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Molecular Modelling Analysis of the Metabolism of Methadone

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Abstract: Methadone (Met) is a synthetic opiate used for analgesis in patients and to treat opioid dependence. Maintenance treatment with Met has contributed to a drop in mortality, reduction in heroin use, decrease in criminal activity and improvement in social relationships, reduction in risk of HIV and hepatitis virus infection. Met is an opiate μ -receptor agonist. It is a chiral molecule that exists in (S)-Met and (R)-Met forms. Except in Germany, Met is therapeutically administered as a racemic mixture. (R)-Met has a higher affinity for the μ -opioid receptor and a longer plasma elimination half-life than (S)-Met. Met undergoes rapid metabolism almost exclusively in the liver by sequential N-demethylation followed by spontaneous cyclisation to form EDDP and EMDP followed by renal and faecal excretion. EDDP and EMDP do not display any analgesic activity. Cytochrome P450 enzymes CYP3A4, CYP2B6 and CYP2C19 are involved in the metabolism of methadone in human liver and intestine. After steady-state administration of Met, a large variation in the plasma (R)-Met to (S)-Met ratio is observed across a population suggesting that Met is metabolized stereoselectively *in vivo*. Whereas N-demethylation through CYP3A4 is not stereo selective, CYP2B6 is found to metabolize (S)-methadone more rapidly than (R)-methadone while CYP2C19 does the reverse. Molecular modelling analyses show that the two enantiomers of methadone differ in their LUMO-HOMO energy separation and hence in their kinetic lability. In both the enantiomers, the centre of most negative electrostatic potential is found to lie close to the tertiary nitrogen, indicating that the position may be most susceptible to electrophilic attack.

Key words: Methadone, metabolites, EDDP, EMDP, toxicity, molecular modelling

Introduction

Methadone (6-dimethylamino-4,4-diphenyl-3-heptanone) is a synthetic opiate used for analgesis in patients and to treat opioid dependence (Rodriguez-Rosas *et al.*, 2005). It is available as methadone hydrochloride that can be used for the preparation of oral, rectal and parenteral solutions. Since its introduction in 1965 (Dole and Nyswander, 1967) its use has increased progressively particularly for the treatment of drug addicts. Maintenance treatment with methadone (Met) has contributed to a drop in mortality, reduction in heroin use, decrease in criminal activity and improvement in social relationships, reduction in risk of HIV and hepatitis virus infection (Brettle, 1991). Met is an opiate μ -receptor agonist. The pharmacological characteristics that support its use as a replacement in the long term treatment of heroin addiction are its high oral bioavailability, long elimination time, lack of behavioural modifications and the availability of a specific antagonist that can be used in the case of overdose (Ferrari *et al.*, 2003). Treating opioid-addicted women with Met in pregnancy has however been found to increase the number of newborns suffering from Neonatal Abstinence Syndrome (NAS) characterized by high-pitch crying, insomnia, tremor, myoclonic jerks, vomiting, diarrhoea and poor weight gain (Langenfeld *et al.*, 2005).

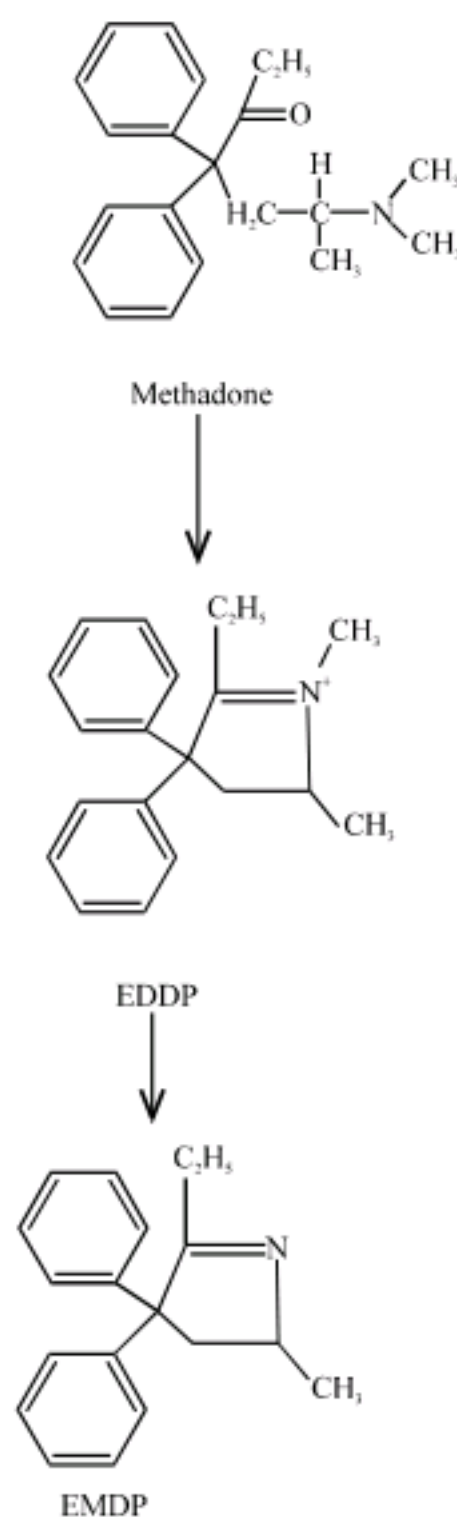


Fig. 1: Metabolic pathways for methadone (Kelly *et al.* 15)

Met is a chiral molecule that exists in (S)-Met and (R)-Met forms. Except in Germany, the drug is therapeutically administered as a racemic mixture (i.e., a 50:50 mixture of both the enantiomers). However, (R)-Met has a higher affinity (50 times greater) than (S)-Met for the μ -opioid receptor (Judson *et al.*, 1976). (R)-Met has a longer plasma elimination half-life than the (S)-Met (Kreek *et al.*, 1979). The two enantiomers also differ in their binding to plasma proteins. Due to its high lipid solubility, 98% of Met reaching the central compartment is rapidly transferred to tissues, particularly liver, kidneys and lungs (Dole and Kreek, 1973). A small portion is transferred to the brain and about 1-2% remains in the blood compartment most of which gets bound to plasma proteins. (R)-Met has a significantly longer elimination half-life than (S)-Met as well as a larger total volume of distribution. Met binds extensively to α 1-acid glycoprotein (AGP), to the AGP variant orosomucoid 2 (ORM2) and to a lesser extent, orosomucoid 1 (ORM1). (S)-Met is bound more extensively to AGP than (R)-Met (Boulton and Devane, 2000).

