RESEARCH ARTICLE OPEN ACCESS

A Hypothetical Study on Structural aspects of Indole-3-carbinol (I3C) by Hyperchem and Arguslab 4 software

K. Laxmi
Department of Chemistry, Chaitanya Bharathi Institute of Technology (CBIT), Gandipet, Hyderabad -500 075, INDIA

ABSTRACT
Indole-3-carbinol (I3C) is a plant compound derived from glucosinolates, found in cruciferous vegetables. Researchers have indicated that I3C shows great promise as a cancer preventative and hormone-balancing agent. HyperChem 7.5 software was used for quantum mechanical calculations. The geometry optimization was carried out using Ab Initio method. QSAR parameters were generated with semi empirical single point AM1 method. The HOMO and LUMO frontier orbital energies were also computed. Conformational analysis and geometry optimization of Indole-3-carbinol (I3C) was performed according to the Hartree-Fock (HF) calculation method by ArgusLab 4.0.1 software .The minimum heat of formation is calculated by geometry convergence function by ArgusLab software. PM3 semi empirical quantum mechanical calculations were carried out on structure of Indole-3-carbinol (I3C) to obtain the geometries, geometric parameters and thermodynamic parameters. The HOMO and LUMO frontier orbital energies were also computed for the optimized molecule. Electron density surface of IDOX is determined using PM3 geometry with PM3 wavefunciton.

Keywords: I3C, Hyperchem 7.5 Software, QSAR, Argus Lab 4.0.1, conformational analysis, geometry optimization, HOMO, LUMO

I. INTRODUCTION

M.P.of **Indole-3-carbinol (I3C)** 96-99 °C (lit.) and storage temperature is 2-8°C.

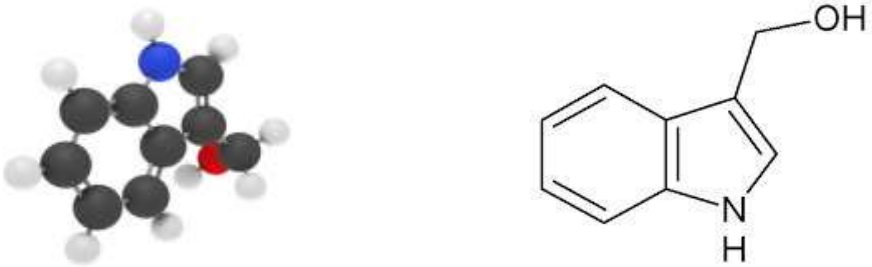


Fig.1 structure of Indole-3-carbinol (I3C)

Between 1990 and the current time, there have been over 120 peer-reviewed scientific studies on indole 3 carbinol with cancers such as breast cancer, prostate cancer, cervical cancer and even respiratory tract cancers. For example, in one (albeit small) double blind study on women with cervical cancer, supplementation of 200 mgs to 400 mgs of I3C reversed the early stages of cancer in 8 of the 17 women. I3C is potent stuff.

Anti-cancer activity:

I3C has been shown in research to inhibit the action of some aflatoxins, which are carcinogenic. It also prevents aflatoxin-induced liver cancer, leukaemia and colon cancer.

I3C can reduce, and even reverse, breast tumour growth

In cell cultures, I3C inhibits human papilloma virus (HPV) proliferation, and the development of cysts and pre-cancerous lesions. There is some evidence that I3C may help prevent recurrences of respiratory papillomatosis. Further research indicates that I3C may well protect your liver, but the most interesting aspect of I3C is its seeming ability to reduce, even reverse, oestrogen-driven tumour growth. The Roman was right!

I3C seems to act in five ways:

- By converting highly active oestrogen forms, and their by-products, to much safer compounds.

- By partially blocking oestrogen receptor sites on cell membranes.
- By returning alpha and beta receptor site expression to normal levels
- By blocking other cancer-enhancing receptor sites
- By directly killing cancer cells.

