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SYNTHESIS AND CONFORMATIONAL STUDIES OF N'-(3Z)-5-FLUORO-2-OXO-2,3-DIHYDRO-1H-INDOL-3-YLIDENE]-3-CARBOHYDRAZIDE WITH SPECIFIC PHARMACOPHORIC FEATURES.

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INTRODUCTION

Isonicotinic acid hydrazide (isoniazid,INH) and isatin are potential bioactive agents[1-5].Tuberculosis (TB) remains a major cause of mortality throughout the world. Resistance of Mycobacterium tuberculosis to antituberculosis drugs becomes very serious problem[6] Isonicotinic acid hydrazide (isoniazid, INH) belongs to the group of the first line antituberculosis drugs being in clinical practice over 50 years. Chemical modifications of isonicotinic acid hydrazide were performed on all parts of the molecule, but the activity of these derivatives against M. tuberculosis has not yet exceeded that of INH[7]. To overcome the resistance, combination of INH molecule with other active molecules is frequently applied[8]. This work was aimed at enhancing the antimycobacterial activity of INH by conjugation with keto group of 5 fluoro isatin and evaluate synergetic effect by conformational analysis using Argus lab, Ligand Scout and Marvin Sketch. The present work describes synthesis and the computer aided conformational analysis that is based on geometry optimization (active conformation) of drug by ArgusLab software. Argus Lab is the electronic structure program that is based on the quantum mechanics, it predicts the potential energies, molecular structures; geometry optimization of

structure, vibration frequencies of coordinates of atoms, bond length, bond angle and reactions pathway [9] Conformational analysis of molecule is based on molecular mechanics, it is method for the calculation of molecular structures, conformational energies and other molecular properties using concept from classical mechanics. A molecule is considered as a collection of atoms held together by classical forces. These forces are described by potential energy function of structural features like bond lengths, bond angles and torsion angles etc.
The energy (E) of the molecule is calculated as a sum of terms as in equation (1).

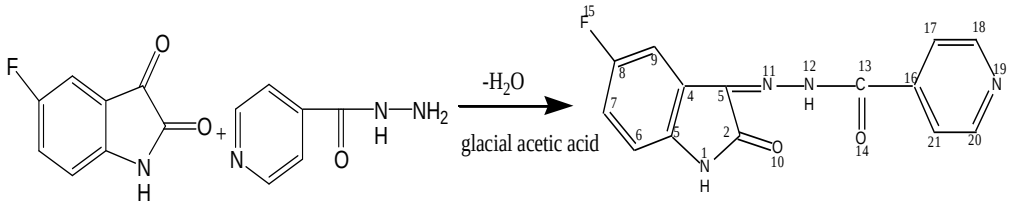
E = E stretching + E bending + E torsion + E Vander Waals + Eelectrostatic + E hydrogen bond + cross term.

These terms are of importance for the accurate calculation of geometric properties of molecules. The set of energy functions and the corresponding parameters are called a force field[10] The molecular mechanics method calculates the energy as a function of the coordinates and energy minimization is an integral part of method. A molecular geometry is constructed by using computer graphics techniques and the atoms moved are iteratively moved (without breaking bonds) using an energy minimization technique until the net forces on all atoms vanish and the total energy of the molecule reaches a minimum. The 3D (3 rotatable bonds) structure of molecule corresponding to this energy minimum is one of the stable conformations of molecule but not necessarily the most stable one [11]. Since the energy minimization methods can not move the molecule across energy barriers, the minimization of a trial molecule continues until the first local energy minimum is found. Other local energy minima including the lowest energy one, the global energy minimum, may be found by repeating the calculation with another start geometry or more efficiently. Conformation search methods random numbers are used to determine how many and which torsion angles and space to be incremented and which directions of the x, y, z, coordinates of each atoms are to be translated [12].

Synthesis Of N'-(3Z)-5-Fluoro-2-Oxo-2,3-Dihydro-1H-Indol-3-Ylidene]-3-Carbohydrazide (5FISO)

The product was synthesized by proceeded smoothly by condensing isoniazid with equimolar ratio of 5 fluoroisatin in presence of glacial acetic acid to form schiff's base[13] The crude solid product was filtered and washed twice with water . The product thus obtained was purified through recrystallization. The pure Sharp orange colour compound was dried in desicator over anhydrous calcium sulphate It showed the M^+ peak at m/z 284.0707 (HR-

EIMS) corresponding to the formula $C_{14}H_9N_4O_2F$ (calc. 284.2454).The purity of compound was checked by TLC using pre-coated silica gel, GF-254 and identified by spectral data with 73% yield Ultraviolet (UV) spectra was recorded in DMSO on a Hitachi U-3200 spectrophotometer. Infra Red (IR) spectra was measured on a Shimadzu IR 460 spectrophotometer using KBR disc. Mass spectra was determined on Massen spectrometer MAT 311A varian Bermen spectrometer (EIMS) and MAT95XP Thermo Finnigan (HR-EIMS). Nuclear magnetic resonance spectra (^{1}HNMR and ^{13}CNMR) was recorded in DMSO on AVANCE AV 300 spectrometer