Met undergoes rapid metabolism almost exclusively in the liver by sequential N-demethylation followed by spontaneous cyclisation to form 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-pyrroline (EMDP) (Fig. 1) followed by renal and faecal excretion. Like Met, both EDDP and EMDP are also chiral molecules but unlike the parent compound, the metabolites do not display any analgesic activity (Sullivan and Due, 1973).

The cytochrome P450 enzyme CYP3AP was identified as the major enzyme involved in the metabolism of methadone in human liver and intestine. CYP3AP is also involved in the metabolism of other drugs such as benzodiazepines, calcium antagonists, macrolide antibiotics and anticonvulsants (Ferrari *et al.*, 2003). Recent studies have suggested that CYP2B6 and CYP2C19 also contribute to the metabolism of Met¹¹. Whereas CYP3AP is a low-affinity, high-capacity enzyme, CYP2B6 and CYP2C19 have a higher affinity towards methadone. CYP2B6 metabolizes (S)-methadone more rapidly than (R)-methadone while CYP2C19 does the reverse (Gerber *et al.* 2004).

After steady-state administration of Met, a large variation in the plasma (R)-Met to (S)-Met ratio was observed across a population suggesting that Met is metabolized stereoselectively *in vivo* (Eap *et al.*, 2000). The elimination of Met and its metabolites occurs mainly through kidneys: 15-60% during the first 24 h (20% as unmodified Met and 13% as EDDP). Elimination in the faeces accounts for 20-40% (Nilsson *et al.*, 1983). When the urinary pH is less than 6, the amount of unmetabolized methadone excreted is three to eight times greater than that at pH higher than 6. The variation in (R)-methadone/(S)-methadone ratio in plasma could be due to the difference in the expression of CYP2B6 and CYP2C19. N-demethylation through CYP3AP is not found to be stereo selective (Foster *et al.*, 1999). Figure 1 summarizes the major metabolic pathways of methadone in humans (Kelly *et al.*, 2002). It should however be noted that complete metabolism of Met has not been worked out yet.

In this study, molecular modelling analyses have been carried out using the programs HyperChem 7.0 (HyperChem 2002) and Spartan '02 (Spartan 2002) to investigate the relative stability of methadone and its metabolites.

Computational Methods

The geometries of (R)-methadone, (S)-methadone, (R)-EDDP, (S)-EDDP, (R)-EMDP and (S)-EMDP have been optimized based on molecular mechanics, semi-empirical and DFT calculations, using the molecular modelling programs Spartan '02 and HyperChem 7.0. Molecular mechanics calculations were carried out using MMFF force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried using the program Spartan '02 at B3LYP/6-31G* level. In optimization calculations, a RMS gradient of 0.001 was set as the terminating condition. For the optimized structures, single point calculations were carried 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 minimized the chances of the structures being trapped in local minima rather than reaching global minima. 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, structures corresponding to global minimum or close to that were reached in most cases. Although RMS gradient of 0.001 may not be sufficiently small for vibrational analysis, it is believed to be so for calculations associated with electronic energy levels. For the optimized structures, single point calculations were carried to give heat of formation, enthalpy, entropy, free energy, dipole moment and solvation energy, energies for HOMO and LUMO.

Results and Discussion

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, dipole moment, energies of HOMO and LUMO as per both PM3 and DFT calculations for (R)-methadone, (S)-methadone, (R)-EDDP, (S)-EDDP, (R)-EMDP and (S)-EMDP. Figure 2-7 give the optimized structures and 2D HOMO orbital plots for (R)-methadone, (S)-methadone, (R)-EDDP, (S)-EDDP, (R)-EMDP and (S)-EMDP. The dotted arrows indicate the positions of more negative electrostatic potential in (a) and the HOMOs with the highest electron density in (b)