I3C gives rise to a broad spectrum of beneficial compounds upon its consumption and digestion. Various researchers and reputable organizations, such as the National Cancer Institute and the Strang Cancer Prevention Center, have interest in I3C as a natural cancer prevention agent, particularly for breast, cervical, endometrial, and colorectal cancer. Several prospective clinical studies have shown that populations that consume higher amounts of cruciferous vegetables have lower incidence of cancer, or improved biochemical indices (e.g., decreased oxidative stress), compared to controls.[1-19]

In fact, epidemiological studies provide evidence that the consumption of cruciferous vegetables protects against cancer more effectively than the total intake of fruits and vegetables.[20,21]

Theoretical studies

The molecule **Indole-3-carbinol (I3C)** was built by Hyperchem tools[22-27] then the geometry optimization was carried out by employing Ab Initio optimized semi empirical single point AM1 method. The minimum potential energy was calculated for **I3C** through the geometry convergence map. Mulliken Atomic Charges , ZDO Atomic Charges of **I3C** and Ground State Dipole (debye) of were determined using PM3 method. Computational advances have generated many tools which are widely used to construct models, minimization and representations of molecular structure.

All conformational analysis (geometry optimization) study was performed on a window based computer using Argus lab software[28-34]. The **I3C** structure is generated by Argus lab, and minimization was performed with the semi-empirical Parametric Method 3 (PM3) parameterization.[35,36]

The minimum potential energy is calculated by using geometry convergence function in Argus lab software. Surfaces created to visualize ground state properties as well as excited state properties such as orbital, electron densities, electrostatic potentials (ESP) spin densities and generated the grid data used to make molecular orbital surfaces to visualize the molecular orbital and making an electro static potential mapped on electron density surface.

II. RESULTS AND DISCUSSIONS

Hypothetical study of Indole-3-carbinol (I3C) by HyperChem 7.5 software The Hyper Chem 7.5 software was used for quantum mechanical calculations to generate spectral data. After building molecule by Hyperchem tools, the geometry optimization was done using Ab Initio method (Figs.1 to 3). The spectral data is generated with single point AM1 method approximation, for **indole 3 carbinol(I3C)**. The calculations are sensitive to the values of input parameters such as molecular geometry, bond lengths and values of coulombic, resonance and overlap integrals ball and cylinders model tubes model From Potentiometric titrations it has been observed that there is only one dissociable proton present in the ligand **I3C**. This is attributable to dissociation of proton from the ligand.

Prospective view and active conformation of **indole 3 carbinol(I3C)** are shown in **Figs. 2&3**

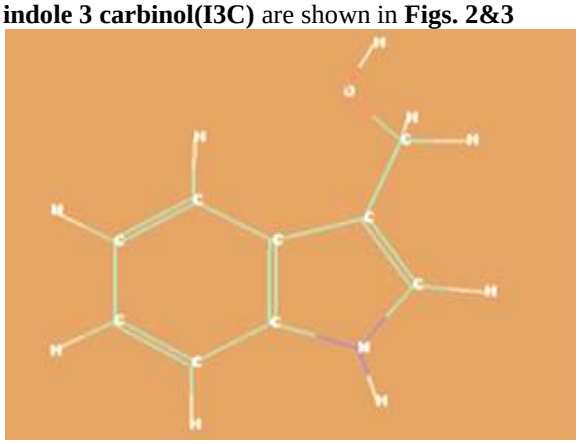


Fig.2 Prospective view of **indole 3 carbinol(I3C)**

Hyperchem data of indole 3 carbinol(I3C) ---- AM1 optimized Single point energy = -2174.122223 Gradient= 2.746174 C1 symmetry

(i) ball & cylinder model **(ii) tubes model**

Fig.3 Active conformations of **indole 3 carbinol(I3C)**

Table 1. QSAR and Molecular properties of indole 3 carbinol (I3C)