5FISO

Spectral Data

^{1}HNMR (MeOD, 300 MHz) δ: 13.94 (s, 1H, NH-12), 11.425 (s,1H, NH-1), 8.87-8.852(m, 2H, H-18, H-20),7.792-7.771(m,2H, H-21,H-17), 7.45-7.42(d, J= 8.1Hz 1H, *H*-9), 7.29-7.225(m, 1H, *H*-6), 6.98-6.94 (m, 1H, *H*-7). **^{13}CNMR (DMSO, 75 MHz)** δ:163.07(C-13), 160.01(C-2), 156.85(C-8), 150.91(C-18),150.91 (C-20), 139.08(C-16),121.20(C-3),120.90(C-5),120.78(C-4),118.73(C-17), 118.42(C-21), 112.54(C-6),112.43 (C-7), 108.50(C-7),108.17(C-9). **EIMS m/z:** 284.1 (M^+ - $C_{14}H_9N_4O_2F$), 257.1, 256.1228.1, 178, 150.1, 122.1, 107. 1**HR-EIMS:** 284.0707 (M^+ - $C_{14}H_9N_4O_2F$) Calculated 284.2454. **IR□$_{max}$(KBr)cm^{-1}**: 3228.6,3163, 3055,2925, 2852.1, 1718.5,1629.7, 1593.1,1473.5,1217, 999.1, 829.3, 752.2, 657.7, 453.2. **UV λ_{max} (DMSO) nm:** 388, 324, 273, 226.

MATERIALS AND METHODS

The three dimensional quantitative structure activity relationships (3D-QSAR) describe the biological activity of molecule with pharmacological potential as a function of their structural properties[14,15]. Computational advances have generated many tools which are widely used to construct models, minimization and representations of molecular structure[16-18]. All conformational analysis (geometry optimization) study was performed on a window based computer using Argus lab and ACD Lab Chem Sketch software's. The chemical structure of showdomycin [19] was refined by X-ray crystallography technique. The showdomycin molecule is utilized to determine 3D structure of molecule. Several computer programs were used to infer the shape of molecule from geometry optimization calculations. The

showdomycin structure is generated by Argus lab, and minimization was performed with the semi-empirical Austin Model 1 (AM1) parameterization [20]. The minimum potential energy is calculated by using geometry convergence function in Argus lab software. In order to determine the allowed conformation the contact distance between the atoms in adjacent residues is examined using criteria for minimum Vander Waal contact distance [21]. 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 and visualized the molecular orbital and making an electro static potential mapped and electron density surface. The minimum potential energy was calculated for drug receptor interaction through the geometry convergence map.

RESULTS AND DISCUSSION

Forthcoming observation and active conformation of 5FISO are shown in Figure1. Figure 2 shows electron density surface of 5FISO molecule. Electron density shows the location of electrons. Large values of electron density show atomic position and chemical bonds, while smaller values will indicate over all size of molecules. Electron density surface gives the shape of the surface of the molecule. An electron density will always show as a positive valued surface. The electron density can not be negative. In compound 5FISO shows blue surface of electron density map. This map shows positive value of electron density. It means no of electron in this molecule shows highest stability for positive test charge. Figure 3 shows electrostatic potential mapped of 5FISO. The molecular electrostatic potential is the potential energy of a proton at a particular location near a molecule. Negative electrostatic potential corresponds to a attraction of the proton by the concentrated electron density in the molecules (from lone pairs, pi-bonds, etc.) Colored in shades of red. Positive electrostatic potential corresponds to repulsion of the proton by the atomic nuclei in regions where low electron density exists and the nuclear charge is incompletely shielded colored in shades of blue. In this figure two red regions are surrounded the carbonyl carbon of this compound that are showed negative electrostatic potential. It means high electron density is present due to carbonyl carbon. And large blue region is covered whole molecule except two carbonyl carbon. It shows positive electrostatic potential where low electron density exists. Electrostatic potential surfaces and electron density map represent a useful computational tools to discuss drug receptor interaction. Electrostatic potential surfaces are valuable in computer-aided drug design because 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 Figure 4 shows molecular orbital of 5FISO compound. Molecular orbital theory, detailed explanation of how electrons are distributed in stable molecules. In the simpler valence theory of the chemical bond, each atom in a molecule is assumed to retain its own electrons. Even when electrons are shared, as in the covalent bond, it is possible to identify which electron came from which atom. The positive and negative phases of the orbitals are represented by two colors, the blue region represent an increase electron density and the red region a decrease in electron density. Atomic coordinates of 5FISO are given in table 1 by using geometry optimization process. It is based on molecular mechanics calculations. Bond angles and bond lengths are given in table 1 and 2. The minimum potential energy is –0.5469 K.cal/mole. Which are taken from geometry optimization process by using molecular mechanics calculation. The minimum potential energy function is used to define drug receptor binding interaction. The minimum potential energy function of 5FISO has shown potent activity. In modern computational chemistry, pharmacophores are used to define the essential features of one or more molecules with the same biological activity. A database of diverse chemical compounds can then be searched for more molecules which share the same features arranged in the same relative orientation. Figure 5 shows pharmacophoric features of 5FISO molecule. In this figure five hydrogen bond acceptor groups (HBA), three hydrogen bond donor groups (HBD) and three aromatic ring are present. These functional groups represent in the molecule for the drug's action. Electron density map , electrostatic potential surface, molecular orbital and pharmacophoric features of 5FISO describe active sites of drug receptor binding interactions, with different functional charged groups.

