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Molecular Modelling Analysis of the Metabolism of Diazepam

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ABSTRACT: Diazepam (DZ) is used as a tranquilizer, anticonvulsant, muscle relaxant and psychostimulant. However, it has a number of side effects including abdominal cramps, dry mouth, increased heart beat, slurred speech, convulsions, hallucinations, memory loss, breathing problems, ataxia, headache, blurred vision, diplopia, confusion, venous thrombosis and phlebitis. It may also potentiate the effects of other drugs that cause drowsiness. DZ is metabolized by hepatic cytochrome P450 enzymes CYP3AP and CYP2C19 by N-demethylation and hydroxylation at C3 to produce three major pharmacologically active metabolites : *N*-desmethyldiazepam (NDZ), temazepam (TMZ), and oxazepam (OXZ) that are excreted in the urine. In addition, two minor metabolites may be produced by hydroxylation at the para-position of the not-fused aromatic ring. Molecular modelling analyses show that DZ and its metabolites have similar kinetic lability but differ in their thermodynamic stability and solubility in water. Based on the results of the analyses neither DZ nor any of its metabolites can be eliminated from being the cause of toxicity and side effects due to the drug. The results of the analyses also indicate that carbonyl oxygen atom and the two nitrogen centres are more likely to be subject to electrophilic attack. Also, commonness in surface locations of the negative regions may mean that DZ and its pharmacologically active metabolites interact with the positive centres of the receptor(s) via these positions leading to the formation of strong hydrogen bonds and may induce similar polarisation effects in receptor(s).

Key words: Diazepam, psychotic disorders, epilepsy, molecular modelling

Introduction

Diazepam (DZ; also known as valium, seduxen, apozepam and diapam) belongs to a class of drugs known as benzodiazepines that are frequently prescribed for treating a wide range of medical and psychotic disorders [1] including relief of anxiety, as hypnotic and sleep promoting agents, and as centrally muscle relaxants and anticonvulsive agents [2]. Chemically DZ is [7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one]. DZ is used as a tranquilizer, anticonvulsant, muscle relaxant and psychostimulant [3]. The use of DZ in epilepsy is indicated when transient high serum and brain concentrations of the drug are needed to control uninterrupted and long-duration tonic-clonic status. Physical dependence can occur with chronic administration of DZ, more commonly with high doses [3] so that abrupt discontinuation of the drug may precipitate seizures.

Anxiety is a complex combination of subjective feelings and characteristic behaviours. The subjective feelings consist of tension, apprehension, fear, worry and difficulty with thinking and concentration. These feelings are usually accompanied by behavioural signs and symptoms of trembling, tremors, muscle tension, restlessness and fatigue with autonomic hyperactivity in the respiratory, cardiovascular, urinary and gastrointestinal systems [2]. The identified anxiety disorders include generalized anxiety disorder, panic disorder, agoraphobia, social phobia, post-traumatic stress syndrome, and obsessive-compulsive disorder. Anxiety is also associated with many organic diseases such as hypoglycaemia, anaemia, vitamin B_{12} deficiency, hyperthyroidism, and coronary heart disease. Highest incidence of seizure disorders occurs in children, particularly in the first and second year of life [4]. Some of these cease with age. However, a small percentage of

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individuals continue to have seizures throughout life either due to a genetic predisposition or due to permanent damage to the developing brain resulting from early-life seizures [4].

DZ can be administered orally, as an intravenous or intramuscular injection or as a suppository. The absorption is rapid when it is given orally and much slower and erratic when it is given as an intramuscular injection. DZ is highly lipid soluble and is believed to cross the blood-brain barrier [3]. The peak blood level of DZ occurs in about 1-2 hours and the blood levels may remain elevated for 6 or more hours. The pharmokinetic half-life of DZ ranges from 20 to 100 hours.

The side effects of DZ include abdominal cramps, blurred vision, dry mouth, increased heart beat, shaking, slurred speech, convulsions, hallucinations, memory loss, problems with breathing, ataxia, headache, blurred vision, diplopia, confusion, venous thrombosis and phlebitis [3]. DZ may also potentiate the effects of other drugs that cause drowsiness, including antidepressants, alcohol, antihistamines, sedatives (used to treat insomnia), pain relievers, anti-anxiety medications, anti-seizure medications and muscle relaxants [1]. DZ has been shown to interfere with chromosome segregation in a number of cell types and cell lines when present in culture medium [5]. The use of DZ has also been identified as a risk factor for transient urinary incontinence [6]. Cardiorespiratory toxicity may occur if DZ is administered to patients who have been taking high doses of other depressant drugs or antiepileptic medications.

