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Der Chemica Sinica, 2011, 2 (4):72-80

Pelagia Research Library

ISSN: 0976-8505
CODEN (USA) CSHIA5

Modelling of more Effective Drug than Thiotepa - an anti-cancer drug, using MESP

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ABSTRACT

Triethylenethiophosphoramide (TTP) has been a very effective anti-cancer drug. Even though its mechanism has not yet been established, it is believed to take place through the breaking up of DNAs'. Thus far, only in-vitro work has been done on this. Since every molecule has its own electric potential, it is found responsible for interaction with other molecules for its activity in many cases. A detailed study is made on the Molecular Electrostatic Potential (MESP) front of thiotepa and a few of its derivatives. To account for the property, chemical reactivity descriptors such as chemical potential, electronegativity, global hardness and softness based on finite and Koopmans' method, local softness, electrophilicity index and local philicity index have been evaluated. The philicity index provided information on the toxicity of the molecule. This is important because the thiotepa molecule may not only inhibit cancerous cell by opening up DNA molecule but also carry out the same thing for other vital cells and organs of the body. About 16 molecules are chosen for this work and some have proved to be more effective than thiotepa.

Keywords: Thiotepa, MESP, DFT and MP2, Reactivity Descriptors.

INTRODUCTION

Molecular recognition and interaction includes attraction between unlike charges, attraction between the dipoles, attraction between cations and π electron clouds of aromatic residues, charge transfer between electron-rich and electron-poor molecules, and the London dispersion attraction between any two electron clouds. Many of the forces that drive molecular recognition are short-range; strong interactions are achieved only if the molecular surfaces of interacting moieties can be close to each other. Few of the forces, such as Coulombic attraction between unlike charges are rather long range, but in the aqueous solution water between interacting charges strongly attenuates the interaction. In summary, tight binding is achieved when the shape and charge distribution of the receptor cavity is optimally matched by the shape and charge distribution of the ligand molecule.

In order to rationally design molecules with good shape complementarity, the question of what determines a shape of the molecular surface must be considered. The shape of a molecule is determined by the electron density of the molecule. In this sense Electrostatic potential surfaces [1] are valuable in computer-aided drug design as they help in optimization of electrostatic interactions between the protein and the ligand. These surfaces can be used to compare different inhibitors with substrates or transition states of the reaction. Electrostatic potential surfaces can be either displayed as isocontour surfaces or mapped onto the molecular electron density. The latter are more widely used because they retain the sense of underlying chemical structure better than isocontour plots. The electrostatic potential at a point is the force acting on a unit positive charge placed as that point. While the electrons give rise to a negative potential, the nucleic are responsible for positive force. The ESP can be determined from using the following equation [2]:

$$V(r) = \sum_A \frac{Z_A}{|r - r_A|} - \int \frac{\rho(r')}{|r - r'|} d^3 r'$$

Z_A is the charge on nucleus A, located at r_A, and ρ(r) is the electronic density. V(r) is a physical observable, which can be determined experimentally, by diffraction methods [3] as well as computationally. Regions of negative V(r) are usually associated with the lone pairs of electronegative atoms, the π electrons of unsaturated hydrocarbons, and strained C–C bonds [4]. It has been well established [5] that since the receptor recognizes the stereo electronic effects and not the atoms, studies of 2D and 3D MESP and its gradient plots have become essential for characterizing pharmacologically active molecules from an electronic point of view. 3D MESP plots have been used to examine a given property within a chemical series and propose a compound with improved features or to investigate the interpretative abilities of some MESP-related parameters for determining certain aspect of the intermolecular interactions involved. A series of nucleoside – hydrolase inhibitors were employed to study the association between the receptor and the macromolecule and correlated to a key and lock model [6]. If the steric, hydrophobic and electrostatic interaction were to be considered for a molecular 'fit', the electrostatic effects were found to predominate [7]. From another study the presence of aromatic ring was found to have an important role in charge distribution and reactivity towards electrophilic reagents [8], where a 'Stacking' type interaction with a ring at the receptor site was found. In the last decade several studies have been centered on probing the features of several drug molecules with their respective receptor sites [9].