QSAR properties	
Surface area appro	**252.32 $^{o}A^{2}$**
Surface area grid	**325.79 $^{o}A^{2}$**
Volume	**490.96$^{o}A^{3}$**
Hydration energy	**-8.18 kcal/mol**
Log P	**-1.09**
Refractivity	**48.31 $^{o}A^{3}$**
Polarisability	**16.96 $^{o}A^{3}$**
Mass	**147.18 amu**
Molecular properties	
Total energy	**-41571.90234 kcal /mole**
Binding energy	**-2174.120605 kcal /mole**
Heat of formation	**5.36645174 kcal/mol**
Electronic energy	**-204110.1563 kcal/mol**
Nuclear energy	**162538.25 kcal/mol**
Dipole moment	**2.762 D**
Dipole X	**2.10625 D**
Dipole Y	**1.66069 D**
Dipole Z	**-0.65878 D**
RMS gradient	**2.755 kcal/A^{o}mol**
Gradient X	**0.16857**
Gradient Y	**1.47497**
Gradient Z	**2.32068**

Quantitative structure activity relationship studies (QSAR studies)

QSAR properties like surface area, volume, hydration energy, log P, refractivity, polarisability, mass, total energy etc. of I3C were determined by single point AM1 method. (Table .1) QSAR properties allows calculation and estimation of a variety of molecular descriptors commonly used in quantitative structure activity relationship (QSAR) studies[7,8].

This analysis represents an attempt to relate structural descriptors of compounds with their physicochemical properties and biological activities.

According to the AM1 calculation binding energy of **I3C** is about **-2174.120605 kcal /mole**. The heat of formation of **I3C** is about **5.36645174 kcal/mol** and it is endothermic. Dipole moment of **indole 3 carbinol (I3C)** is **2.762D**

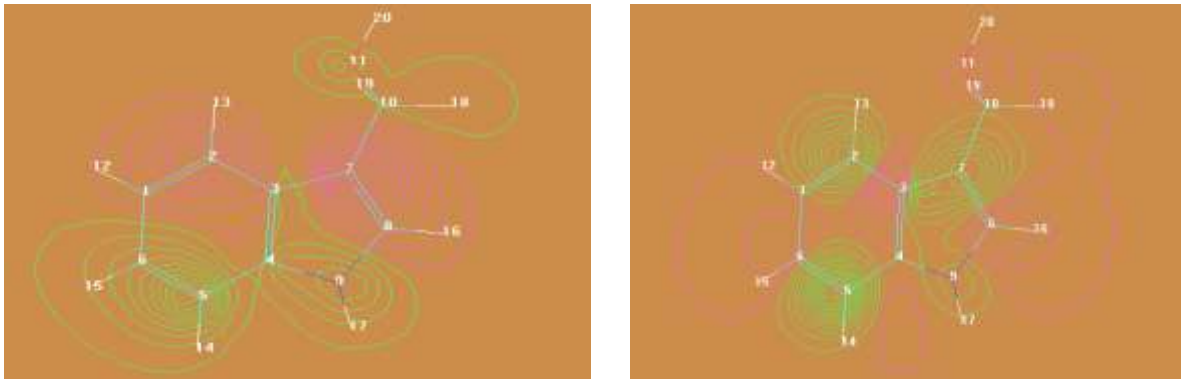
The trends of the molecular properties (Table.1) obtained by calculations are in good agreement with the experimental results[7-12.]

This analysis represents an attempt to relate structural descriptors of compounds with their physicochemical properties and biological activities.

Quantum Chemical Studies of indole 3 carbinol (I3C)

The frontier molecular orbital energies (*i.e.*, E_{HOMO} and E_{LUMO}) [9,10] are significant parameters for the prediction of the reactivity of a chemical species. The E_{HOMO} is often associated with the electron donating ability of a molecule. The E_{LUMO} indicates the ability of the molecule to accept electrons. Therefore higher value of E_{HOMO} indicates higher tendency for the donation of electron(s) to the appropriate acceptor molecule with low energy and empty molecular orbital.