DZ is commonly used as a premedicant in endoscopic procedures [7,8]. It has been found that there is a wide variation in the residual cognitive effects of the DZ in subjects given 5 mg DZ before gastrointestinal endoscopy [9] which is believed to be due to variations in pharmacodynamic factors rather than pharmacokinetic factors.

DZ is metabolized by hepatic cytochrome P450 enzymes CYP3AP and CYP2C19 by N-demethylation and hydroxylation at C3 to produce three major pharmacologically active metabolites: *N*-desmethyldiazepam (NDZ), temazepam (TMZ), and oxazepam (OXZ) [9-11] that are excreted in the urine. Two other metabolites namely *p*-hydroxydiazepam (PHDZ) and *p*-hydroxy-*N*-desmethyldiazepam (PHNDZ) have also been identified [12]. In humans, the metabolism of DZ is mediated mainly by cytochrome P450 enzymes CYP2C19 and CYP3A4 [9,14]. The established pathways for DZ metabolism are shown in Figure 1. Terminal metabolites TMZ, OXZ, PHDZ and PHNDZ are conjugated with glucuronic acid before being excreted via the kidneys into the urine.

The rate of elimination of DZ and the major metabolic pathways in DZ metabolism have been found to differ in different rat strains due to polymorphic expression of the enzymes involved in DZ metabolism [12].

It has been found that DZ impaired platelet function which may be due the inhibition of the cyclooxygenase pathway of the arachidonic acid metabolism in platelet [15]. No increase in the production of reactive oxygen species (ROS) from the metabolism of DZ has been observed suggesting that ROS may not be involved in the toxicity of DZ [16].

In this study, molecular modelling analyses have been carried out using the program Spartan '02 [17] to investigate the relative stability of DZ and its metabolites with the aim of providing a better understanding on the relative toxicity due to DZ and its metabolites. Although a number of theoretical studies have been carried out on different aspect s of drugs including structure-activity relationships of drugs [18], hydrogen-bonding [19] and virtual screening [20], no systematic study has been carried out on the thermodynamic stability, kinetic lability and solubility of DZ and all its metabolites.

Computation methods

The geometries of DZ and its metabolites DZ, NDZ, TMZ, OXZ, PHDZ and PHNDZ 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 0.01 was set as the terminating condition. For the optimised structures, single point calculations were carried to give heat of formation, enthalpy, entropy, free energy, dipole moment, 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 DZ and its metabolites NDZ,

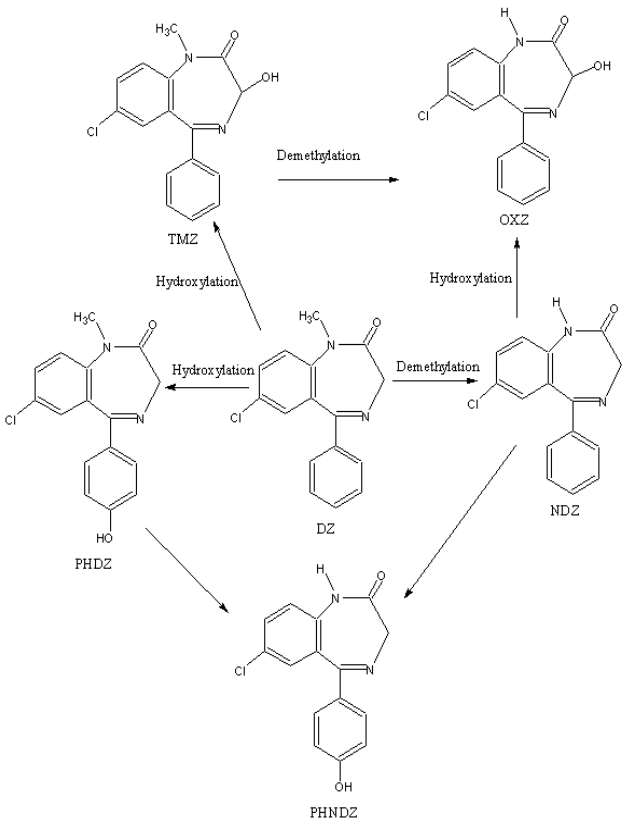


Figure 1: Proposed metabolic pathways for diazepam and its metabolites [Based on reference 10]

TMZ, OXZ, PHDZ and PHNDZ. Figures 2, 3, 4, 5, 6 and 7 give the optimised structures of DZ and its metabolites NDZ, TMZ, OXZ, PHDZ and PHNDZ as per DFT calculations. The structures also give regions of negative electrostatic potential (greyish-white envelopes) and HOMOs (blue and red where red indicates HOMOs with high electron density).