The technique of using only segments of the drug molecules for a family of compounds in establishing a quantitative relationship was provided by Politzer *et al.* [10]. They used three groups of drugs, two acting on reverse transcriptase and one on HIV Protease. Segmental analysis was found beneficial and provided insight into the nature of the process involved in interactions. The molecules chosen for the study were *N-hydroxy-N'-amino guanidine*, *carboxanilide*, *cyanoguanidine* and their derivatives. The effect of different segments on the substrate molecule was quantitatively established using MESP. As a result the segment that had phenyl links yielded a distinctly better correlation than the entire molecule. They considered $Vs_{,min}$ and $Vs_{,max}$ as a key feature of Vs(r) as it was correlated with empirically developed scales of hydrogen bond basicity and acidity respectively [11]. Some 'strong' conclusions [12] generalized from various studies suggested that the biological activity appeared to require negative potential above all or most of the lateral positions with optimum values of minima at 1.75 A^o above the plane [13], and the negative regions of V(r) above the lateral position of the molecule should be separated by a large central region of positive V(r). Negative region of V(r) associated with central oxygen are not necessary for high activity; on the contrary, it is important that the oxygen potentials be relatively weak and small.

Thiotepa is a cytotoxic agent of the polyfunctional alkylating type related chemically and pharmacologically to nitrogen mustard [14]. An alkylating agent reacts with DNA phosphate groups to produce cross-linking of DNA strands leading to inhibition of DNA, RNA and protein synthesis. The mechanism of action has not been explored as thoroughly but it is presumed that the aziridine rings open and react as nitrogen mustard do. The reactivity is enhanced at a lower pH [15]. Since thiotepa is stable in aqueous biological systems at physiological pH, it penetrates cells rapidly and then releases NN'N" triethylenephosphoramide to disrupt the manufacture of DNA. [16]. One of the principal bond disruptions is initiated by alkylation of guanine at the N-7 position, which severs the linkage between the purine base and the sugar and liberates alkylated guanines [17].

As the mechanism of action is uncertain, molecular modeling might help in the designing of a better drug in this class. Different scaffolds or segments of a drug may be cut or attached to a tested drug and its property calculated using computational methods. This might help locate active centre in the drug which interacts with the substrate based on key and lock model. Molecular modeling might provide 'stitching' of the required fragment or induction of the desired scaffold. The job would then be to identify a derivative which might have lesser side effects or toxicity. While proteins might be stitched with desired scaffolds to suite the ligand [18], a ligand might as well be tailored to suit an interaction. For evaluation, the subject molecule was built form a basic and untested compound. Changes were made at different centres with the incorporation of hetero atoms and at each stage the properties were evaluated.

MATERIALS AND METHODS

Methodology and Computational Details

The molecules were first optimised at the semi-empirical level followed by 6-31 G (d) level using MP2 and DZV d+ level of the Density Functional Theory available in GAMESS [19]. But for the computation of the MESP the MP2 level calculations were chosen as this was the usual method that involved SCF-RHF [20]. Even though the program produced electron density over the atoms sufficient to produce MEP, it was not considered for generating surfaces, as it does not use the common ChelpG [21] or Merz-Kollman (MK) [22] charges. The output was then visualised using Molekel [23]. The MEP was used to generate surfaces and the maximum and minimum surface potentials were evaluated as $V_{s,max}$ and $V_{s,min}$.. The poses pertaining to these pictures are those with high negative MEP values. The negative regions have been identified with red colour and the positive with blue.

Condensed Fukui function and local softness was calculated via the formulations as per standard methods, using Mulliken, Löwdin and Natural Population Analysis (NPA) [24]. NPA was chosen because it uses natural atomic and natural bond orbitals (NBO). Properties like global and local hardness (GH & LH) and softness (GS), global and local electrophilicity, and electronegativity have been computed using standard methods. The restricted HF method has been used for energy calculations and for the corresponding anionic and cationic systems the Restricted Open shell HF method was employed both at MP2 and DZV (d) level. Short names have been assigned for the molecules in the IUPAC format and are given in Table 1.

RESULT AND DISCUSSION

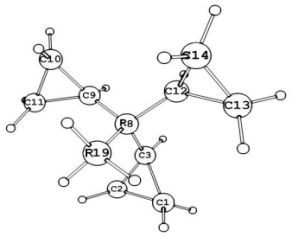


Fig. 1: General structure of Thiotepa molecule

The general skeletal structure of the thiotepa is shown in the Fig. 1. The MESP pictures are given in the Plate 1. The global softness values are tabulated in Tables 1.

