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Tautomers of Ethosuximide and their Interaction with Calcium Cation - A DFT Treatment

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Abstract

Ethosuximide is an imide which is often used in the treatment of typical epilepsy. Ethosuximide affects neuronal excitability by blocking T-type calcium channels. It may exhibit 1,3-type tautomerism (amide-iminol type tautomerism). All those possible tautomeric forms are considered. Additionally, interactions of those tautomers with calcium cation have been investigated. All the calculations have been performed within the realm of density functional theory with the constraints of B3LYP/6-311++G(d,p) level. The tautomers and their composites with calcium cation are found to be electronically stable and thermodynamically favorable structures. Ethosuximide tautomers have relatively much lower Boltzman distributions compared to ethosuximide. However, they form electronically stable and thermodynamically favorable composites with calcium cation. Also some quantum chemical and spectral properties of those systems have been obtained and discussed.

1. Introduction

Ethosuximide (sold under the brand name Zarontin) is an anticonvulsant frequently used in the treatment of typical generalized absence (petit mal) epilepsy [1,2]. In fact, its anticonvulsant action is proved to be specific for this type of epilepsy. It is ineffective in the treatment of other types of generalized seizures and against all types of partial seizures [l, 2]. However, few cellular actions of ethosuximide have been described that are consistent with its specific anticonvulsant effects [3-5]. Researches on characterization of the cellular mechanism of action of ethosuximide could have direct

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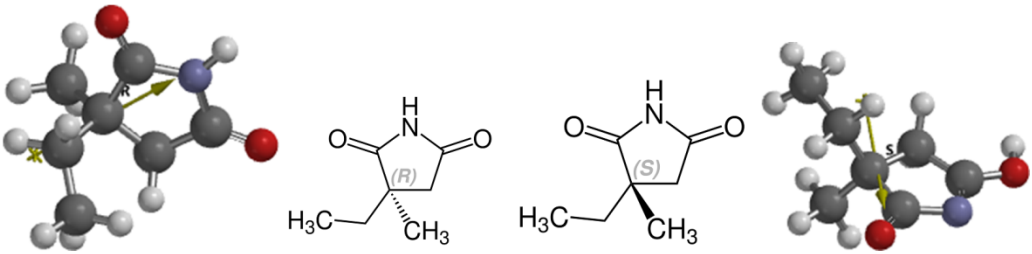
bearing on understanding of the pathogenesis of petit mal epilepsy, and also could aid in the development of other anticonvulsant drugs.

The mechanism by which ethosuximide affects neuronal excitability includes block of T-type calcium channels, and may include effects of the drug on other classes of ion channel. The primary finding that ethosuximide is a T-type calcium channel blocker gained widespread support, but initial attempts to replicate the finding were inconsistent. Subsequent experiments on recombinant T-type channels in cell lines demonstrated conclusively that ethosuximide blocks all T-type calcium channel isoforms [6-9]. Significant T-type calcium channel density occurs in dendrites of neurons, and recordings from reduced preparations that strip away this dendritic source of T-type calcium channels may have contributed to reports of ethosuximide ineffectiveness.

Ethosuximide, commercially available and administered to the patients, is a racemic mixture of two enantiomers, each of them exist in different conformations. This drug is used clinically as the racemate. It was shown that the ratio of the two enantiomers of ethosuximide ranged from 1.00 to 1.36 and was apparently unaffected by pregnancy, passage into breast milk, and transfer over the placenta. Hence, it has been accepted that determination of total ethosuximide concentrations appear to be sufficient for therapeutic drug monitoring during pregnancy and lactation [10].



Drewniak et al., not only investigated those enantiomers by various experimental methods [11,12] but also aided by DFT model calculations and molecular dynamics [11,13]. Serdaroğlu and Ortiz performed some *ab ınitio* calculations on some antiepileptic drugs, including ethosuximide [14].

In the present study, tautomers of ethosuximide (in the S form) and their interaction with calcium cation have been investigated (for pure chemical interest) computationally using density functional theory.

2. Method of Calculation

All the present structures of interest were subjected to the geometry optimizations leading to energy minima. The optimizations have been achieved first by applying MM2 method which has been followed by semi-empirical PM3 self consistent fields molecular orbital (SCF MO) method [15,16] at the restricted level [17,18]. Subsequent optimizations have been managed at Hartree-Fock level employing various basis sets hierarchically. Afterwards, geometry optimizations have been achieved within the framework of density functional theory [19,20] at the level of B3LYP/6-311++G(d,p) [17,21]. Note that the exchange term of B3LYP consists of hybrid Hartree-Fock and local spin density (LSD) exchange functions with Becke's gradient correlation to LSD exchange [20,22]. The correlation term of B3LYP consists of the Vosko, Wilk, Nusair (VWN3) local correlation functional [23] and Lee, Yang, Parr (LYP) correlation correction functional [24]. Also, vibrational analyses have been done on the optimized structures. The total electronic energies were corrected for the zero point vibrational energy (ZPE). The normal mode analysis for each structure yielded no imaginary frequencies for the 3*N*–6 vibrational degrees of freedom, where *N* stands for the number of atoms in the system. This has indicated that the structure of each molecule corresponds to at least a local minimum on the potential energy surface. All these calculations have been done by using the Spartan 06 package program [25].