The calculated solvation energies of DZ and its metabolites NDZ, TMZ, OXZ, PHDZ and PHNDZ from PM3 calculations in kcal mol^{-1} are respectively -7.03, -10.24, -8.79, -12.14, -11.41 and -14.32 and their dipole moments from DFT calculations are 2.89, 2.87, 4.36, 4.15, 3.41 and 3.57 respectively. The values indicate that DZ would be least soluble in water than any of its metabolites. It was noted earlier that DZ is actually lipophilic. As expected, OXZ, PHDZ and PHNDZ, each of which has an OH group either at C3 or the *para*-position of the non-fused aromatic ring, have larger solvation energies and therefore greater solubility in water.

The calculated heat of formation of TMZ is -13.19 k cal mol^{-1} as compared to that for DZ of 29.30 k cal mol^{-1} suggesting that the reaction: DZ $\rightarrow$ TMZ would be spontaneous thermodynamically. Likewise, the conversion of NDZ to OXZ is also expected to be spontaneous. DZ and its five metabolites have similar but rather large LUMO-HOMO energy differences (about 4.7-4.8 eV from DFT calculations) indicating DZ and all its metabolites have low kinetic lability.

In the case of DZ and all its metabolites NDZ, TMZ, OXZ, PHDZ and PHNDZ the electrostatic potential is found to be more negative around the carbonyl oxygen atom and a nitrogen atom of the seven-membered heterocyclic ring, indicating that the positions may be subject to electrophilic attack.

Figure 8 gives surface electric charge distribution of DZ and its pharmacologically active metabolites NDZ,

Table 1
Calculated thermodynamic and other parameters of DZ and its metabolites

Molecule	*Calculation type*	*Total energy (kcal mol^{-1})*	*Heat of formation (kcal mol^{-1})*	*Enthapy (kcal $mol^{-1}K^{-1}$)*	*Entropy (cal mol^{-1} K^{-1})*	*Solvation energy (kcal mol^{-1})*	*Free energy (kcal mol^{-1})*	*Dipole moment (debye)*	*HOMO (eV)*	*LUMO (eV)*	*LUMO HOMO (eV)*
DZ	PM3	22.27	29.30	169.44	131.72	-7.03	130.17	3.41	-9.20	-0.73	8.47
	DFT	-1262.46		170.23	130.36	-8.23	131.38	2.89	-6.32	-1.65	4.67
NDZ	PM3	18.48	28.72	151.60	125.39	-10.24	114.21	3.44	-9.22	-0.76	8.46
	DFT	-1223.15		152.45	124.67	-9.12	115.30	2.87	-6.44	-1.70	4.74
TMZ	PM3	-21.97	-13.19	173.25	136.82	-8.79	132.46	4.08	-9.27	-0.84	8.43
	DFT	-1337.68		174.57	136.00	-8.88	134.04	4.36	-6.54	-1.75	4.79
OXZ	PM3	-26.00	-13.86	155.36	130.69	-12.14	116.39	4.00	-9.30	-0.85	8.45
	DFT	-1298.37		156.45	129.23	-10.12	117.94	4.15	-6.65	-1.80	4.85
PHDZ	PM3	-27.45	-16.04	173.66	136.79	-11.41	132.87	3.05	-9.17	-0.73	8.44
	DFT	-1337.68		172.84	136.78	-11.13	132.08	3.41	-6.26	-1.51	4.75
PHNDZ	PM3	-31.66	-16.50	155.81	130.66	-14.32	116.85	3.40	-9.20	-0.77	8.43
	DFT	-1298.37		154.76	130.02	-15.45	116.14	3.57	-6.33	-1.53	4.80