As the target molecule (thiotepa) was built up gradually from hydrocarbons, the descriptive properties revealed interesting insights. The softness values increased gradually with the insertion of nitrogen atoms from 2.72 to 2.83 at the DFT level using finite method. But the Koopmans' method did not show any such trend. The reason for this is due to the consideration of HOMO-LUMO orbitals. The principle of maximum hardness is based on the band gap and in a chemical reaction hardness and band gap tends to increase and a change in the electron density should be primarily electron withdrawal from or addition to the HOMO and LUMO, the frontier orbitals of Fukui [25]. With the introduction of Phosphorus atom the softness value further increased (3.9 to 5.5) making the molecule further reactive. This trend continued even after the removal of all the three N atoms (TTP6). This suggested that with the introduction of a hypervalent atom such as P, the global reactivity of the molecule increased. The same trend was shown by the finite method values. There was a great decrease in global softness (GS) values on substituting S-atom directly over P. As further atoms were added to the P, the softness values decreased further and when the thiotepa molecule was attained on atom incorporations, the values were the lowest. Substitution of O in the place of S decreased the value further. The molecule TEPA (TTP 12) may be considered less reactive than thiotepa. Upon substitution with F-atom on one of the cyclopropane ring of the thiotepa, the lowest softness value (4.16) was seen. Substitution of chlorine and -OCH_3 on cyclopropane ring showed slightly increased values. The nitric oxide (NO) substituted derivative had the highest value in the series. This molecule was considered for the study because 'NO' happens to be a biologically active 'fragment'. The highest value of this derivative might mean that this was the highest reactive molecule among the series.

The surface value ranges have been indicated in graphs (Fig.2 & 3). As the values (GS) increased the MESP value ranges were also found to vary between random limits. Hence it may be concluded that while Koopmans' softness was accountable at the DZV level, the MESP range was not. However, the range for the thiotepa molecule was interestingly found between 0.0639 and -0.1000 as the latter value may be fixed as standard. Without any hetero atom the range was from 0.0463 to -0.0332. The negative value limit increased with introduction of hetero atoms in accordance with Koopmans' softness values. For oxygen substituted molecules (TTP 11 & 12) the -0.1 limit was found crossed and with substituents the values went below -0.1. The oxygenated derivatives (TTP11 & 12) offered the widest range of surface values.

At the MP2 level the GS values decreased gradually both for the finite and Koopmans' method (Fig. 2 & 3). The lowest value of GS was shown by TTP4, another symmetrical molecule. The MESP value range was changed for $V_{s,min}$ from 0.0463 to 0.0635 and $V_{s,max}$ from -0.032 to -

0.1000. If the MESP values were considered, the change was shorter and hence Koopmans' method for GS may be considered ideal for reactivity or property studies. The finite method GS values provided more reactivity pattern for other intermediate derivatives. For instance, TTP6 molecule showed the highest GS value that decreased upon 'S' substitution over P. A steady change in values was found at the MP2 level using Koopmans' method. This way the highest reactive molecule may be TTP 16, where the thiotepa molecule was substituted with NO. For this molecule the inside range of MEP surface value was found between 0.0899 to -0.0695.

The hardness concept presents the resistivity to change. If these values were considered they were just the reverse of the GS values. Briefly a change in GH values may be presented here for the finite method using DZV d+ level. The range of value was 0.183 to 0.168 (finite), while it was from -0.134 to -0.117 (Koopmans'). If the stability of a molecule is the criteria for less toxicity or more reactivity then hardness values at the MP2 level using finite method would provide rich insight into the study.

At the MP2 level, the local electrophilicity values ω- for R8 and R19 were found to vary between -0.00021 to -0.00027 from TTP1 and TTP10. The highest value was shown by the molecule TTP16 which had a NO substitution on one of its cyclopropane ring. For R19 the values were between -0.0001 and 0.00449. There was no good resolution in the values and hence this atom cannot be accounted for variation in properties.

The ω+ values for R8 lied between 0.00002 and 0.00285 and those of R19 between 0.00036 and 0.00122. Many values for R8 were close to zero and hence due to low resolution in values the ω+ values could not be considered for property evaluation. The MESP surface values may also be referred for a quantitative analysis. With the substitution of 'NO' on one of the CP ring, the Sulphur atom had the most negative surface with a small hole at the apex, which was not present with any of the substituents. Such a surface would be available for all types of interactions. The oxygen atom of the NO had negative surface in its periphery, while the apex had more negative small region. The base of the molecule had an uneven surface distribution. Only a small portion of the Nitrogen atom on the cyclopropane ring had the negative value. Positive surface was found on all the interfaces of these atoms with the Central axis P=S. The most positive region was found at the interface of the cyclopropane with -NO.