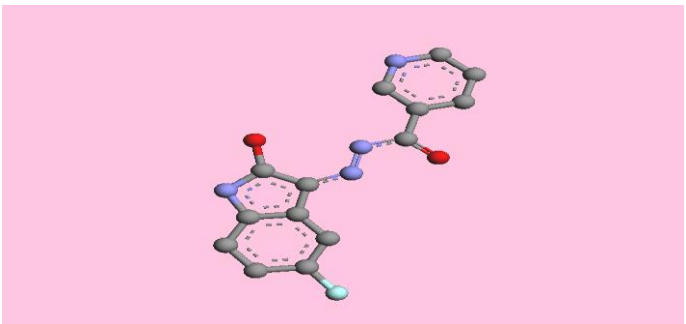


Figure 1. Prospective view of

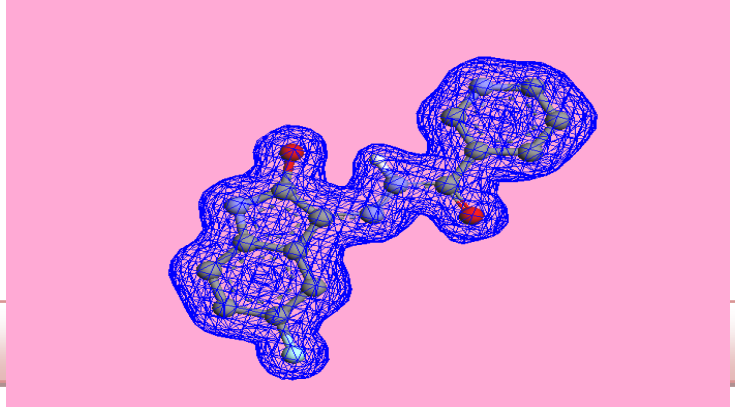


Figure 2.Electron Density Surface of 5FISO Molecule Active 5FISO

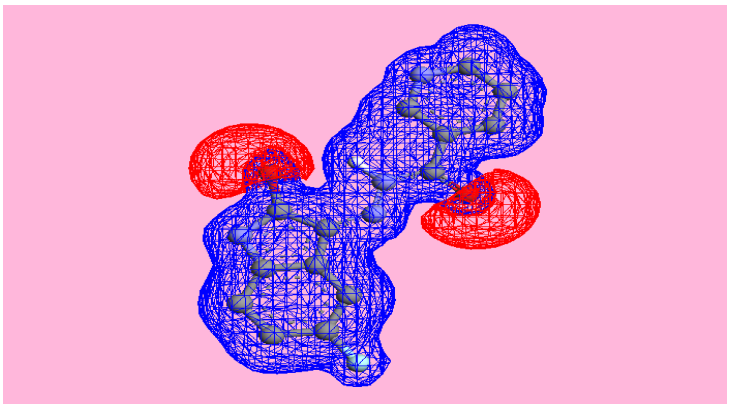


Figure 3: Electrostatic Potential Map of 5fiso.