3. Results and Discussion

Having an imide structure, ethosuximide can exhibit 1,3-type proton tautomerism (amide-iminol type tautomerism). Since tautomers having different structures possess dual reactivity, it is anticipated that ethosuximide exhibits 1,3-proton tautomerism and it should display variable biological properties (beside others) depending on its tautomer content (allelotropic mixture [26,27]). It is to be noted that substances which are isomeric under certain conditions are tautomeric under more drastic conditions [26,27].

Figure 1 shows the optimized structures of ethosuximide tautomers (I-III), as well as their composites with calcium cation (labeled as primed numbers). The calcium cation is one of the most important biological ion participating various metabolic processes. Ethosuximide affects neuronal excitability including the block of T-type calcium channels. The interaction of ethosuximide with calcium cation in nonliving conditions may spell some scientific light for pure chemical purposes.

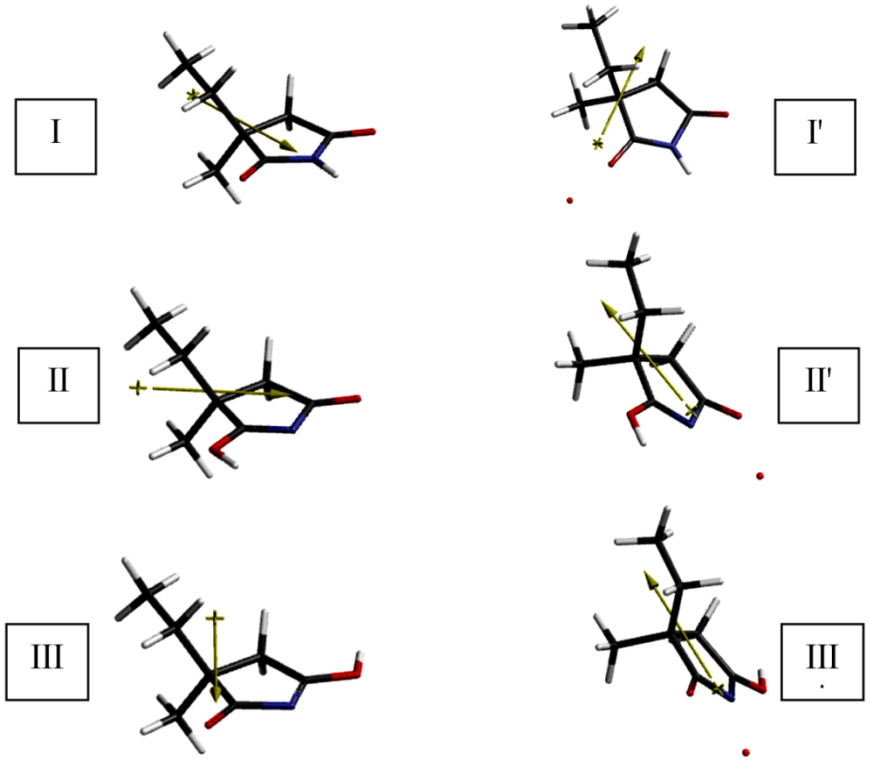


Figure 1. Optimized structures of the systems considered.

Table 1 shows the relative energies and the Boltzman distribution values for the considered ethosuximide tautomers in vacuum as well as in water. The data indicate that tautomers-II and III (possess iminol moiety) have relatively negligible concentration at room temperature. It should be noted that the "energy aqueous (Eaq)" is the sum of the base energy and the energy of solvation. The program calculates the solvation energy using SM54A method (Ghose-Crippen method) [25,28].

Table 1. Relative energies (Rel E) and the Boltzman distribution values (K) for ethosuximide tautomers considered.

System	Rel E	K	Rel E(aq)	K(aq)
I	0.00	1.000	-17.43	0.999
II	77.57	0.000	24.61	0.000
III	99.43	0.000	0.000	0.001

Energies in kJ/mol.