TTP7 was similar to TTP2 but with P and S atoms. As a result of these two atoms there was a reduction of overall negative surface of the molecule. The surface of S atom has three regions with the highest surface value in the entire sphere, with two different regions at the top of the sphere, indicating a more positive small hole, which is otherwise often referred as Coulomb hole - characteristic of its reactivity towards the nucleophilic centre. The other negative centre in the molecule was the lone N-atom at the base of the molecule. Among the series, the highest negative values was shown for TTP 16 & TTP 6 (-0.00153 and -0.00152). Since higher ω- and ω+ values would mean more toxicity, lower ω- value would just be opposite of this. This would indicate that a thiotepa like molecule without Sulphur atom (TTP6) and a common alkyl group substituent would be toxic or more reactive. Other derivatives that had less significance as drug include TTP2, TTP3 and TTP4. A molecule with higher global softness (finite) value at MP2 was TTP7. This molecule was due to the substitution of Sulphur atom in the place of methylene molecule. The molecule may prove to be toxic in view of the local electrophilicity values.

Table 1: The molecules optimized and short names assigned to the IUPAC names along with Global softness values compared on two levels.

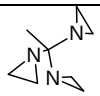
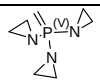
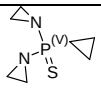
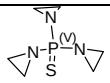
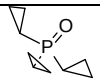
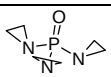
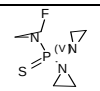
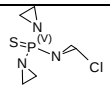
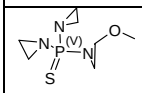
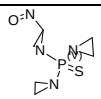
Molecule	Short name	IUPAC name	Global Softness (MP2)	
			Finite	Koopmans'
	TTP1	1,1',1''-ethane-1,1,1-triyltricyclopropane	1.85377	1.66667
	TTP2	1-(1,1-dicyclopropylethyl)aziridine	2.09835	1.73491
	TTP3	1,1'-(1-cyclopropylethane-1,1-diyl)diaziridine	2.02745	1.69635
	TTP4	1,1',1''-ethane-1,1,1-triyltriaziridine	1.90003	1.61005
	TTP5	1,1',1''-(methylidene-l5-phosphanetriyl)triaziridine	2.50414	2.03749
	TTP6	tricyclopropyl(methylidene)-l5-phosphane	1.54251	2.09161
	TTP7	tricyclopropylphosphane sulfide	2.30195	1.92456
	TTP8	1-(dicyclopropylphosphorothioyl)aziridine	2.28542	1.88395
	TTP9	1,1'-(cyclopropylphosphorothioyl)diaziridine	2.22121	1.84638
	TTP10	1,1',1''-phosphorothioyltriaziridine (THIOTEPA)	2.18758	1.81719
	TTP11	tricyclopropylphosphane oxide	2.07209	1.67813
	TTP12	1,1',1''-phosphoryltriaziridine	1.94512	1.65920
	TTP13	(2S)-1-[bis(aziridin-1-yl)phosphorothioyl]-2-fluoroaziridine	2.24382	1.86498
	TTP14	(2S)-1-[bis(aziridin-1-yl)phosphorothioyl]-2-chloroaziridine	2.35273	1.90259
	TTP15	(2S)-1-[bis(aziridin-1-yl)phosphorothioyl]-2-methoxyaziridine	2.26015	1.87406
	TTP16	(2R)-1-[bis(aziridin-1-yl)phosphorothioyl]-2-nitrosoaziridine	3.35429	2.37304

