hydroxylation and N-dealkylation. Hydroxylation produces 5-HM-TTD and is catalysed by CYP2D6 while the N-dealkylation to produce ND-TTD from tolterodine and ND-5-HM-TTD from 5-HM-TTD is catalysed by CYP3A. Oxidation of 5-HM-TTD produces TTDA. Delakylation of TTDA produces ND-TTDA. The major portion of administered dose is excreted as TTDA and ND-TTDA. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that TTD and its primary metabolite M1 have moderately large to large LUMO-HOMO energy differences so that they would be kinetically inert. Thus, although TTD and its metabolites have some electron-deficient regions on their molecular surfaces so that they could react with glutathione and nucleobases in DNA, the rates of such adverse reactions are expected to be low.

Key words: Tolterodine, antimuscarinic drug, overactive bladder, CYP2D6, molecular modelling

INTRODUCTION

Tolterodine (TTD; (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenyalpropanamine is a new antimuscarinic drug used for the treatment of patients with overactive bladder presenting urinary frequency, urgency and urge incontinence (Rentzhog *et al*., 1998; Abrahams *et al*., 1998 *in vitro*, TTD has high affinity and specificity for muscarinic receptors and shows selectivity for the urinary bladder over salivary glands *in vivo* Nivebrant *et al*. (1997). TTD is a weak base that is rapidly absorbed in humans and eliminated mainly by metabolism. Two oxidative metabolic pathways of TTD involve hydroxylation and N-dealkylation Brynne *et al*. (1998) Hydroxylation produces 5-hydroxymethyltolterodine (5-HM-TTD; PNU-200577) and is catalysed by CYP 2D6 Brynne *et al*. (1998), while the N-dealkylation to produce N-dealkylated tolterodine (ND-TTD) from tolterodine and ND-5-HM-TTD from 5-HM-TTD is catalysed by CYP3A (Postlind *et al*., 1998). Oxidation of 5-HM-TTD produces tolerodine acid (TTDA). Delakylation of TTDA produces N-delakylated tolterodine acid (ND-TTDA) Brynne *et al*. (1998) The major portion of administered dose is excreted as carboxyl metabolites TTDA and ND-TTDA Brynne *et al*. (1997). 5-HM-TTD is seven times more active than the parent drug.

In this study, molecular modelling analyses have been carried out using the program Spartan (2002) to investigate the relative stability of TTD and its metabolites with the aim of providing a better understanding on their relative toxicity.

COMPUTATIONAL METHODS

The geometries of TTD and its metabolites ND-TTD, 5-HM-TTD, TTDA, ND-TTDA and ND-5-HM-TTD have been optimised based on molecular mechanics, semi-empirical and DFT calculations, using the molecular modelling program Spartan '02. Molecular mechanics calculations were carried out using MMFF force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried at B3LYP/6-31G* level. In optimization calculations, a RMS gradient of 0.001 was set as the terminating condition. For the optimised structures, single point calculations were carried out to give heat of formation, enthalpy, entropy, free energy, dipole moment, solvation energy, energies for HOMO and LUMO. The order of calculations: molecular mechanics followed by semi-empirical followed by DFT ensured that the structure was not embedded in a local minimum. To further check whether the global minimum was reached, some calculations were carried out with improvable structures. It was found that when the stated order was followed, structure corresponding to the global minimum or close to that could ultimately be reached in all cases. Although RMS gradient of 0.001 may not be sufficiently low for vibrational analysis, it is believed to be sufficient for calculations associated with electronic energy levels.