Table 2 displays the dipole moment and polarizability values of the systems considered. Note that the primed structures are actually composites of the ethosuximide tautomers with calcium cation. The polarizability is defined according to the formula [25].

$$\text{Polarizability} = 0.08*V - 13.0353*h + 0.979920*h2 + 41.3791$$

where V and h are the Van der Waals volume and hardness, respectively. Hardness is defined as,

$$\text{Hardness} = -(\varepsilon_{HOMO} - \varepsilon_{LUMO})/2$$

where ε_{HOMO} and ε_{LUMO} are the molecular orbital energies of the highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbital energies.

Table 2. Dipole moment and polarizability values of the systems considered.

System	Dipole moment	Polarizability	System	Dipole moment	Polarizability
I	2.53	51.91	I'	29.52	53.69
II	5.00	52.03	II'	28.80	53.25
III	6.85	52.05	III'	24.77	53.19

Dipole moments in debye units. Polarizabilities in 10^{-30} m^3 units.

Tables 3 and 4 show some energies of the parent systems as well as the composites considered, where E, ZPE and E_C stand for the total electronic energy, zero point vibrational energy and the corrected total electronic energy, respectively. The data reveal that all the structures considered are electronically stable. The E_C values follow the order of I<II<III for the parent tautomers and III'<II'<I' for their composites with the calcium cation. The systems considered are all structurally stable. Although, ethosuximide structure is electronically more stable than its tautomers, in the composite form with the calcium cation, III' is the most stable of the composites considered. However, note that it has the lowest Boltzman distribution value.

Table 3. Some energies of the ethosuximide tautomers considered.

System	E	ZPE	E_C
I	-1256965.79	461.91	-1256965.79
II	-1256951.03	461.35	-1256951.03
III	-1256866.36	460.22	-1256866.36

Energies in kJ/mol.

Table 4. Some energies of the calcium(+2) composites of ethosuximide tautomers considered.

System	E	ZPE	E_C
I'	-3034593.87	463.72	-3034130.15
II'	-3034613.02	464.80	-3034148.22
III'	-3034628.31	464.74	-3034163.57

Energies in kJ/mol.

Tables 5 and 6 show some thermo chemical properties of the ethosuximide tautomers and their composites with the calcium cation. As seen in Tables 5 and 6, ethosuximide tautomers and their calcium cation composites have negative heats of formation values and favorable G^o values. Therefore, all the systems considered are not only electronically stable but thermodynamically favored as well.

Table 5. Some thermo chemical properties of the ethosuximide tautomers considered.

System	H^o	S^o (J/mol^o)	G^o
I	-1256489.351	384.29	-1256603.929
II	-1256412.216	385.10	-1256527.038
III	-1256391.222	386.59	-1256506.485

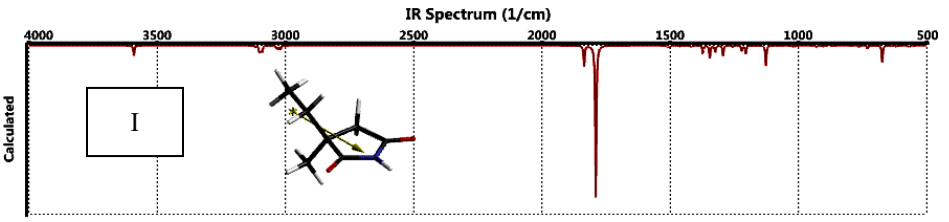
Energies in kJ/mol.

Table 6. Some thermo chemical properties of the composites of ethosuximide tautomers considered.

System	Hº	Sº (J/molº)	Gº
I'	-3034128.431	418.05	-3034253.064
II'	-3034146.678	415.56	-3034270.576
III'	-3034162.037	416.65	-3034286.251

Energies in kJ/mol.

Figure 2 shows the calculated IR spectra of the systems considered. The N-H stretching of structure-I (ethosuximide) occurs at 3591 cm^{-1}. The O-H stretchings of tautomers-II and III happen at 3740 cm^{-1} and 3811 cm^{-1}, respectively. The symmetrical and asymmetric imide C=O stretchings of I occur at 1836 cm^{-1} and 1790 cm^{-1}, respectively. Whereas C=O stretchings of the others happen at 1823 cm^{-1} (II) and 1821 cm^{-1} (III). The presence of calcium cation shifts the N-H stretching of ethosuximide (I) from 3591 cm^{-1} to 3533 cm^{-1} in composite-I'. Similarly, O-H stretchings of tautomers-II and III shift and occur at 3727 cm^{-1} and 3711 cm^{-1}, respectively in composites-II' and III'. The two carbonyl stretchings of composite-I' are at 1927 cm^{-1} and 1621 cm^{-1} (due to presence of the cation, they are further apart from each other as compared to the respective values of I). Whereas in the cases of composite-II' and III', the carbonyl stretchings occur at 1653 cm^{-1} and 1654 cm^{-1}, respectively. Figure 2 comparatively displays the IR spectra of ethosuximide tautomers as well as their composites with the calcium cation. It is clear that the cation affects the IR spectrums of tautomers of ethosuximide to a certain extent (probably changing the bond constants via perturbation of the electron distribution) which is one of the major factors dictating IR frequencies [29].