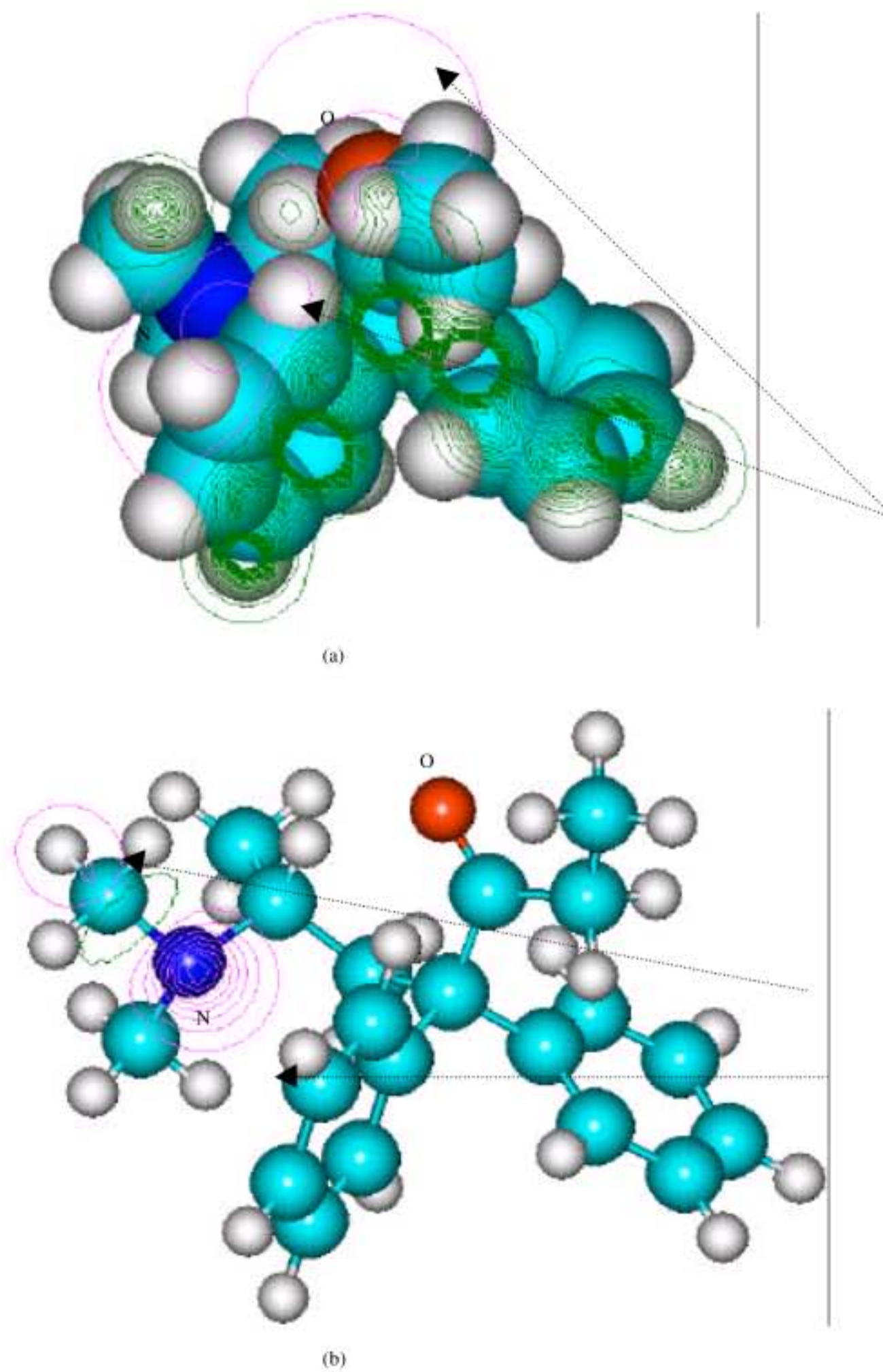
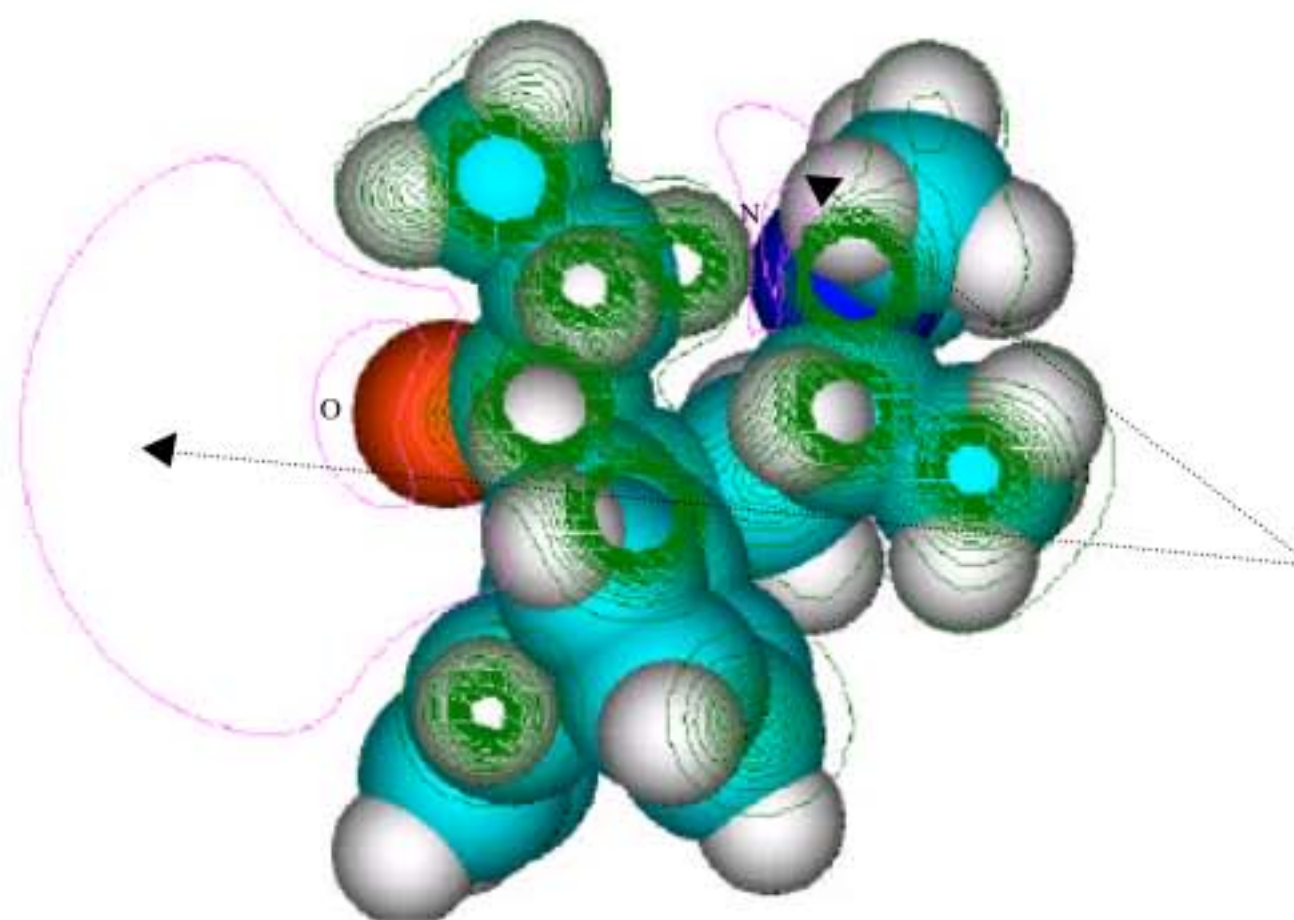
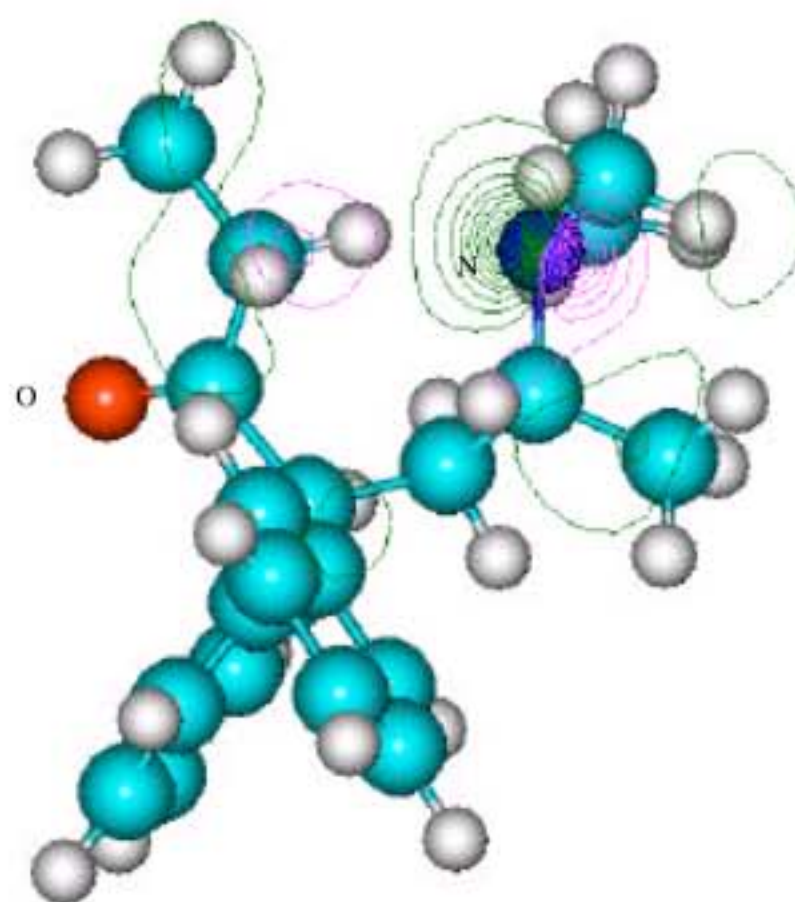