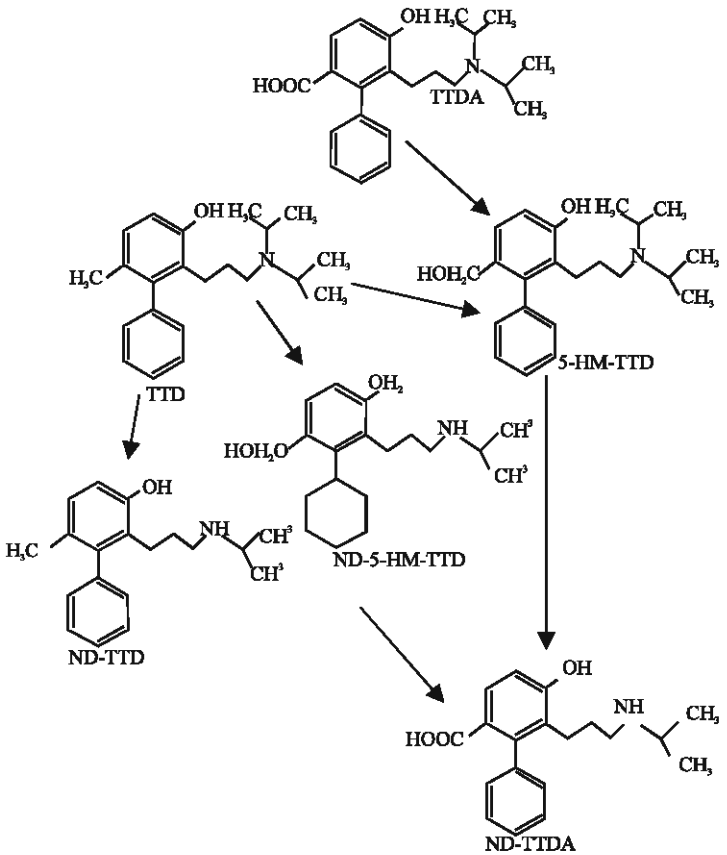


Fig. 1: Metabolic pathways for TTD based on Brynne *et al.* (Brynne *et al.*, 1999)

Table 1: Calculated thermodynamic and other parameters of tolterodine and its metabolites

Molecule	Calculation type	Total energy (kcal mol^{-1}/ atomic unit*)	Heat of formation (kcal mol^{-1})	Enthalpy (kcal mol^{-1} K^{-1})	Entropy (cal mol^{-1} K^{-1})	Free energy (kcal mol^{-1})
TTD	PM3	-41.21	-38.16	317.84	163.84	268.99
	DFT	-986.98		318.91	163.05	270.32
ND-TTD	PM3	-26.95	-20.00	244.19	144.25	201.19
	DFT	-829.74		245.48	143.00	202.87
5-HM-TTD	PM3	-128.62	-117.53	325.60	176.69	272.92
	DFT	-1062.19		326.71	175.89	274.29
TTDA	PM3	-128.36	-118.68	311.12	170.96	260.15
	DFT	-1136.23		312.06	169.99	261.40
ND-TTDA	PM3	-122.97	-108.74	256.34	161.08	208.32
	DFT	-1018.31		257.56	160.03	209.87
ND-5-HM-TTD	PM3	-69.58	-79.95	266.58	158.58	219.29
	DFT	-944.26		267.67	157.60	220.71

	Solvation Calculation type	energy (kcal mol^{-1})	Area (Å^2)	Volume (Å^3)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
TTD	PM3	-3.05	391.11	382.48	2.3	-8.81	0.18	8.99
	DFT	-2.89	402.17	385.79	2.3	-5.03	-0.29	4.77
ND-TTD	PM3	-6.95	328.67	310.97	2.6	-9.11	0.13	9.24
	DFT	-6.08	332.16	312.61	2.7	-5.55	-0.33	5.22
5-HM-TTD	PM3	-11.09	408.28	397.23	3.4	-8.93	0.01	8.94
	DFT	-9.78	417.18	394.86	3.2	-4.77	-0.38	4.39
5-HM-TTD	PM3	-9.68	396.58	391.40	4.4	-9.06	-0.62	8.44
	DFT	-8.89	411.71	395.11	5.0	-5.28	-1.16	4.12
TTDA	PM3	-14.23	359.06	338.63	5.4	-9.35	-0.59	8.76
	DFT	-13.08	349.53	338.10	5.8	-6.22	-0.70	5.52
ND-5-HM-TTD	PM3	-10.37	357.43	336.58	3.6	-9.12	0.04	9.16
	DFT	-9.45	361.29	338.74	3.8	-5.36	-0.41	4.95