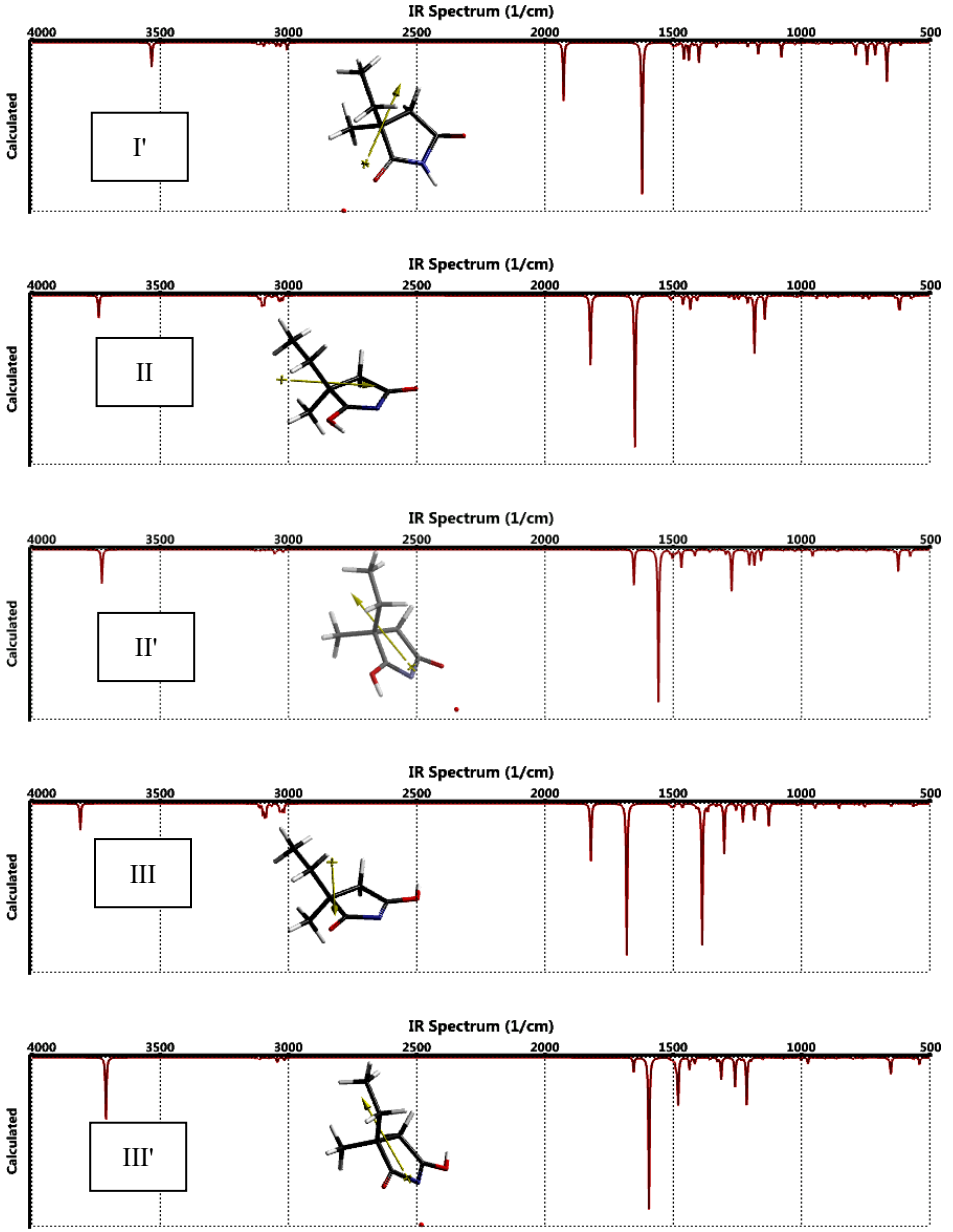


Figure 2. Calculated IR spectra of the systems considered.

Figure 3 displays the electrostatic potential (ESP) charges on the atoms of the systems considered. It is worth noting that the ESP charges are obtained by the program based on a numerical method that generates charges that reproduce the electrostatic potential field from the entire wavefunction [25]. As seen in the figure, in all the cases the

nitrogen atom originating from imide structure is negatively charged. The charge order of that nitrogen atom is III<II<I (algebraic order) for the parent tautomers but in the composites it turns out to be I'<III'<II'. Whereas the charge order of calcium atom is II'<III'<I'. So, the interaction is not merely charge-dipole type but some electron population has been transferred from ethosuximide originating species to the calcium component, therefore decreasing the formal charge of the cation. As it is seen, the charge order of nitrogen atoms (negatively charged), originating from the imide structure and the calcium cation (positively charged) are the same in absolute values.