Fig. 2: Structure of (R)-Met giving (a) 2D contours of total electrostatic potential and (b) 2D plot of HOMO

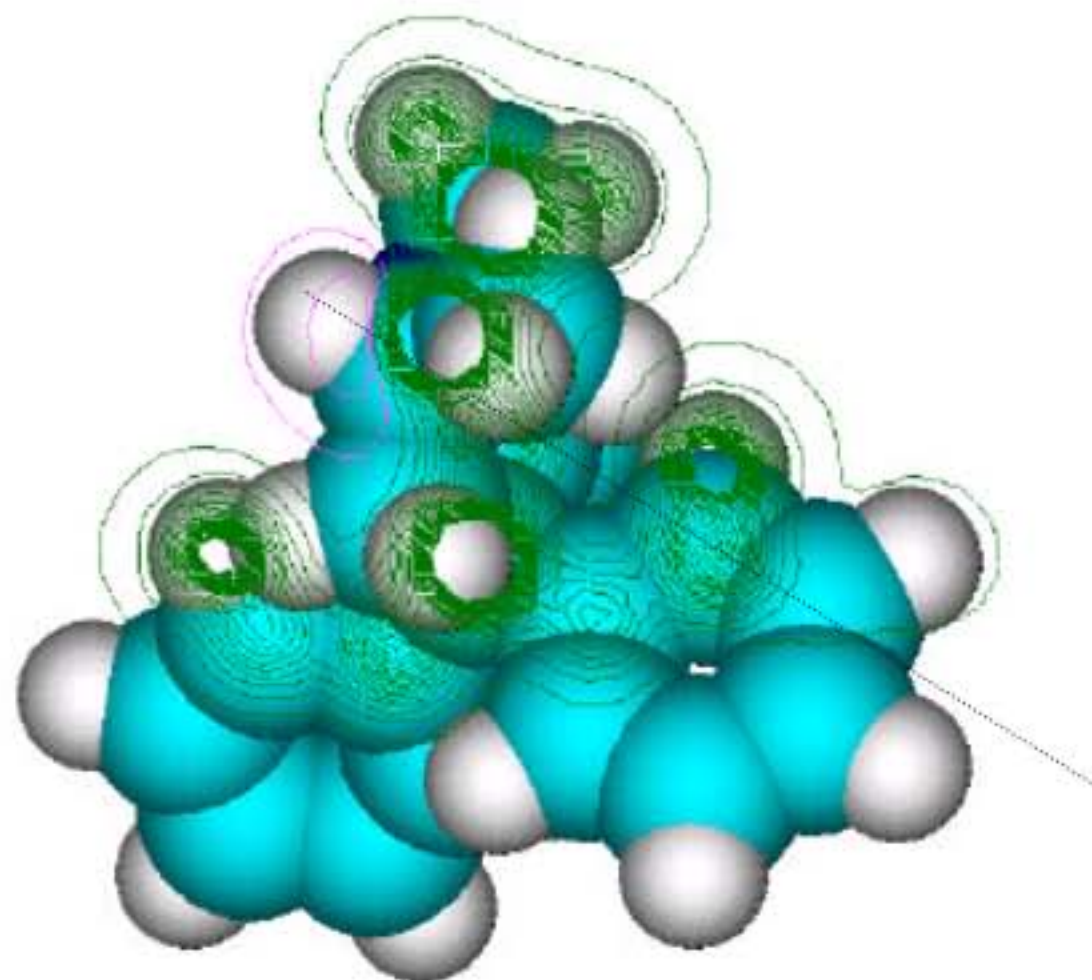


(a)