*in atomic units from DFT calculations

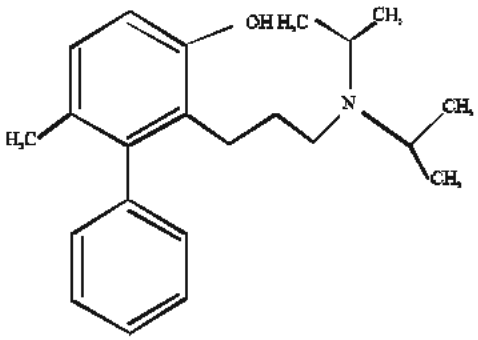
RESULTS AND DISCUSSION

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, surface area, volume, dipole moment, and energies of HOMO and LUMO as per both PM3 and DFT calculations for TTD and its metabolites ND-TTD, 5-HM-TTD, TTDA, ND-TTDA and ND-5-HM-TTD. Figures 2-7 give the regions of negative electrostatic potential (greyish-white envelopes) in (a), HOMOs (where red indicates HOMOs with high electron density) in (b), LUMOs in (c) and density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to the optimised structures of TTD and its metabolites ND-TTD, 5-HM-TTD, TTDA, ND-TTDA and ND-5-HM-TTD.

The calculated solvation energies from PM3 calculations of TTD and its metabolites ND-TTD, 5-HM-TTD, TTDA, ND-TTDA and ND-5-HM-TTD are respectively -3.05, -6.95, -11.09, -9.68, -14.23, -10.37 and the corresponding dipole moments from DFT calculations are 2.3, 2.7, 3.2, 5.0, 5.8 and 3.8. The values suggest that TTD and its metabolites would vary in their solubility in water with TTD being least water-soluble one and ND-TTDA being most water-soluble.

The LUMO-HOMO energy differences for TTD and its metabolites from DFT calculations are found to be high or moderately high ranging from 4.1 to 5.5 eV indicating that TTD and its metabolites would vary significantly in their kinetic lability with ND-TTDA being most inert and TTDA being least inert.

In the case of TTD, ND-TTD and 5-HM-TTD and ND-5-HM-TTD, the electrostatic potential is found to be more negative around the hydroxyl oxygen and the amino nitrogen atoms, indicating that the positions may be subject to electrophilic attack. In the case of TTDA and ND-TTDA, the electrostatic potential is found to be more negative around the amino nitrogen, hydroxyl and carboxyl oxygen atoms, indicating that the positions may be subject to electrophilic attack.