The values of $E_{HOMO,}$ E_{LUMO} & $E_{LUMO\text{-}HOMO}$ of **indole 3 carbinol (I3C)** were found to be-8.209828 eV , 0.3334137 eV & 7.8764143 eV respectively. (**Fig.4)**



HOMO of I3C E= -8.209828 eV **LUMO of I3C E= 0.3334137 eV**

Fig.4 Highest occupied molecular orbital (HOMO) and Lowest unoccupied molecular orbital (LUMO) of Indole-3-carbinol (I3C)

Hypothetical study of Indole-3-carbinol (I3C) by Argus lab software

The molecule **indole 3 carbinol(I3C)** is build using molecule builder of Argus lab[28-35].

The "Molecule Settings of **I3C** are Atoms20, Net charge 0 and Valence electrons 56

Table 2: Atomic coordinates of Indole-3-carbinol (I3C) - Input Atomic Information Input Atomic Information

1	C	-4.419712	1.176028	-0.038441	11	O	-7.771394	1.706704	0.653845
2	C	-3.248180	1.934787	0.229083	12	H	-1.092112	1.923473	0.557322
3	C	-1.991192	1.329215	0.367836	13	H	-0.973873	-0.559656	0.336998
4	C	-1.936657	-0.045440	0.249555	14	H	-3.003144	-1.900003	-0.056468
5	C	-3.094923	-0.810901	0.009585	15	H	-5.230879	-0.812586	-0.339659
6	C	-4.332643	-0.218316	-0.142322	16	H	-5.567933	4.321335	0.081686
7	C	-5.519115	2.105379	-0.146828	17	H	-3.079943	4.014336	0.662225
8	C	-5.024194	3.375012	0.070837	18	H	-6.970489	0.786059	-1.012787
9	N	-3.623602	3.301020	0.244919	19	H	-7.401234	2.524818	-1.107603
10	C	-6.929221	1.753715	-0.474530	20	H	-7.352095	1.138744	1.290776

The atomic input information for above calculation is given in **Table no.2.** The final geometrical energy and SCF energy was found to be -61.0179909654 au/-38289.4020 kcal/mol as calculated by RHF/ PM3method, as performed by ArgusLab 4.0.1 suite**.(Table no.3)**

Table 3: Calculating SCF using one electron matrix---Performing SCF

Cycle	Energy (au)	Difference	Cycle	Energy (au)	Difference
1	-30.350762		21	-61.017990937	-2.80369e-008
2	-41.774967396	-11.4242	22	-61.017990952	-1.48481e-008
3	-43.905230305	-2.13026	23	-61.017990958	-6.55427e-009
4	-49.865539442	-5.96031	24	-61.017990962	-3.64935e-009
5	-52.732016517	-2.86648	25	-61.017990964	-1.76834e-009
6	-54.515449090	-1.78343	26	-61.017990964	-8.81982e-010
7	-57.849009142	-3.33356	27	-61.017990965	-4.42924e-010
8	-60.490932094	-2.64192	28	-61.017990965	-2.21917e-010
9	-60.964263769	-0.473332	29	-61.017990965	-1.13175e-010
10	-61.015844362	-0.0515806	30	-61.017990965	-5.67297e-011
11	-61.017754626	-0.00191026	31	-61.017990965	-2.92744e-011
12	-61.017924300	-0.000169675	32	-61.017990965	-1.38698e-011
13	-61.017971338	-4.70379e-005	33	-61.017990965	-7.78755e-012
14	-61.017984259	-1.29208e-005	34	-61.017990965	-3.97904e-012
15	-61.017988429	-4.17012e-006	35	-61.017990965	-2.16005e-012
16	-61.017989924	-1.4949e-006	36	-61.017990965	-2.84217e-013
17	-61.017990460	-5.35952e-007	37	-61.017990965	-6.25278e-013
18	-61.017990726	-2.66212e-007	38	-61.017990965	-6.82121e-013
19	-61.017990857	-1.3061e-007	39	-61.017990965	1.7053e-013
20	-61.017990909	-5.18908e-008	40	-61.017990965	5.68434e-014