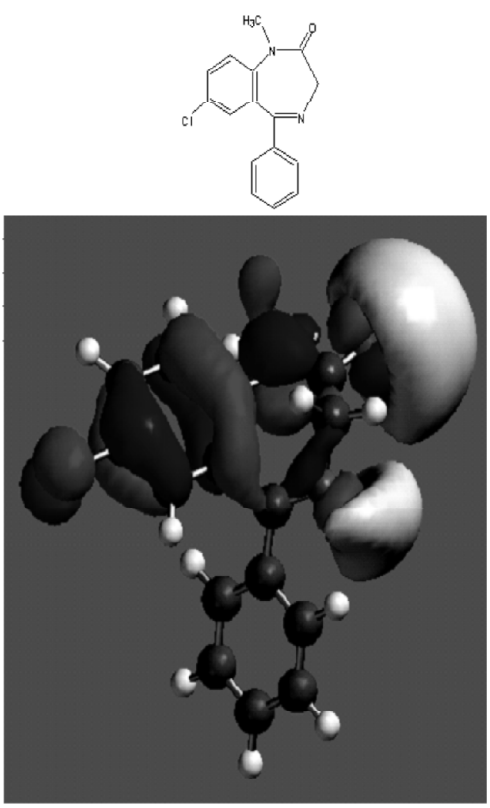


Figure 2: Structure of DZ giving the electrostatic potential and the HOMOs

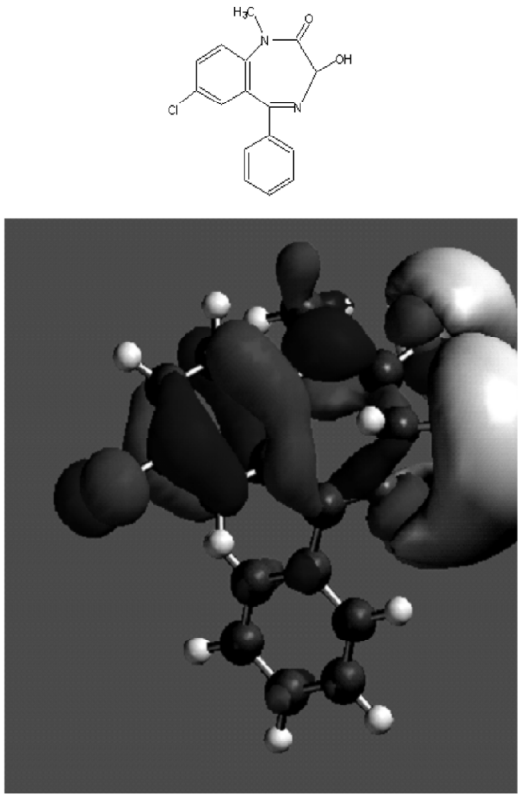


Figure 4: Structure of TMZ giving the electrostatic potential and the HOMOs

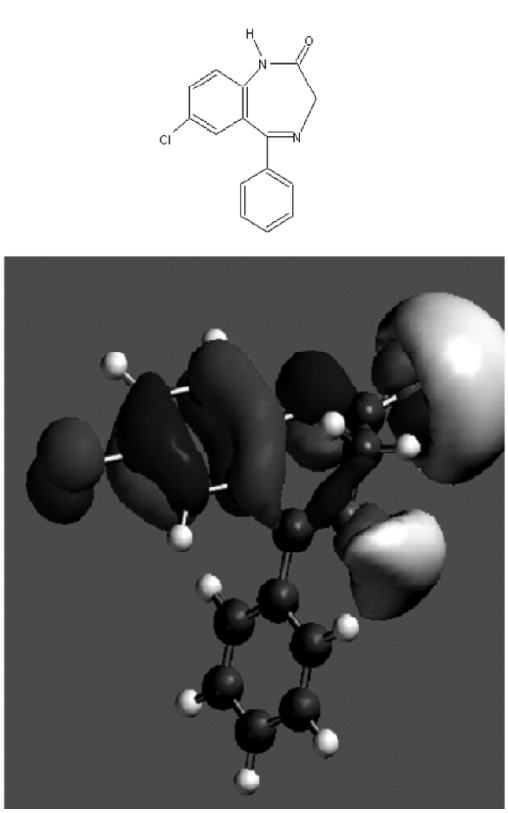


Figure 3: Structure of NDZ giving the electrostatic potential and the HOMOs

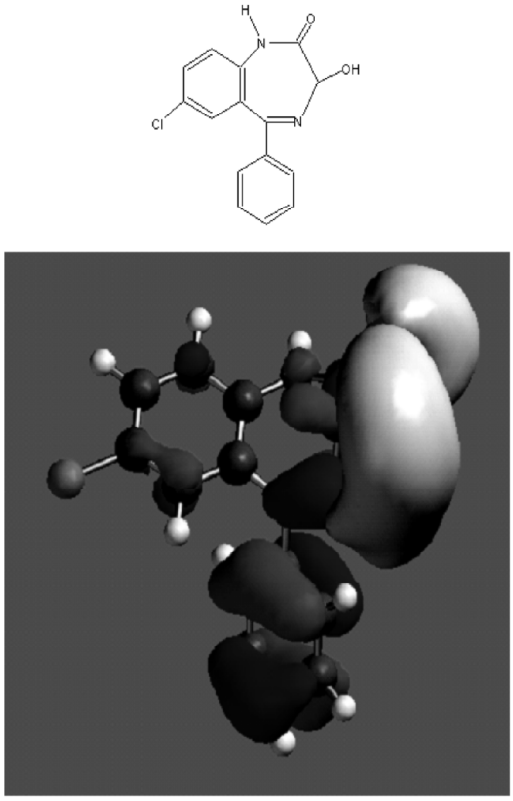


Figure 5: Structure of OXZ giving the electrostatic potential and the HOMOs

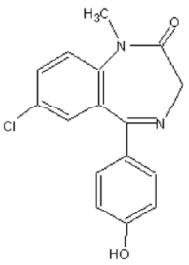
TMZ and OXZ where red indicates negative, blue indicates positive and green indicates neutral. It can be seen that there is a great deal of similarity in the locations of negative regions on the molecular surfaces of the four compounds.

The commonness in the positions of negative regions on the surface may mean that DZ and its pharmacologically active metabolites may interact with positive centres of the receptor(s) via these positions leading to the formation of strong hydrogen bonds. In an earlier study it was found that for a number of anticonvulsant drugs there was a striking similarity in their local polarity [21] that may be a strong determinant of their activity.

It has been shown that in a number of cases the affinity of a particular molecule for a specific receptor depends upon the degree to which the electrostatic potential of the former possesses certain characteristics that are deemed to be necessary for effective interaction with the receptor [22,23]. Besides the formation of hydrogen bonds, similarly