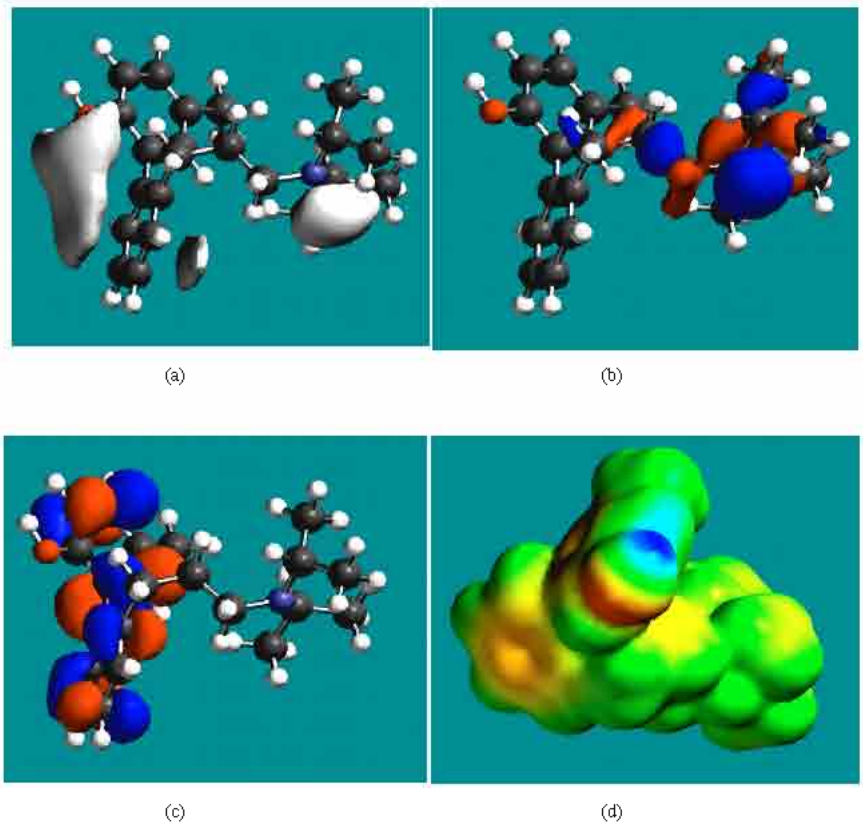


Fig 2 Structure of TTD giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

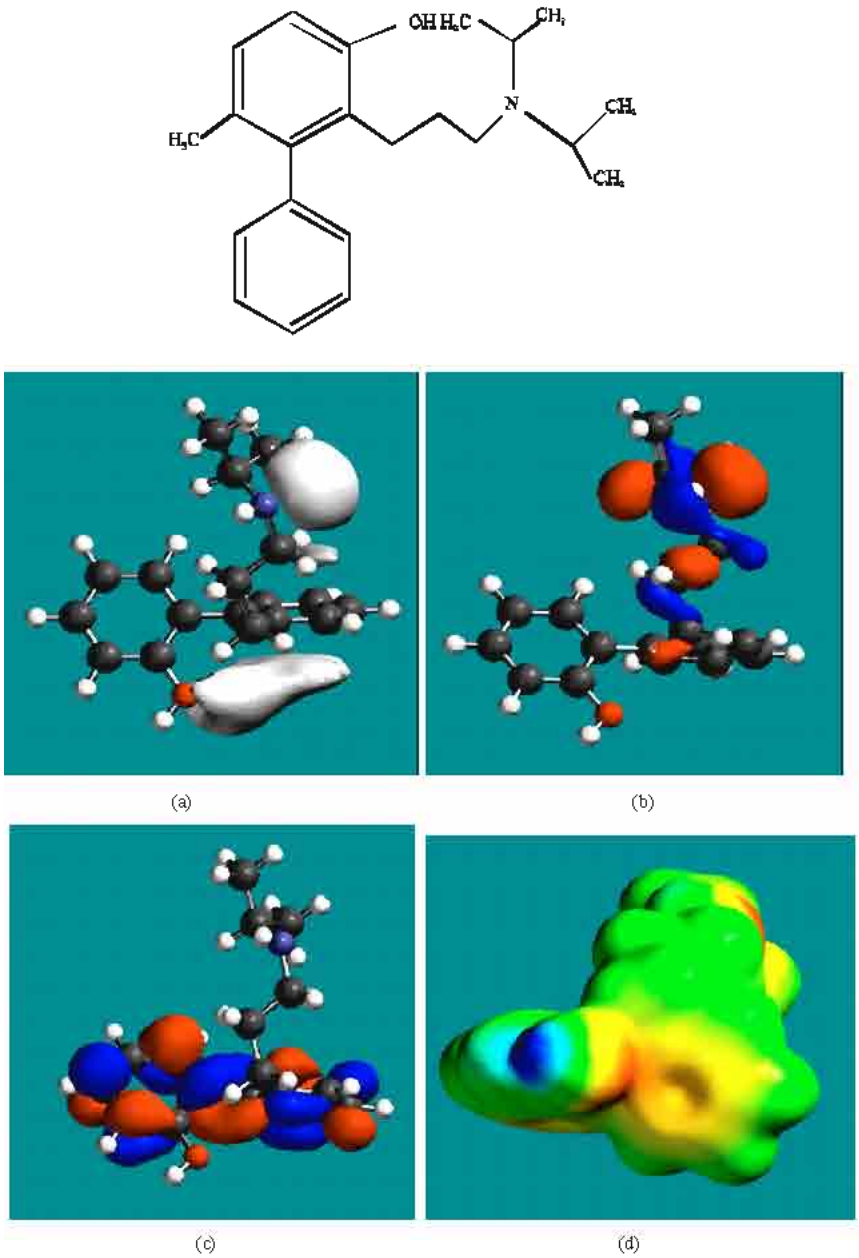
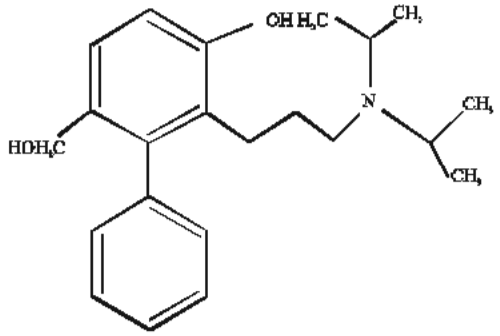