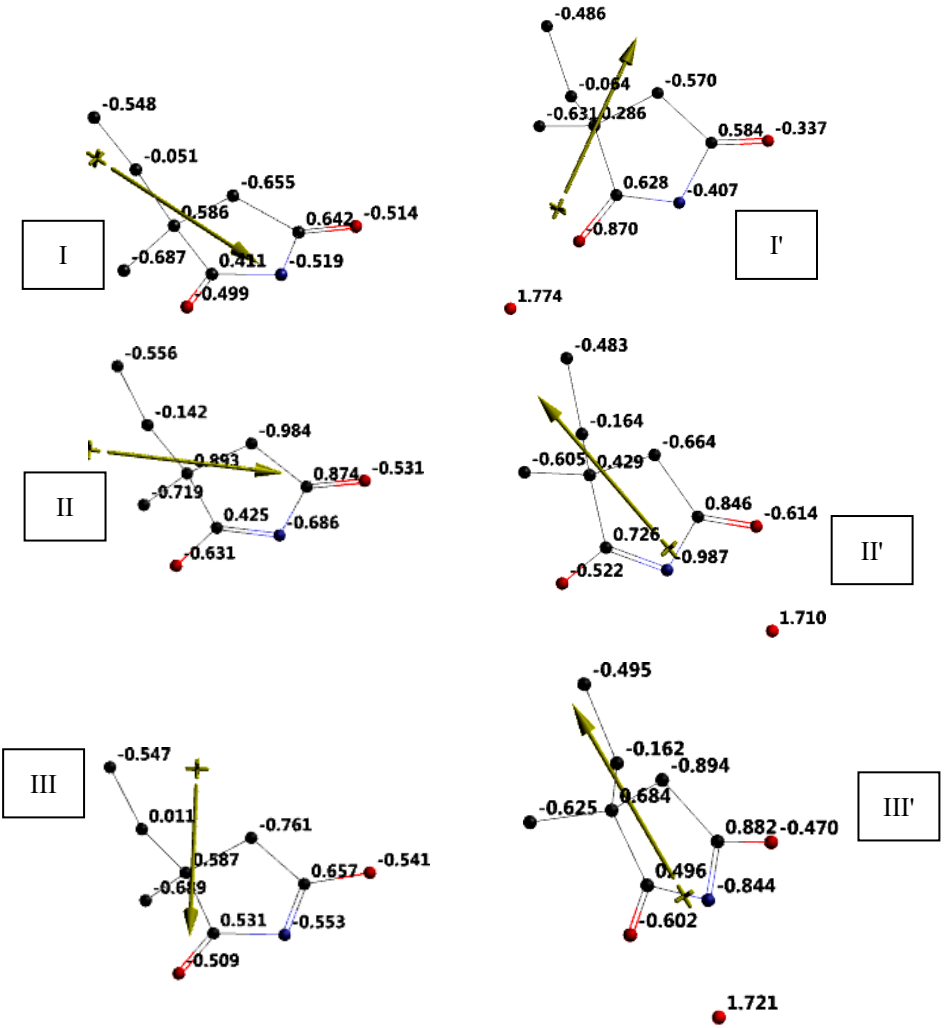


Figure 3. The ESP charges on the atoms of the systems considered (hydrogens not shown).

Figure 4 displays the electrostatic potential maps of ethosuximide tautomers considered. As seen in the figure, negative (red/ reddish regions) and positive potential (blue/bluish regions) parts vary depending on the position of the tautomeric hydrogen and the structural form of the ring. The highest negative potential region in all the cases covers the carbonyl oxygen and its vicinity.

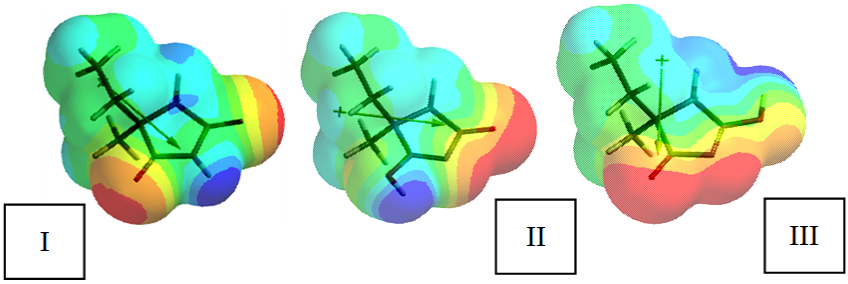


Figure 4. Electrostatic potential maps of ethosuximide tautomers considered.