localised negative surface charges may induce similar polarisation effects in receptor(s). It has been suggested among electronic properties that must be considered to understand how chemicals affect living systems or their parts are polarizability [24].

In the case of DZ and NDZ, the HUMOs with large electron density are found to be centred on most of the carbon atoms of the aromatic ring that is fused with the seven-membered heterocyclic ring, the two nitrogen atoms and the carbonyl oxygen belonging to the seven-membered heterocyclic ring and the chlorine atom. In the case of TMZ, the HUMOs with large electron density are found to be centred on most carbon atoms of the aromatic ring that is fused with seven-membered heterocyclic ring, the two nitrogen atoms and carbonyl oxygen of the seven-membered heterocyclic ring, two carbon atoms of the non-fused aromatic ring and the chlorine atom. In the case of OXZ, the HUMOs with large electron density are found to be centred on the two nitrogen atoms, carbonyl and hydroxyl oxygen atoms bonded to two carbon atoms of the seven-membered heterocyclic ring, two carbon atoms of the fused aromatic ring and most carbon atoms of the non-fused aromatic ring.

In the case of PHNDZ, the HUMOs with large electron density are found to be centred on a number of atoms including a nitrogen atom of the seven-membered heterocyclic ring, the chlorine atom and the most of the carbon atoms of the non-fused aromatic ring.



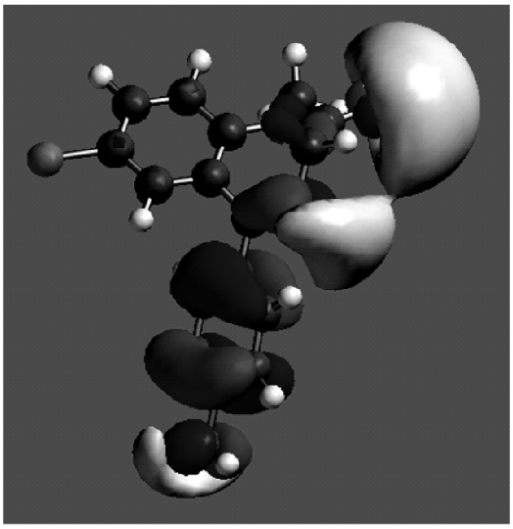
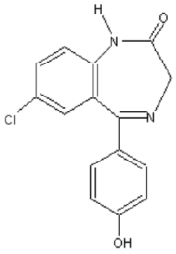