SCF converged
Final SCF Energy = -61.0179909654 au
Final SCF Energy = -38289.4020 kcal/mol
Final Geometrical Energy = -61.0179909654 au
Final Geometrical Energy = -38289.4020 kcal/mol

Table 4 : List of Mulliken Atomic Charges and ZDO Atomic Charges of Indole-3-carbinol (I3C) using ArgusLab software ZDO Atomic Charges of I 3 CAR

Atom no.	atom	ZDO Atomic Charges	Atom no.	atom	ZDO Atomic Charges
1	C	-0.0684	11	O	-0.3167
2	C	-0.1692	12	H	0.1065
3	C	-0.0953	13	H	0.1003
4	C	-0.0907	14	H	0.1018
5	C	-0.1228	15	H	0.1058
6	C	-0.0566	16	H	0.1422
7	C	-0.2027	17	H	0.0694
8	C	-0.1888	18	H	0.0298
9	N	0.2660	19	H	0.0526
10	C	0.1496			

ZDO Atomic Charges) of **Indole-3-carbinol (I3C)** were given in **Table. 4**

Heat of Formation: The atomic heat of formation is the heat that released during the formation of the stable form of the element from individual atoms at standard conditions. It should be noted that thermodynamical corrections (e.g., zero-point energies) should not be added to the formation energy, as these are implicitly included by the parametrization. The most energetically favorable conformation of **I3C** is found to have a heat of formation of -6.6437 kcal/mol via use of the Argus Lab software.

HOMO and LUMO orbitals of I3C

The HOMO and LUMO orbitals are commonly known as *Frontier Orbitals* and were found to be extremely useful in explaining chemical reactivity. Electrophilic attacks were shown to correlate very well with atomic sites having high density of the HOMO orbital, whereas nucleophilic attacks correlated very well with atomic sites having high density of the LUMO orbital (Kunichi Fukui was awarded the Nobel prize in chemistry in 1981 for developing this concept). HOMO (Highest Occupied Molecular Orbital i.e MO 28) and LUMO (Lowest Unoccupied Molecular Orbital i.e MO 29) of I3C are shown in **fig 5 and fig.6** respectively. This was done theoretically using PM3.

Electronic properties: It is important to examine the E HOMO and E LUMO so as to explain the electronic properties of the complex. This was done theoretically using PM3. The positive and negative phases of the orbital are represented by the two colors, the blue regions represent an increase in electron density and the red regions a decrease in electron density.
However, these calculations were examined in the ground state and also in vacuum. It is possible to use them to get information by comparing them with similar compounds.