Fig 3 Structure of ND-TTD giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)



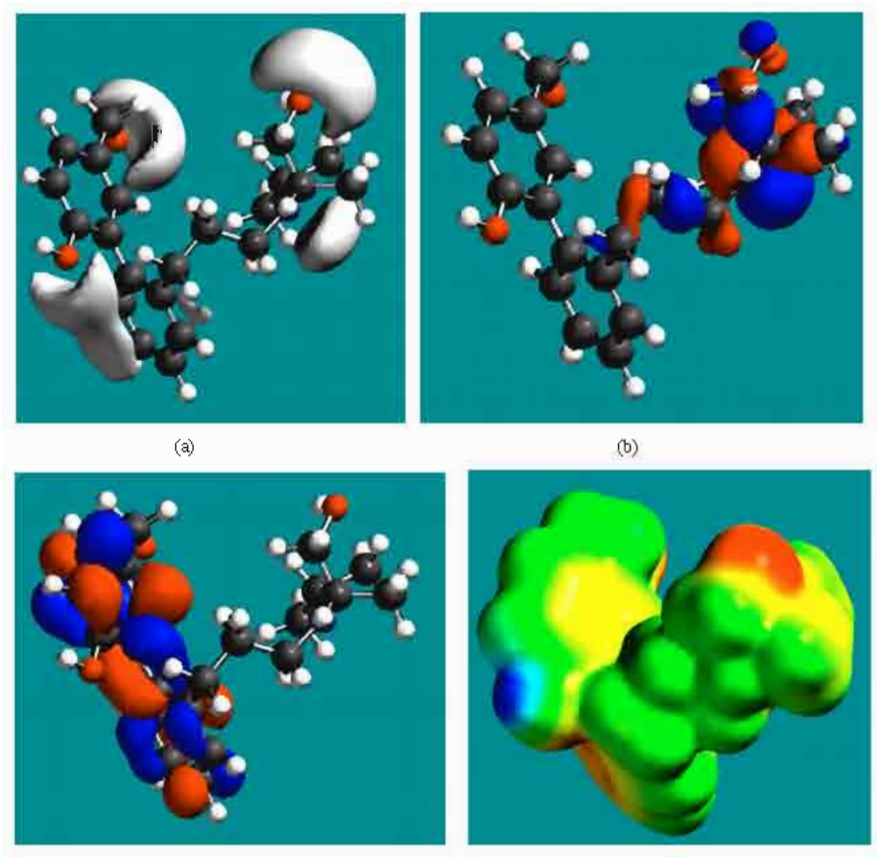
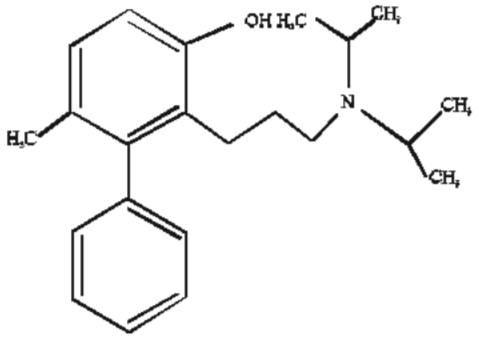