Figures 5 and 6 display some of the molecular orbital energies of the parent

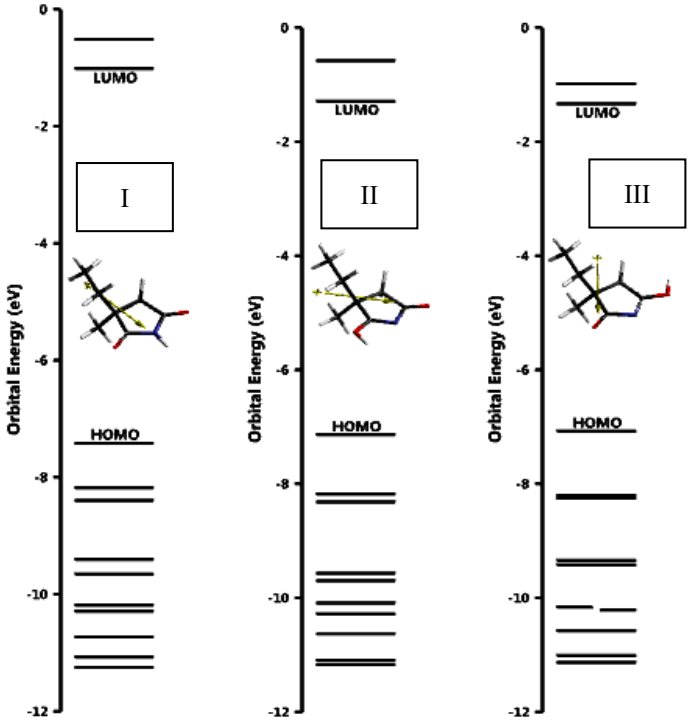


Figure 5. Some of the molecular orbital energies of the ethosuximide tautomers considered.

tautomers and their composites with the calcium cation, respectively. Whereas Table 7 lists the HOMO, LUMO energies and the interfrontier molecular orbital energy gaps Δε ($\Delta\varepsilon = \varepsilon_{LUMO}-\varepsilon_{HOMO}$) values of the systems considered. The data in the table indicate that the HOMO energy order of the parent tautomers is I<II<III but the LUMO energy order is II<II<I. On the other hand, the presence of calcium cation, changes the order of both the HOMO and the LUMO energies. The orders are I<II<III and III'<II'<I' for the HOMO energies and III< II< I < and I'< II'< III' for the LUMO energies, respectively. So, the presence of calcium cation lowers both the HOMO and LUMO energies of the parent tautomers. Consequently, Δε values for the parent tautomers and the composites become III<II<I and I<II<III, respectively.