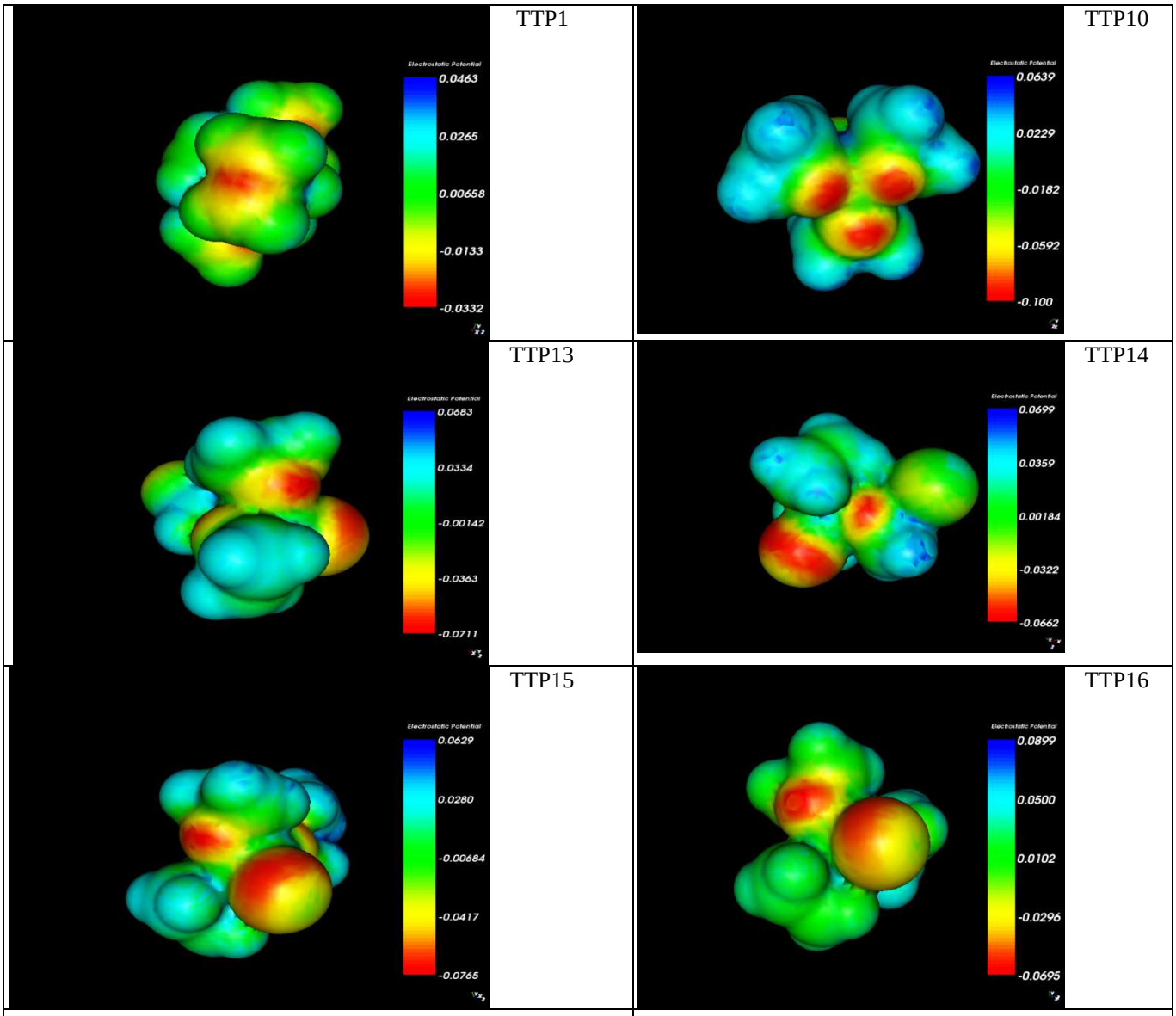
Table 2:: Local Philicity for R8 (Fig. 1) using NPA at DFT and MP2 levels

Molecule	DFT (Koopmans')		MP2 (Koopmans')	
	ω-=f-* ω	ω +=f+* ω	ω -=f-* ω	ω +=f+* ω
TTP1	0.00058	0.00102	-0.00016	0.00001
TTP2	-0.00028	0.00035	-0.00021	-0.00001
TTP3	-0.00120	0.00035	-0.00022	-0.00001
TTP4	-0.00122	0.00057	-0.00018	-0.00003
TTP5	-0.00003	0.00126	0.00000	0.00036
TTP6	-0.00121	0.00277	-0.00003	0.00023
TTP7	-0.00205	0.00336	-0.00021	0.00094
TTP8	-0.00133	0.00244	-0.00021	0.00150
TTP9	-0.00114	0.00071	-0.00010	0.00131
TTP10	-0.00078	0.00057	-0.00016	0.00164
TTP11	-0.00211	0.00446	-0.00084	0.00211
TTP12	-0.00090	0.00167	-0.00047	0.00164
TTP13	-0.00107	0.00069	-0.00006	0.00205
TTP14	0.00006	0.00080	-0.00008	-0.00009
TTP15	0.00047	0.00018	-0.00006	0.00167
TTP16	-0.00239	-0.00198	-0.00051	-0.00042