Fig 4 Structure of 5-HM-TTD giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)



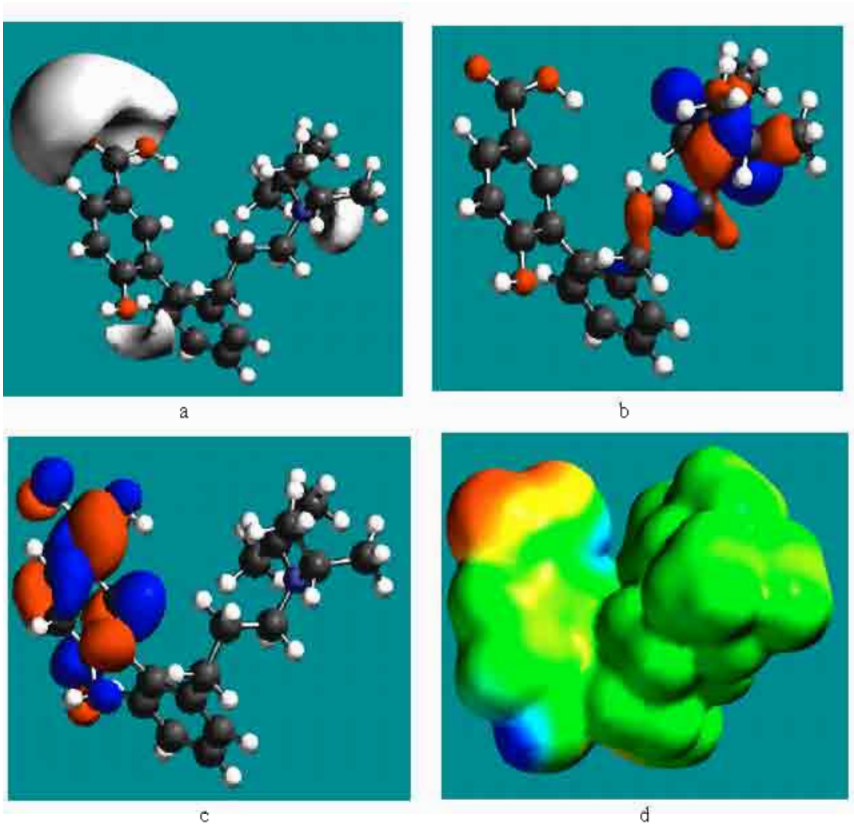


Fig 5 Structure of TTDA giving in (a)the lectrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