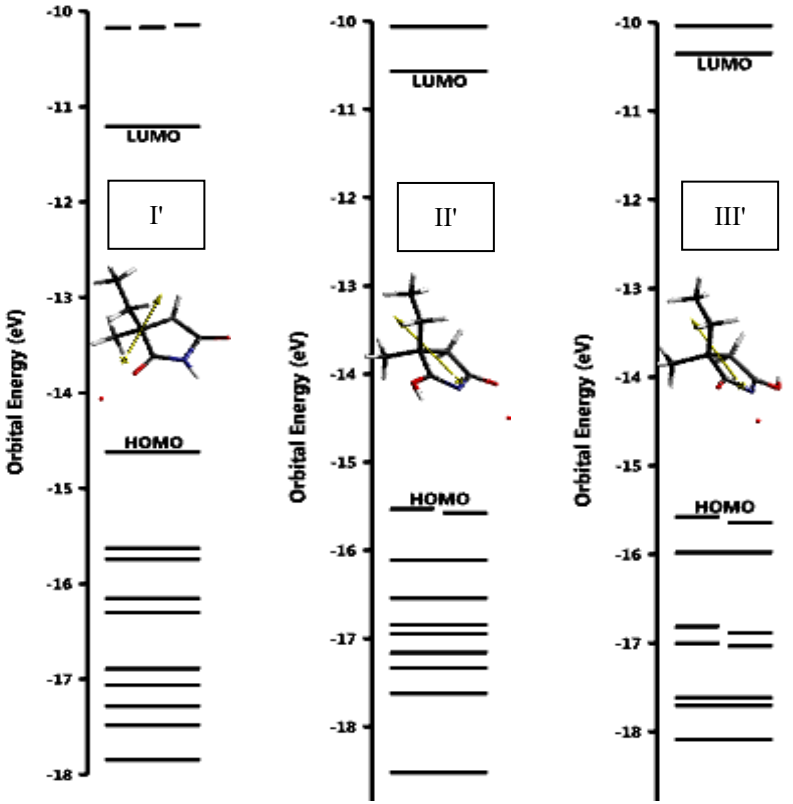


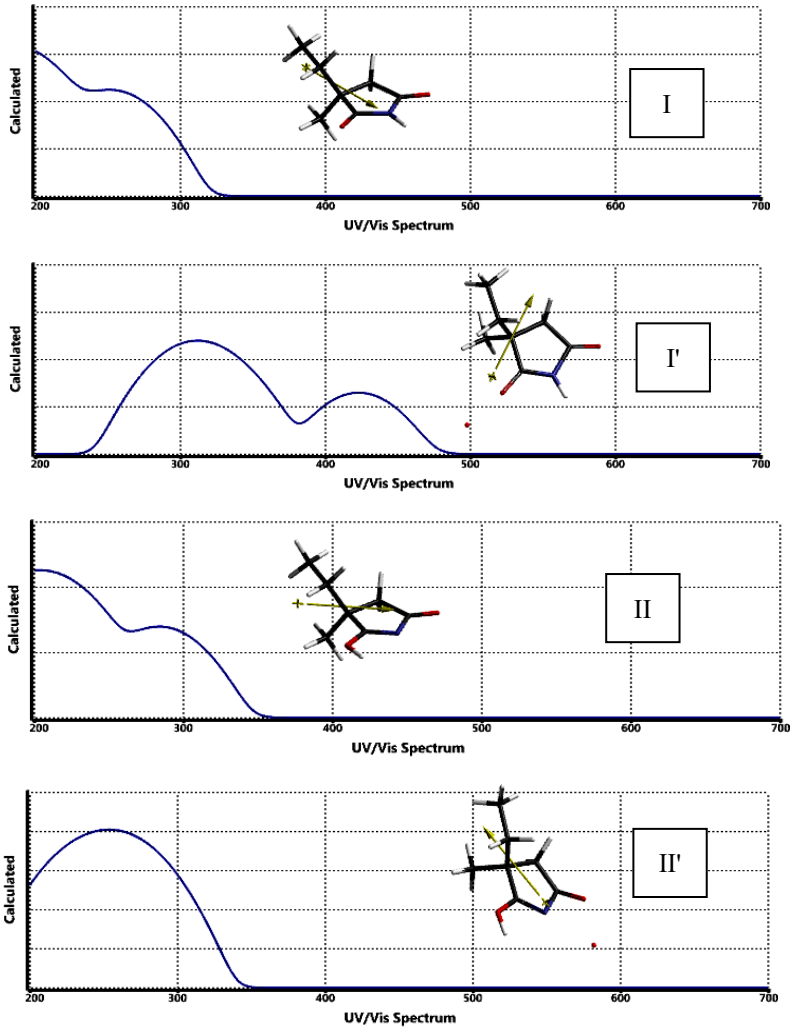
Figure 6. Some of the molecular orbital energies of the calcium(+2) composites of the tautomers considered.

Table 7. The HOMO, LUMO energies and Δε values of the systems considered.

System	HOMO	LUMO	Δε	System	HOMO	LUMO	Δε
I	-715.74	-98.18	617.56	I'	-1410.51	-1081.17	329.34
II	-688.86	-124.42	564.44	II'	-1498.17	-1019.29	478.88
III	-682.81	-129.03	553.78	III'	-1502.54	-998.92	503.62

Energies in kJ/mol.

Figure 7 shows the calculated UV-VIS spectra (time dependent DFT) of the systems



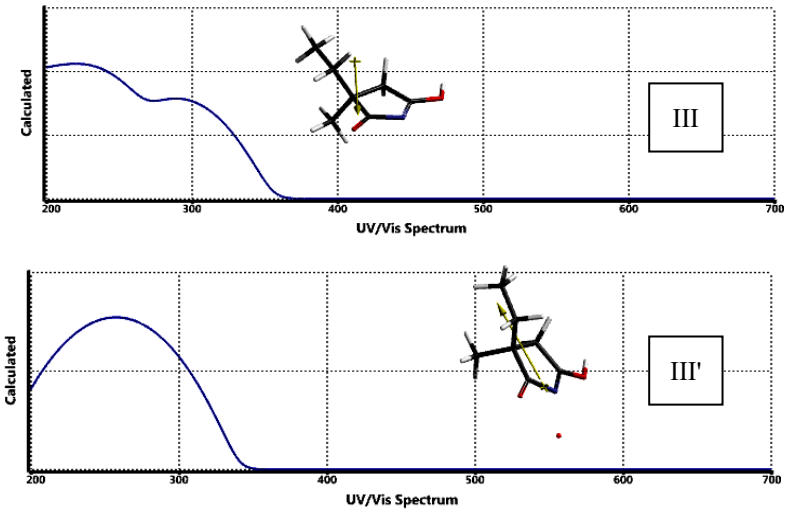


Figure 7. Calculated UV-VIS spectra of the systems considered.