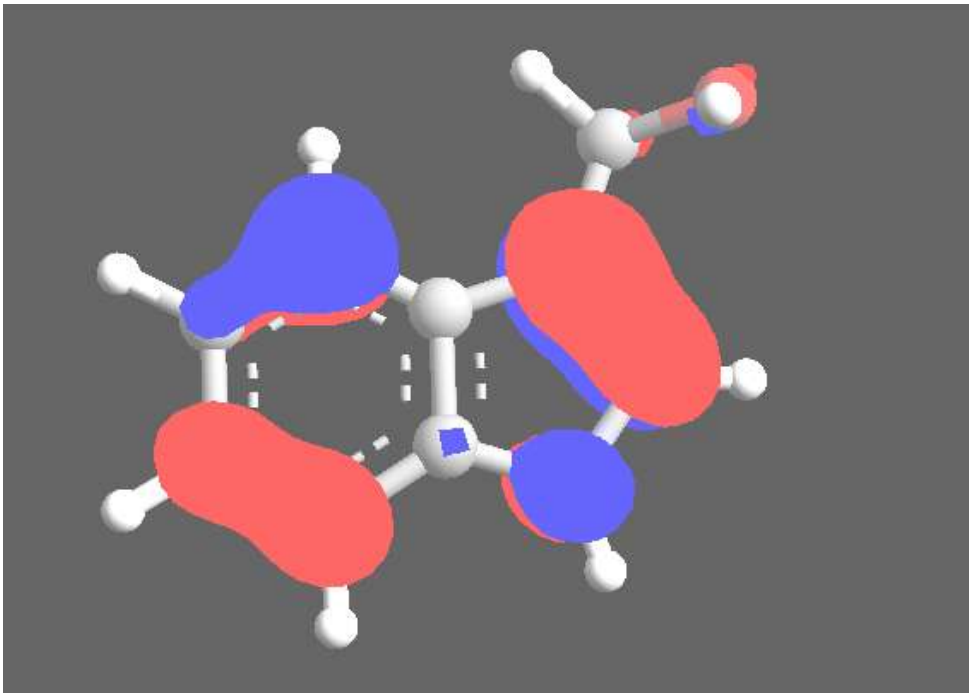


Fig.5 I3C HOMO--**Visualize the HOMO (Highest Occupied Molecular Orbitals) (MO 28) of** I3C**, blue shows positive and red shows negative.**

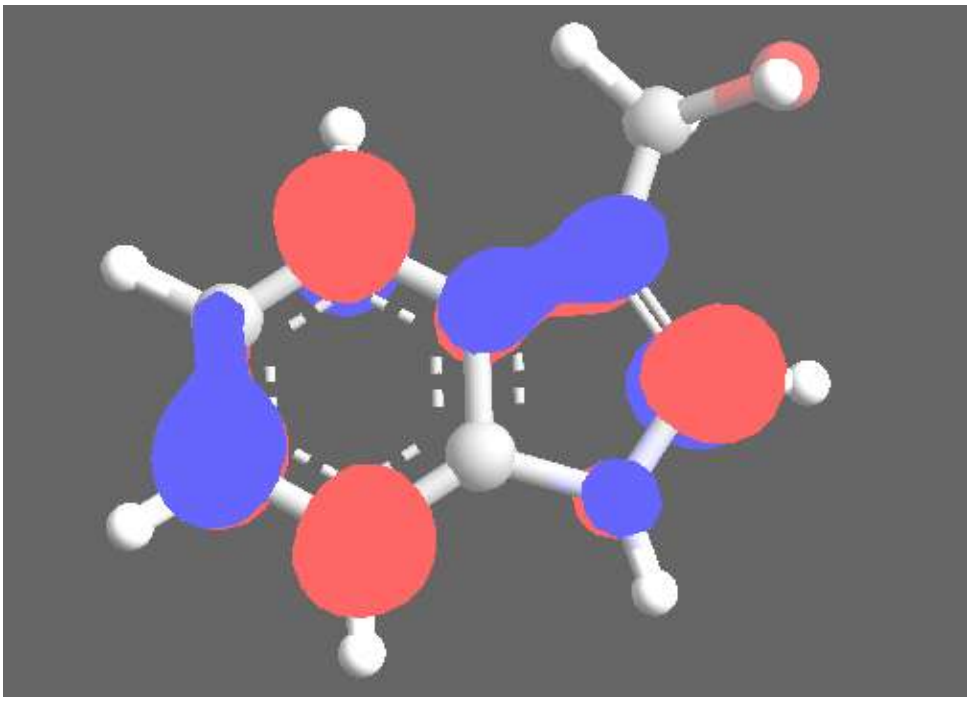


Fig. 6 Visualize the LUMO (Lowest Unoccupied Molecular Orbitals) (MO 29) of I3C, blue shows positive and red shows negative.

Electrostatic Potential (ESP) of Indole-3-carbinol (I3C)

Fig.7 shows the complete surface of **Indole-3-carbinol (I3C)** with the color map. This figure uses a clipping plane showing a cutaway of the same surface revealing the underlying molecular structure. The color map shows the ESP energy (in hartrees) for the various colors. The surface color reflects the magnitude and polarity of the electrostatic potential. The red end of the spectrum shows regions of highest stability for a positive test charge, magenta/ blue show the regions of least stability for a positive test charge.