Figure 6: Structure of PHDZ giving the electrostatic potential and the HOMOs



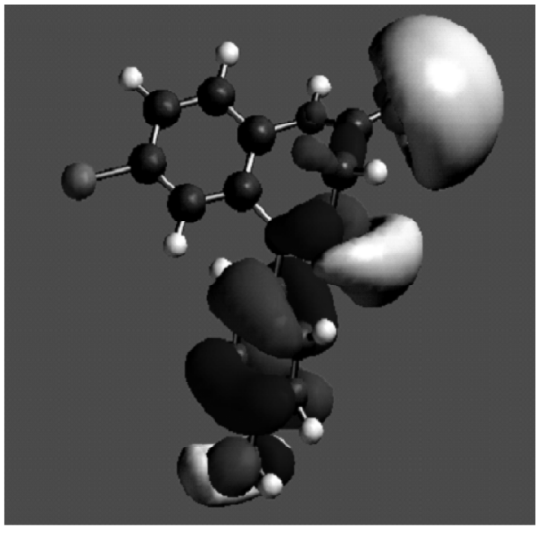


Figure 7: Structure of PHNDZ giving the electrostatic potential and the HOMOs

When the regions of negative electrostatic potential are compared with the positions of HUMOs with high electron density it can be seen that there is a convergence of the two on the carbonyl oxygen and at least one of the two nitrogen atoms, in the case of DZ and all its metabolites, further providing support to the idea that the positions would be subject to electrophilic attack.

It was noted earlier that reactive oxygen species are not produced in the metabolism of DZ. Relatively low lability of DZ and its metabolites and the absence of any free radical among the metabolites may explain why reactive oxygen species are not produced in the metabolism of DZ.

Conclusion

Molecular modelling analyses show that DZ and its metabolites have similar kinetic lability but differ in their thermodynamic stability and solubility in water so that they may have different clearance rates. The analyses also suggest that the carbonyl oxygen atom and the nitrogen centres are likely to be subject to electrophilic attack. Also, commonness in surface locations of the negative regions may mean that DZ and its pharmacologically active metabolites interact with the positive centres of the receptor(s) via these positions leading to the formation of strong hydrogen bonds and may induce similar polarisation effects in receptor(s). The results of the analyses however do not exclude DZ and any of its metabolites from being the cause of toxicity due to the drug.

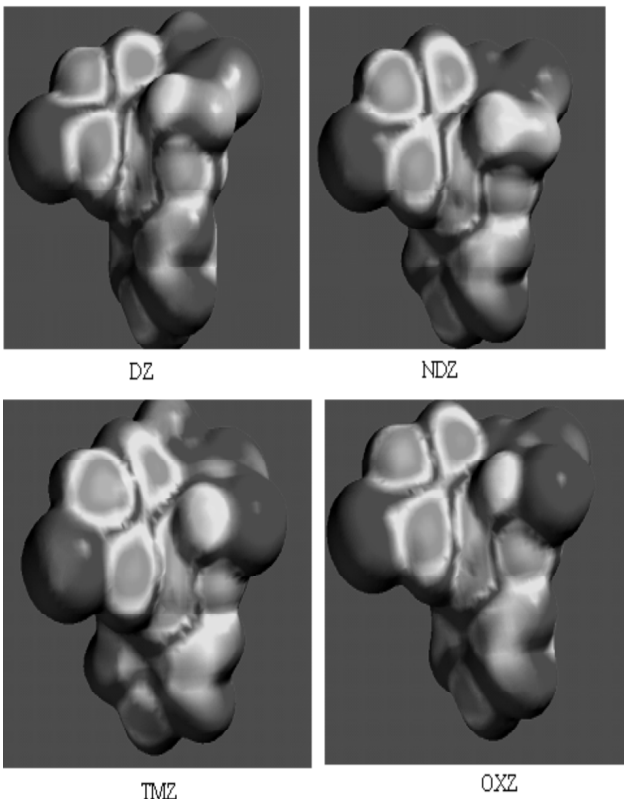


Figure 8: Surface electric charges of DZ and its pharmacologically active metabolites NDZ, TMZ and OXZ and OXZ where red indicates negative, blue indicates positive and green indicates neutral.

Abbreviations

DZ: Diazepam

NDZ: *N*-desmethyldiazepam

TMZ: Temazepam

OXZ: Oxazepam

PHDZ: *p*-Hydroxydiazepam

PHNDZ: *p*-Hydroxy-*N*-desmethyldiazepam

ROS: Reactive oxygen species

LUMO: Lowest energy unoccupied molecular orbital

HOMO: Highest energy occupied molecular orbital

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