Table 3:: Local Philicity for R19 (Fig. 1) using NPA at DFT and MP2 levels

Molecule	DFT (Koopmans')		MP2 (Koopmans')	
	ω -=f-* ω	ω +=f+* ω	ω -=f-* ω	ω +=f+* ω
TTP1	-0.00034	0.00641	-0.00008	0.00027
TTP2	-0.00108	0.00941	0.00000	0.00025
TTP3	-0.00057	0.00605	-0.00001	0.00018
TTP4	-0.00034	0.00427	-0.00005	-0.00001
TTP5	0.01304	0.00127	0.00059	-0.00001
TTP6	0.01578	0.00043	0.00040	0.00002
TTP7	0.02352	0.00238	0.00298	0.00143
TTP8	0.01804	0.00173	0.00290	0.00054
TTP9	0.01670	0.00141	0.00249	0.00059
TTP10	0.00441	0.00169	0.00288	0.00070
TTP11	0.01265	0.00127	0.00534	0.00070
TTP12	0.00261	0.00010	0.00059	0.00021
TTP13	0.01582	0.00054	0.00370	0.00092
TTP14	-0.00270	0.00173	0.00441	0.00051
TTP15	-0.00209	0.00125	0.00308	0.00077
TTP16	0.02048	0.00336	0.01687	0.00151

Plate 1: MESP pictures for some important molecules mentioned against their simple names



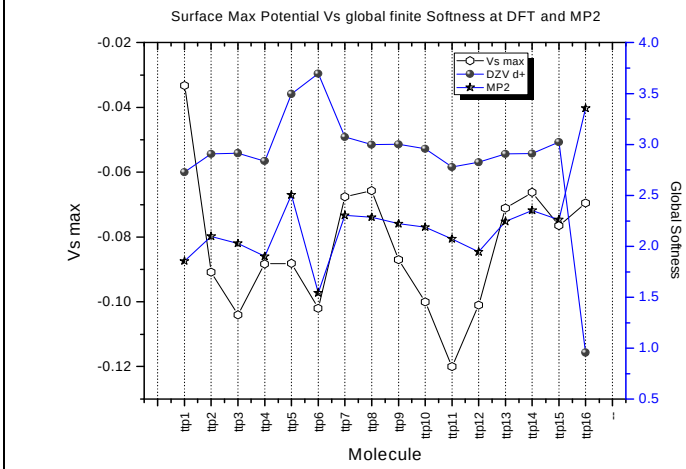


Fig. 2: Surface Potential ($V_{s,max}$) Vs global finite softness at DFT and MP2 using NPA

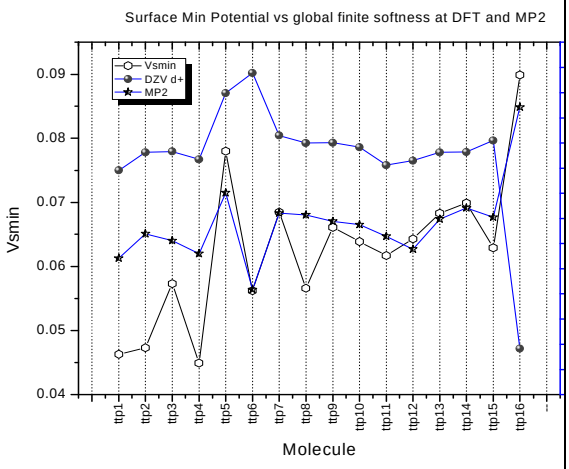


Fig. 3: Surface Potential ($V_{s,min}$) Vs global finite softness at DFT and MP2 using NPA

CONCLUSION

Based on the observations it may be concluded that global hardness, local softness and philicity index are useful for establishing the reactivity pattern of a series of analogues. The reactivity studies at MP2 level provided good correlation with the observed MESP using MK potential and the descriptive properties. The 'finite' method of computing the reactivity descriptors was useful over the Koopmans' approximation method. Without the presence of some hetero atoms such as P, S and N there was more toxicity and the inclusion of these atoms gradually raised the effectiveness and drug-likeness of the molecules. The local descriptive properties have provided insight into the nature of the molecule and have proved beyond doubt that the surface potential calculated at or beyond the van der Waals' radii are helpful in establishing the efficacy of the drug molecules. The way in which the drug molecule was evolved, tested with the MESP and descriptive values should provide an idea on how similar molecules might be computed, synthesised and tested biologically.

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