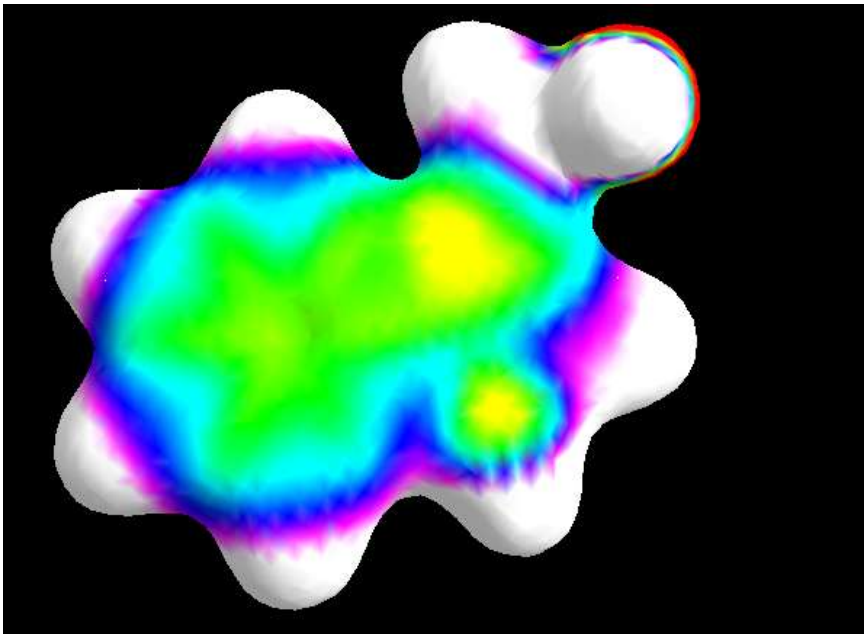


Fig.7 The complete surface with the color map of ESP of I3C---ESP MAPPED DENSITY

	0.0500 to 0.0409		**-0.0045 to -0.0136**
	0.0409 to 0.0318		**-0.0136 to -0.0227**
	0.0318 to 0.0227		**-0.0227 to -0.0318**
	0.0227 to 0.0136		**-0.0318 to -0.0409**
	0.0136 to 0.0045		**-0.0409 to -0.0500**
	0.0045 to -0.0045		

III. CONCLUSION

Hyperchem study on Indole-3-carbinol (I3C) compound is informative in understanding various physicochemical aspects of compounds. The HOMO and LUMO frontier orbital energies computed for the optimized molecule of Indole-3-carbinol (I3C) indicated that the above compound possess potential electron donor atoms.

The most energetically favorable conformation of conformation of **I3C** is found to have a heat of formation of -6.6437 kcal/mol via use of the Argus Lab software. The present work indicates that the best conformation of **I3C** is found to be at **-61.0179909654 au /-38289.4020** kcal/mol which is the minimum potential energy by using Argus Lab software. At this point **I3C** will be more active as anti cancerous agent.

Mulliken Atomic Charges and ZDO Atomic Charges and thermodynamic parameters were calculated and the data obtained from the calculated parameter are analysed and is found to be well within the accuracy of computational results.

In conclusion, these studies does not only presented us the opportunity to take a critical look at this novel compound but has also given us the opportunity to compile fundamental result on properties that cannot be calculated in the laboratory.

References

[1.] Cover CM et al.Cancer Res. 1999; 59 1244-51
[2.] McDonnell R., Food Chain Toxicol 1998; 26.59-70.
[3.] Rosen et al Otolaryngol Head Neck Surgery 1998. 118:810-815.
[4.] Shertzer et al. Biochem Pharmacol 1988; 37. 333-8
[5.] Bradlow, Ann. N Y Acad Science 1999; 889. 204-13
[6.] Bell et al, Gynecol oncol 2000; 78. 123-9
[7.] Wong GYC, Bradlow HL, Sepkovic DW, et al. J Cell Biol. 1988; 28:111-116