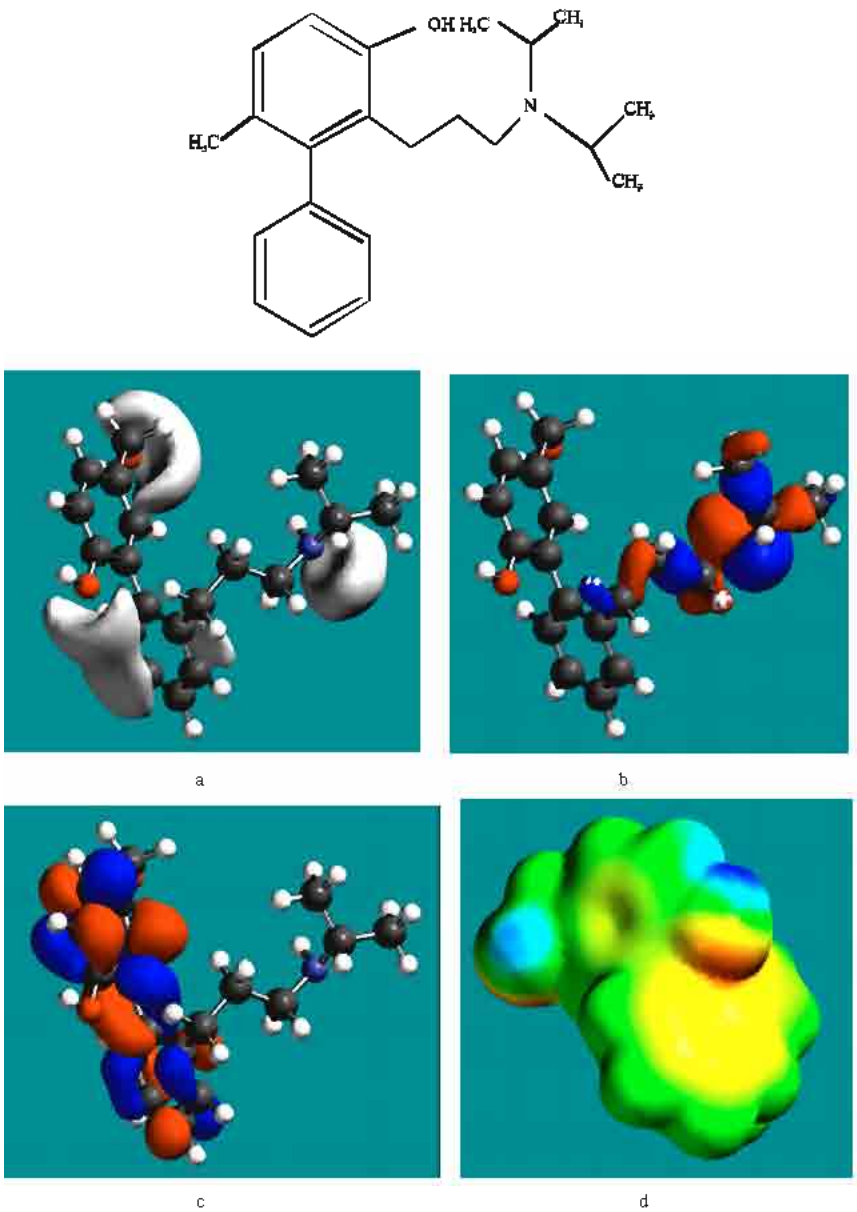
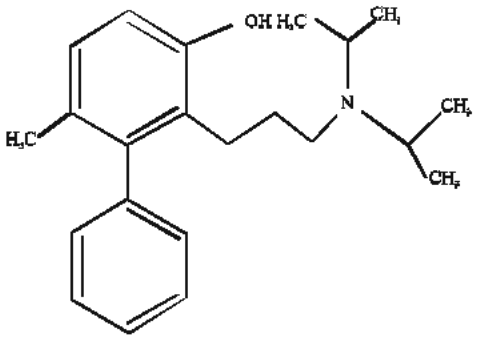


Fig 6 Structure of ND-5-HM-TTD giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)