considered. As seen in the figure, tautomers of ethosuximide exhibit some bathochromic effect. In the ethosuximide tautomers, due to tautomerism some extended conjugation occurs narrowing the interfrontier molecular orbital gap, thus causing a bathochromic effect to emerge. In the composites considered, effect of the calcium cation does not operate in parallel. It affects ethosuximide (which possesses an imide group) electronic structure so a bathochromic effect occurs in I'. Whereas in the cases of composites II' and III' (they have both an amide and an iminol moieties embedded) some degree of hypsochromic effect happens. It is most probably due to interaction with the calcium cation which partially disturbs the extended conjugation present in the parent system. Indeed, $\Delta\varepsilon$ values of II' and III' is greater than I'.

Medicines in living organisms are subjected to various enzymatic and/or chemical attacks which may be directed mainly by the frontier molecular orbitals (HOMO and LUMO) and energies of the interacting/reacting species [30]. Thus, sites of reactivity of ethosuximide tautomers can be predicted via their local ionization potential maps and LUMO maps.

Figure 8 is the local ionization potential map of ethosuximide tautomers, where conventionally red/reddish regions (if any exists) on the density surface indicate areas from which electron removal is relatively easy, meaning that they are subject to electrophilic attack.

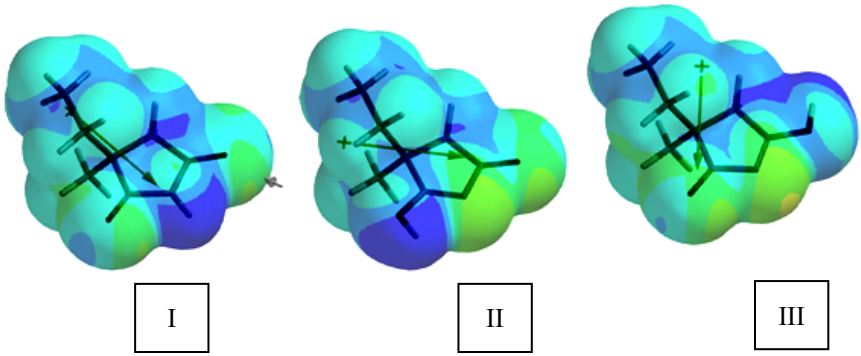


Figure 8. The local ionization maps of the ethosuximide tautomers considered.

Figure 9 shows the LUMO maps of the ethosuximide tautomers considered. A LUMO map displays the absolute value of the LUMO on the electron density surface. The blue color (if any exists) stands for the maximum value of the LUMO and the red-colored region, associates with the minimum value.

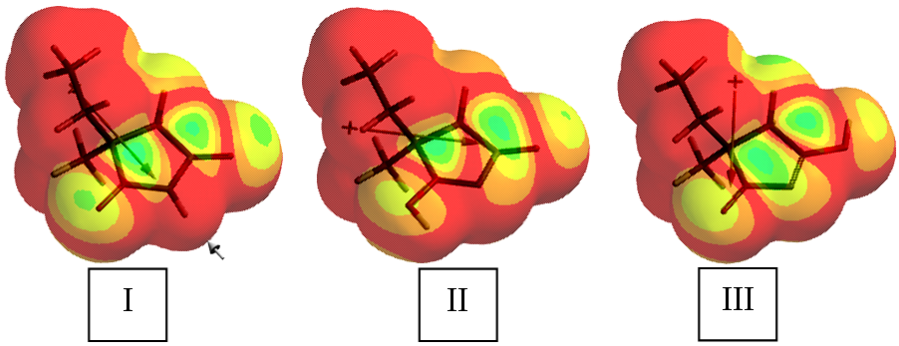


Figure 9. The LUMO maps of the ethosuximide tautomers considered.

4. Conclusion

The present DFT study at the level of B3LYP/6-311++G(d,p) indicates that in vacuum and aqueous conditions the concentrations of iminol tautomers of ethosuximide are relatively low. However, those tautomers and their composites with the calcium cation are electronically stable and thermodynamically favorable. All the results indicate that a certain extent of interaction between ethosuximide tautomers and the calcium cation occurs. The composites may increase the equilibrium distribution of ethosuximide originating species. However, the effect of hydrogen-bond donor and acceptor solvents should be rather different. All these may affect the potential of ethosuximide on the neuronal excitability through which blocks certain type of calcium channels.

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