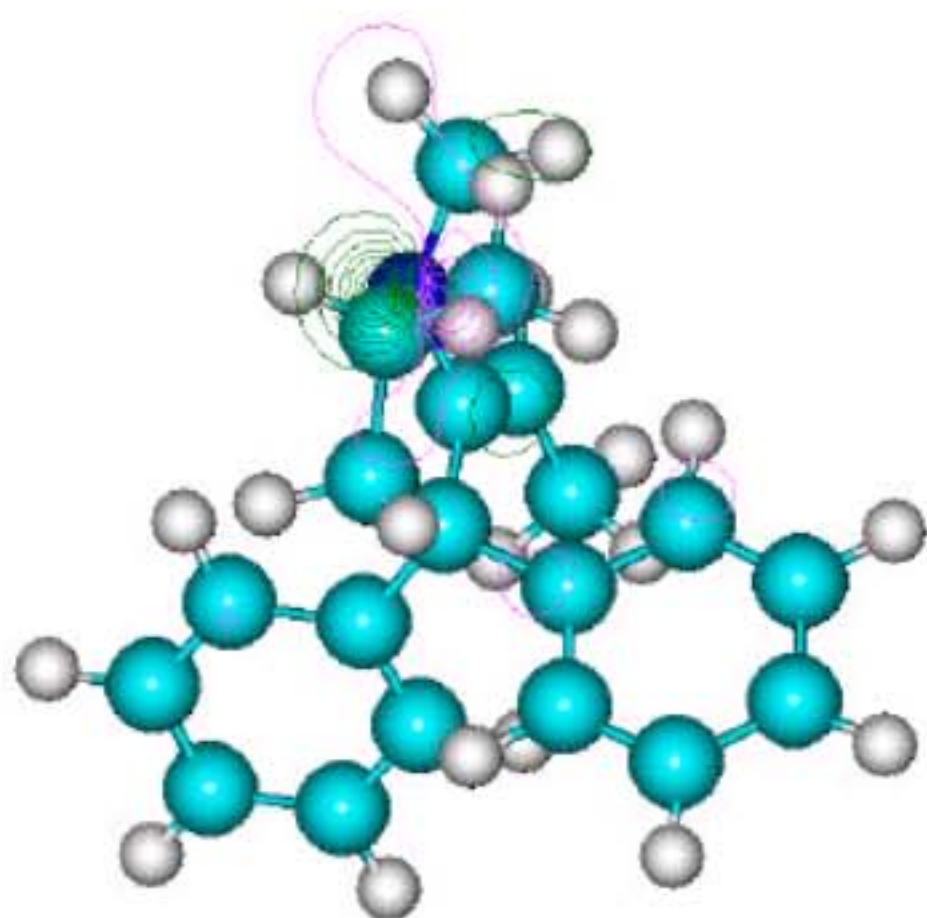


(b)

Fig. 3: Structure of (S)-Met giving (a) 2D contours of total electrostatic potential and (b) 2D plot of HOMO



(a)



(b)

Fig. 4: Structure of (R)-EDDP giving (a) 2D contours of total electrostatic potential and (b) 2D plot of HOMO

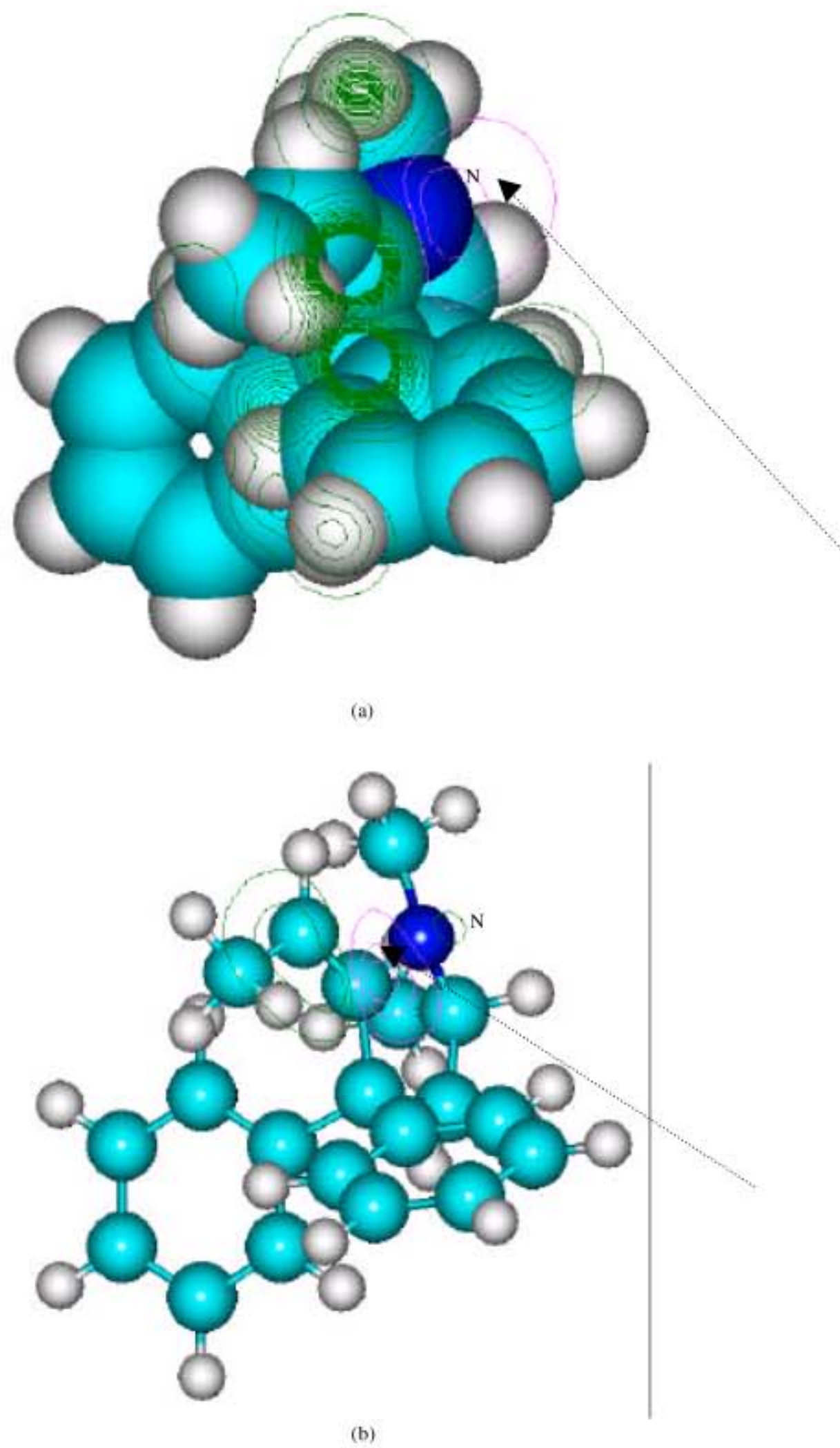


Fig. 5: Structure of (S)-EDDP giving (a) 2D contours of total electrostatic potential and (b) 2D plot of HOMO