[8.] Bradlow HL, Michnovicz JJ, Wong GYC, et al. Cancer Epidemiol Biomarkers Prev. 1994; 3 :591-595.
[9.] Bradlow HL, Sepkovic DW, Telang NT, Osborne MP. Ann NY Acad Sci. 1999; 889:204-213.
[10.] Custodio JB, et al. 1994. Biochem Phearmacol 47:1989-98.
[11.] Devanaboyina U, et al. 1997. Proc Annu Meet am Assoc. Cancer Res 38:A2427. Grubbs CJ, et al. 1995. Clin Cancer Res 2:1439-44.
[12.] He Y-H, Freisen MD, Ruch RJ, Schut HAJ. Food Chem Toxicol. 2000; 38:15-23.
[13.] Michnovicz JJ, Bradlow HL. J Natl Cancer Inst. 1990; 50:947-950.
[14.] Niwa T, Swaneck G, Bradlow HL. Steroids. 1994; 59:523-527.
[15.] Shertzer HG, et al. 1988. Biochem Pharmacol 37:333-38.
[16.] Telang NT, et al. 1997. Proc Soc Exp Biol Med 216:246-52.
[17.] Thangaraju M, et al. 1994. Cancer 74:78-82.
[18.] Verhagen H, et al. 1995. Cancer Res 38:1410-13.
[19.] Yang JH, et al. 1999. Carcinogenesis 20:13-18.
[20.] Terry P, Wolk A, Persson I, Magnusson C. Brassica vegetables and breast cancer risk. J Am Med Assoc 2001; 285: 2975–77.
[21.] Keck AS, Finley JW. Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. Integr Cancer Ther 2004;3(1):5-12.
[22.] A.Broo, Per Lincoln, Ab- initio and Semi empirical Calculations of Geometry and Electronic Spectra of Ru complexes and Modeling DNA Binding, *Inorg. Chem*., 1997, 36, 2544- 2553.
[23.] RK.Gilpin , Predicting ID NMR Spectra – HyperNMR, Analytical Chemistry.,1995, 67, Iss 17, A 541.
[24.] O.vanciuc, Hyperchem Release 4.5 for Windows, Journal of Chemical Information and Computer Sciences.,1996., 36 , Iss 3 , 612-614.
[25.] WR.Winchester and MP-Doyle, Hyperchem, Journal of the American Chemical Society, 1992, 114, Iss 23, 9243.
[26.] W.Witanowski et al ,Solvent effects on the nitrogen NMR shieldings in oxadiazole systems, J .Magnetic Resonance .,1996, 120,Iss 2, 148-154
[27.] (a)HyperChem Software, Hypercube Inc Florida Science and Technology Park,1115 NW,4th Street, Gainesvilla, Florida, 326001, USA., 2006, 12 (b) HyperChem® Computational Chemistry, Part 1 &Part 2 Hypercube, Inc. Publication ,HC50-00-03-00,1996,21-67
[28.] Peng C, Ayali PY, Schlegel HB and Frisch MJ, (1995) J. Comp Chem., 16: 49-51.
[29.] Cramer CJ and Truhlar DG(1992), Computer-Aided Mol. Design, 6: 629-666.
[30.] Merz JKM and Kollaman PA (1989), . J. Am. Chem. Soc., 11(1): 5649-5658.
[31.] Still WC, Tempczk A, Hawley RC and Hendrickson T(1990) . J. Am. Chem. Soc.,112: 6127- 6129.
[32.] Martin YC (1998). Perspective in drug discovery and design. Springer Publisher, USA, 12th Volume, pp.3- 23.
[33.] Cruciani G, Clementi S and Pastor M (1998). GOLPEguided region selection. *Perspectives in Drug Discovery and Design*, 12-14(16): 71-86.
[34.] Dunn III and Hopfinger AJ (1998). Drug Discovery, Kluwer Academic Publishers. Chapter 12, pp.167-182.
[35.] Dewar MJS, Zoobisch EG, Healy EF and Stewart JJP(1985).). AM1: A new general purpose quantum mechanical molecular model. J. Am. Chem. Soc. 107: 3902-3910.
[36.] James J. P. Stewart, J. Comp. Chem., 10, 209-220 (1989) and 221-264, (1989)