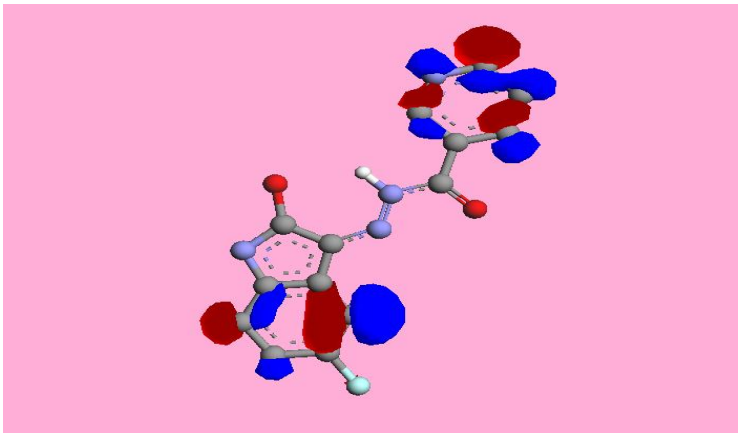


Figure 4: Visualize Molecular Orbitals

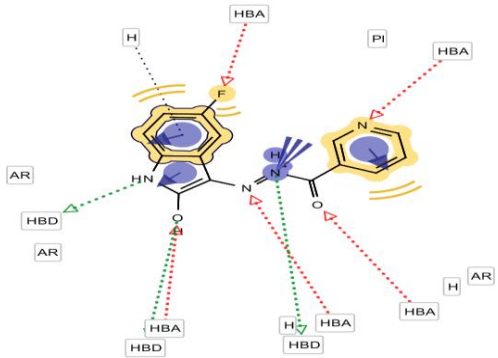


Figure 5. Pharmacophoric Features of 5FISO.

Table1: Atomic Coordinates of 5FISO.

Atoms	x	y	z
C1	-3.194760	-5.455608	0.997571
C2	-3.769340	-6.719346	1.213495
C3	-5.155052	-6.845438	1.451449
C4	-5.984982	-5.709584	1.475617
C5	-5.390209	-4.467896	1.257331
C6	-4.041918	-4.350739	1.027419
N7	-6.011216	-3.258950	1.258679
C8	-3.772529	-3.016730	0.866763
C9	-5.002937	-2.367433	1.016284
O10	-5.226375	-0.976990	0.972273
N11	-2.531109	-2.508716	0.650070
N12	-2.254613	-1.302638	0.467148
C13	-0.958270	-0.920372	0.302352
C14	-0.597577	0.499033	0.061784
015	-0.035062	-1.784475	0.364144
C16	0.738656	0.911515	0.154854
C17	-1.569228	1.452415	-0.270646
N18	-1.213057	2.757807	-0.490633
C19	0.091574	3.165900	-0.392869
C20	1.087333	2.244469	-0.068769
F21	-2.937313	-7.892623	1.201347
H22	-3.005416	-0.575402	0.438836

Table 2: Bond length of 5FISO

S.No.	Atoms	Bond length
1.	C1…C2	1.379256
2.	C1…C6	1.379256
3.	C2…C3	1.379256
4.	C2…F21	1.439434
5.	C3…C4	1.379256
6.	C4…C5	1.379256
7.	C5…N7	1.356681
8.	C5…C6	1.379256
9.	C6…C8	1.379256
10.	N7…C9	1.356681

11.	C8…C9	1.379256
12.	C8…N11	1.343384
13.	C9…O10	1.407689
14.	N11…N12	1.243512
15.	N12…C13	1.346235
16.	N12…H22	1.048529
17.	C13…C14	1.461000
18.	C13…O15	1.260307
19.	C14…C16	1.379256
20.	C14…C17	1.379256
21.	C16…C20	1.379256
22.	C17…N18	1.356681
23.	N18…C19	1.356681
24.	C19…C20	1.379256

Table 3: Bond Angles of 5FISO.

S.No.	Atoms	Bond Angles
1.	C2…C1…C6	120.000000
2.	C1…C2…C3	120.000000
3.	C1…C2…F21	120.000000
4.	C1…C6…C5	120.000000
5.	C1…C6…C8	120.000000
6.	C3…C2…F21	120.000000
7.	C2…C3…C4	120.000000
8.	C3…C4…C5	120.000000
9.	C4…C5…N7	120.000000
10.	C4…C5…C6	120.000000
11.	N7…C5…C6	120.000000
12.	C5…N7…C9	120.000000
13.	C5…C6…C8	120.000000
14.	C6…C8…C9	120.000000
15.	C6…C8…N11	120.000000
16.	N7…C9…C8	120.000000
17.	N7…C9…O10	120.000000
18.	C9…C8…N11	120.000000
19.	C8…C9…O10	120.000000
20.	C8…N11…N12	120.000000
21.	N11…N12…C13	120.000000
22.	N11…N12…H22	120.000000
23.	C13…N12…H22	120.000000
24.	N12…C13…C14	120.000000
25.	N12…C13…O15	120.000000
26.	C14…C13…O15	120.000000

27.	C13…C14…C16	120.000000
28.	C13…C14…C17	120.000000
29.	C16…C14…C17	120.000000
30.	C14…C16…C20	120.000000
31.	C14…C17…N18	120.000000
32.	C16…C20…C19	120.000000
33.	C17…N18…C19	120.000000
34.	N18…C19…C20	120.000000

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