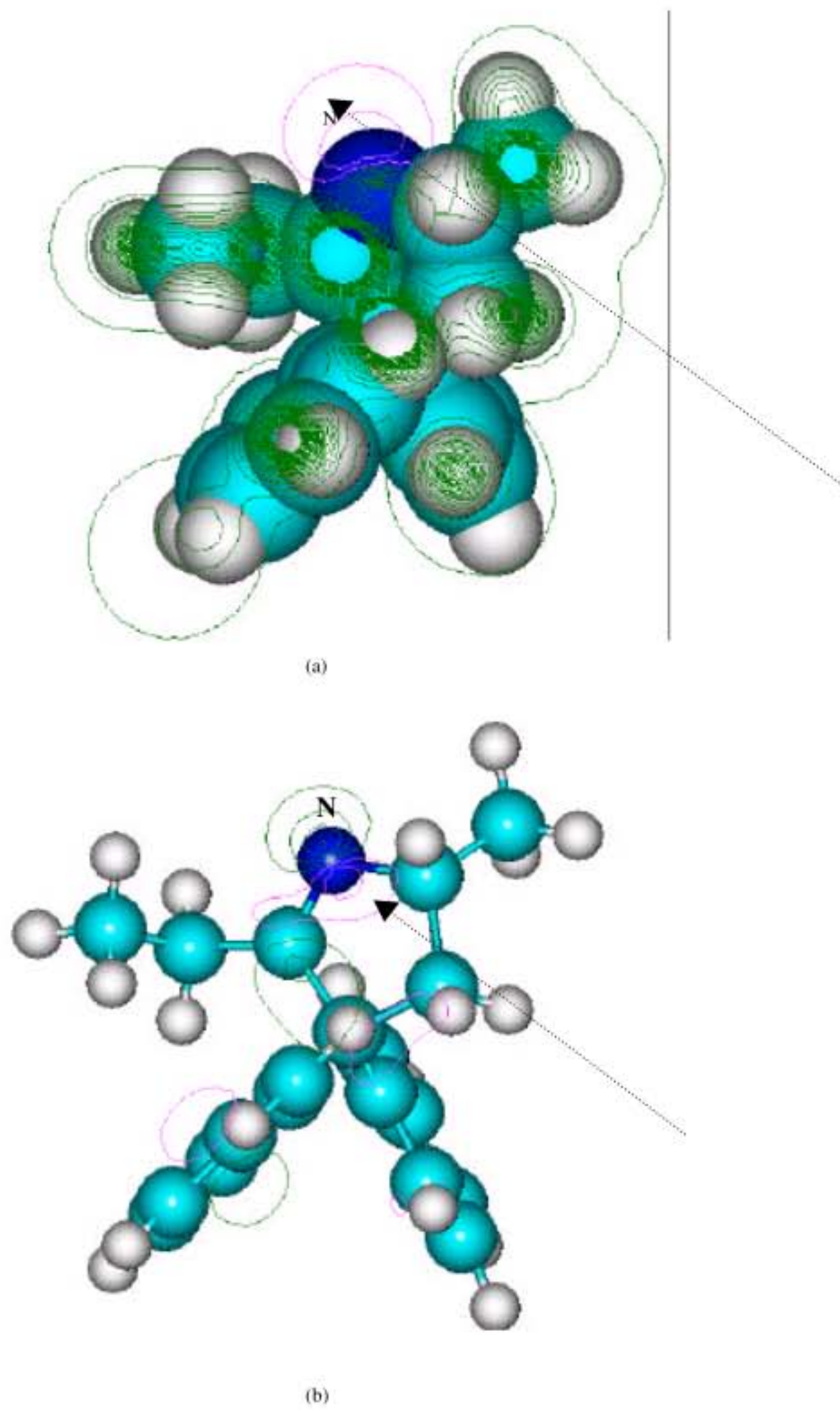
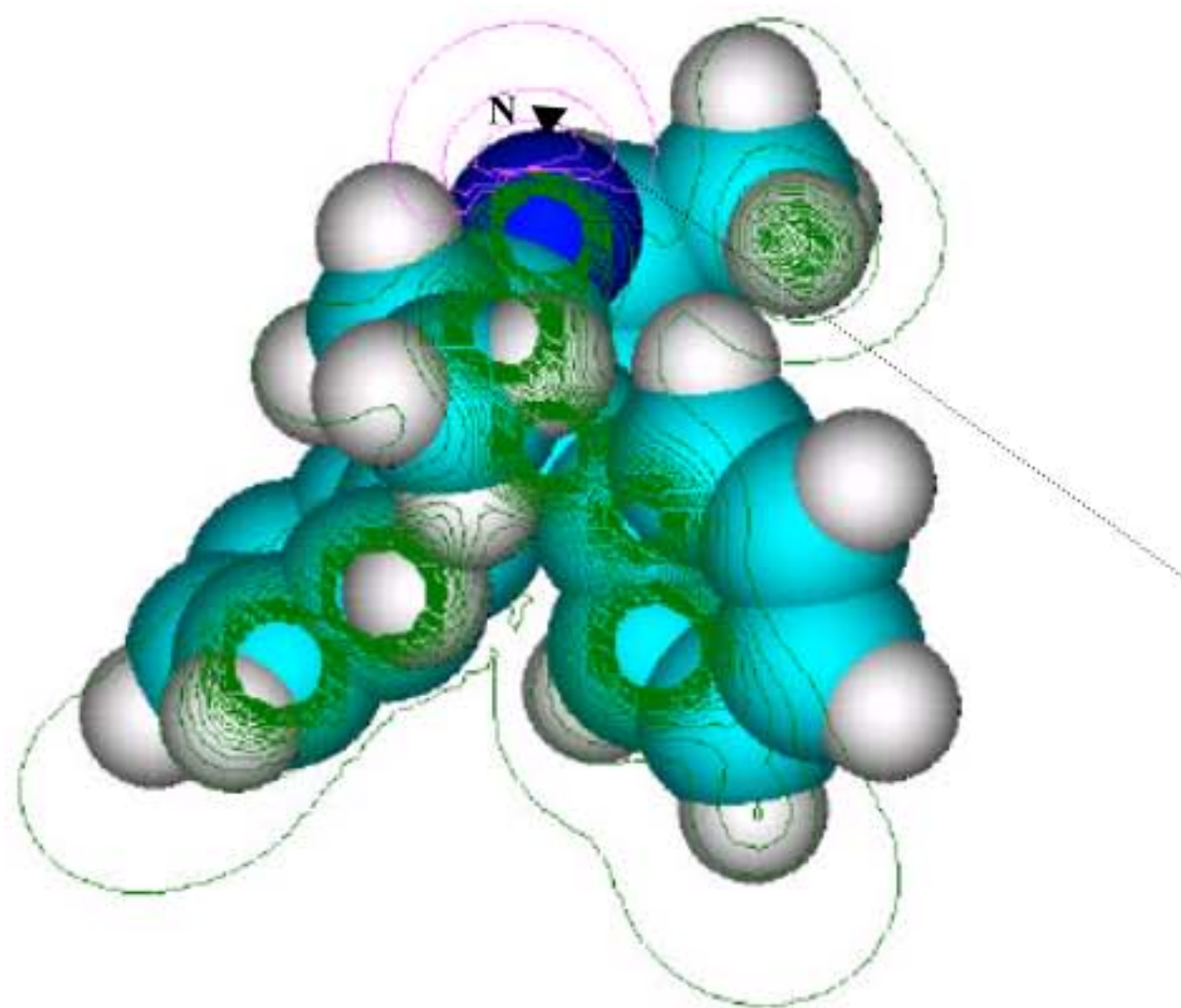