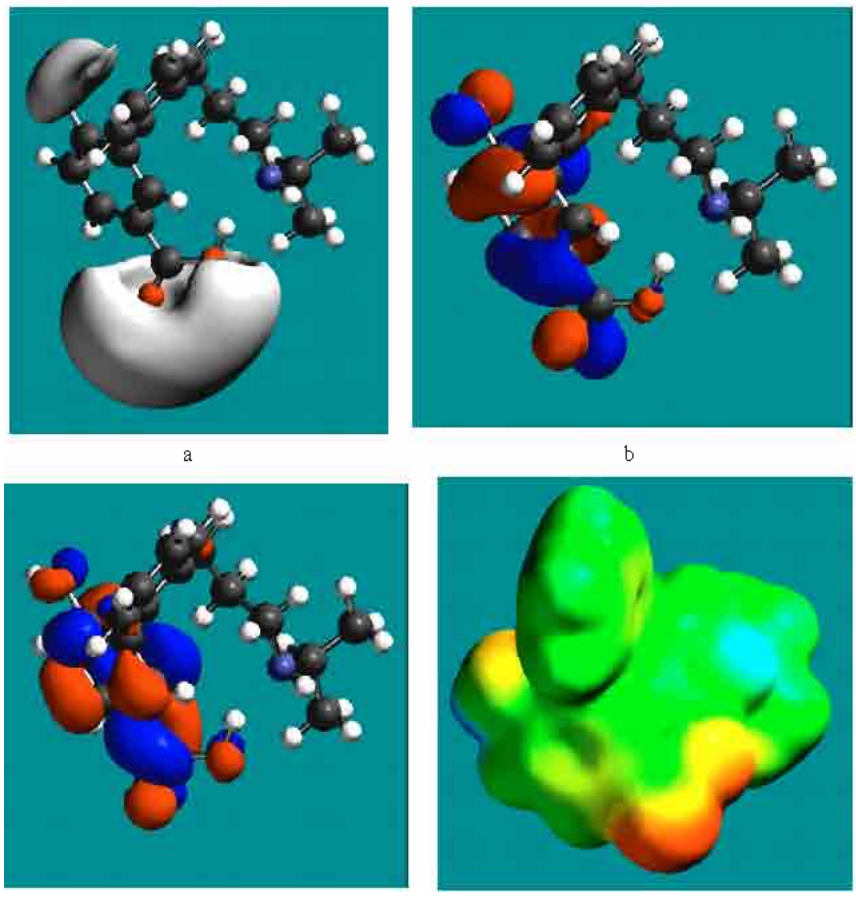


Fig 7 Structure of ND-TTDA giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

In the case of TTD, ND-TTD, 5-HM-TTD, ND-5-HM-TTD and TTDA, the HOMOs with high electron density are found to be centred mostly on the non-hydrogen atoms of N, N-diisopropyl side chain whereas the LUMOs are found to be close to the non-hydrogen atoms of one or both of the phenyl rings.

In the case of ND-TTDA, both the HOMOs with high electron density and the LUMOs are found to be close to the non-hydrogen atoms of the phenyl rings. The overlap of HOMO with high electron density and region of negative electrostatic potential at some positions, gives further support to the idea that the positions may be subject to electrophilic attack.

The molecular surfaces of TTD and its metabolites are found to abound in neutral (green) regions so that it is more likely to be hydrophobic. However, its molecular surface also have electron-rich (red and yellow) and electron-deficient (blue) regions so that TTD may also be subject to electrophilic and nucleophilic attacks. Nucleophilic attack may be due to glutathione and nucleobases in DNA. The former would result into glutathione depletion, thus producing oxidative stress and hence cellular toxicity whereas the latter would cause oxidation of nucleobases in DNA and hence DNA damage. However, because of relative kinetic inertness of TTD and its metabolites and the presence of only a small amount of electron-deficient regions on their molecular surfaces, the rates of such adverse reactions are expected to be low (except perhaps for TTDA which has the smallest LUMO-HOMO energy difference) unless offcourse these are speeded up enzymatically.

When surface area and volume of TTD are compared with those of its metabolites, it is found that the values for TTDA correspond closely with those of TTD (Table 1) so that the metabolites may bind to the same site of the receptor as TTD does unless such binding is disfavoured by differences in their chemical nature.

CONCLUSION

Molecular modelling analyses based on semi-empirical and DFT calculations show that TTD and its metabolites have moderately large to large LUMO-HOMO energy differences so that they would be kinetically inert. This means that although the molecular surfaces of TTD and the metabolites are found to possess some electron-deficient regions so that they could react with glutathione and nucleobases in DNA, in actual fact the rate of such adverse reactions may not be low significant except in the case of TTDA which has the smallest LUMO-HOMO energy difference.

Abbreviations

TTD:	Tolterodine; (R)-N, N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenyalpropanamine
5-HM-TTD:	5-Hydroxymethyltolterodine; PNU-200577
ND-TTD:	N-Dealkylated tolterodine
ND-5-HM-TTD:	N-Dealkylated-5-hydroxymethyltolterodine
TTDA:	Tolerodine acid
ND-TTDA:	N-Delakylated tolterodine acid
DFT:	Density functional theory
LUMO:	Lowest unoccupied molecular orbital
HOMO:	Highest occupied molecular orbital

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