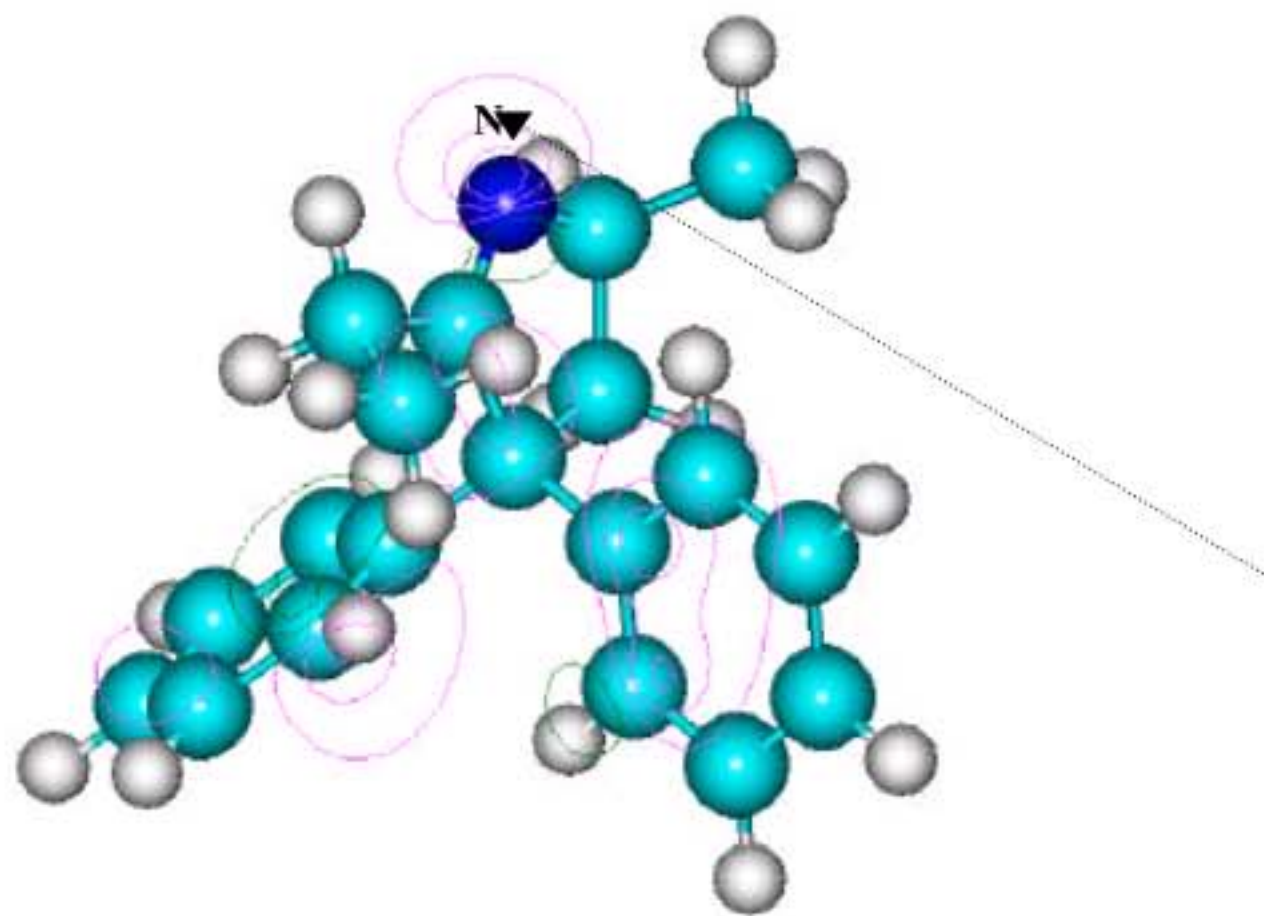


Fig. 6: Structure of (R)-EMDP giving (a) 2D contours of total electrostatic potential and (b) 2D plot of HOMO



(a)



(b)

Fig. 7: Structure of (S)-EMDP giving (a) 2D contours of total electrostatic potential and (b) 2D plot of HOMO

Table 1: Calculated thermodynamic and other parameters for methadone and its metabolites ('DM' stands for dipole moment)

Molecule	Calculation type	Total energy (kcal mol ⁻¹ / atomic unit*)	ΔH_f (kcal mol ⁻¹)	Enthalpy (kcal mol ⁻¹)	Entropy (cal mol ⁻¹ K ⁻¹)	Solvation energy (kcal mol ⁻¹)	Free energy (kcal mol ⁻¹)	DM (debye)	HOMO (eV)	LUMO (eV)	HOMO-LUMO (eV)
(R)-Methadone	PM3	7.98	12.80	265.35	143.90	-1.12	222.44	1.61	-8.91	0.04	8.95
	DFT	-907.13		267.94	145.31	-1.03	224.64	1.99	-5.34	-0.59	4.75
(S)-Methadone	PM3	-3.32	5.98	283.82	150.48	-2.66	238.96	2.83	-9.04	-0.07	8.97
	DFT	-946.44		285.98	153.34	-2.42	240.28	2.45	-5.78	-0.38	5.40
(R)-EDDP	PM3	51.69	52.14	248.66	138.14	-0.45	207.47	1.16	-8.43	0.21	8.64
	DFT	-830.71		250.34	139.88	-0.43	208.66	1.09	-4.83	-0.11	4.72
(S)-EDDP	PM3	51.33	53.46	248.35	139.39	-2.12	206.80	1.22	-8.34	0.17	8.51
	DFT	-830.71		250.36	140.24	-2.22	208.57	1.38	-4.65	-0.10	4.55
(R)-EMDP	PM3	47.00	51.23	230.62	135.01	-4.22	190.37	1.98	-9.51	0.16	9.67
	DFT	-791.42		232.56	136.03	-4.21	192.02	1.69	-6.18	-0.13	6.05
(S)-EMDP	PM3	46.72	51.16	230.60	134.69	-4.44	190.44	1.97	-9.52	0.16	9.68
	DFT	-791.41		233.45	136.23	-4.22	192.85	1.74	-6.28	-0.27	6.01

* in atomic unit from DFT calculations

Met and its metabolites have low solvation energy values indicating that they would have a higher solubility in lipid than in water. As expected, the two enantiomers of EMDP (the doubly N-demethylated metabolite of methadone) have the largest solvation energy values resulting from the loss of both the methyl groups.

It can be seen that the two enantiomers of methadone differ in their HOMO-LUMO energy difference values. (R)-Met has a smaller LUMO-HOMO energy difference than (S)-Met (4.75 eV for the former as against 5.40 eV for the latter as per DFT calculations), indicating that the (R)-Met would be more kinetically labile than (S)-Met. It was noted earlier that the two enantiomers of methadone differ in receptor affinity, metabolism and binding with protein. It was also noted that (R)-Met has a higher affinity (50 times greater) than (S)-Met for the μ -opioid receptor (Judson *et al.*, 1976). The difference in HOMO-LUMO energy values may partly explain the difference in metabolism and receptor binding affinity of the two enantiomers. It was also noted earlier that three cytochrome P450 enzymes CYP3A4, CYP2B6 and CYP2C19 are involved in the metabolism of methadone in human liver and intestine. Whereas CYP3A4 is a low-affinity, high-capacity enzyme, CYP2B6 and CYP2C19 have a higher affinity towards methadone. CYP2B6 metabolizes (S)-Met more rapidly than (R)-Met while CYP2C19 does the reverse. This difference in activity of the enzymes as applied to the two enantiomers is more likely to be related to steric factors rather than the size of the LUMO-HOMO energy difference.

The metabolite (S)-EDDP has a slightly lower HOMO-LUMO energy difference than (R)-EDDP whereas for the values for (R)-EMDP and (S)-EMDP are found to be similar.

In the case of both (R)-Met and (S)-Met, the positions of most negative electrostatic potentials are found to be close to the carbonyl oxygen and the tertiary nitrogen and the HOMOs with the high electron densities are centered on the tertiary nitrogen and a methyl group bonded to the nitrogen. The high negative electric potential around carbonyl oxygen and the tertiary nitrogen atom suggest that the positions would be most susceptible to electrophilic attack.

In the case of both (R)-EDDP and (S)-EDDP, the positions of most negative electrostatic potential as well as the HOMOs with the high electron density are found to be close to the tertiary nitrogen. The high negative electric potential around the tertiary nitrogen suggest that the position would be most susceptible to electrophilic attack.

In the case of both (R)-EMDP and (S)-EMDP, the positions of most negative electrostatic potential are found to be close to the tertiary nitrogen and the HOMOs with the high electron density lie close to the tertiary nitrogen and some aromatic ring carbons. The high negative electric potential around the tertiary nitrogen suggest that the position would be most susceptible to electrophilic attack.

The calculated heat of formation from PM3 calculations of (R)-Met is 12.80 kcal mol⁻¹ as against 5.98 kcal mol⁻¹ for (S)-Met. The values suggest that (S)-Met has a slightly higher thermodynamic

stability than (R)-Met. In contrast, the heat of formation values of (R)-EDDP and (S)-EDDP are found to be more similar (52.14 kcal mol⁻¹ for the former and 53.46 kcal mol⁻¹ for the latter) with (R)-EDDP being marginally more stable than (S)-EDDP. The heat of formation values for (R)-EMDP and (S)-EMDP are found to be almost the same (51.23 kcal mol⁻¹ for the former and 51.16 kcal mol⁻¹ for the latter). The large increase in heat of formation in going from Met to EDDP indicates the conversion of Met to EDDP may not be spontaneous. Low kinetic lability, relatively high thermodynamic stability and high lipid solubility indicate that both Met would have a low clearance rate.

Conclusions

Molecular modelling analyses show that the Met and its metabolites have low solvation energy values, indicating their lipid solubility. The two enantiomers of methadone are found to differ in their thermodynamic stability, HOMO-LUMO energy separation. The differences in thermodynamic stability and kinetic lability may partly explain why the two enantiomers have different metabolic rates and receptor binding capacity.

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