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Molecular properties prediction, docking studies and antimicrobial screening of ornidazole and its derivatives

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ABSTRACT

5-Nitroimidazole derivatives have been focused in the past decades due to their remarkable biological and pharmacological activities.Ornidazole is a 5-nitroimidazole derivative drug which has antimicrobial action. It is used in the treatment of protozoal infections, and also in the treatment of amebiasis, giardiasis, trichomoniasis and prophylaxis of anaerobic bacterial infections. This study presents DFT study on bio-active Nitro heterocyclic compounds -orinadazole and with modified orinadazole. For the compounds, initial geometry optimizations were carried out with DFT. The lowest energy conformations of the compounds obtained by the Density Functional Theory [DFT] method by employing Becke's three-parameter hybrid functional [B3LYP] and 6-311++G [d,p] basis set. In this study, physicochemical, pharmacological and pharmacokinetic properties in addition to bioavailability of ornidazole and its derivatives are discussed. Ornidazole and its derivatives were subjected to Molinspiration, Molsoft and Osiris programs to predict their molecular properties that are important for drug candidates. Subsequently, all of them were docked into the active sites of proteins namely 1ucf [Anti-Parkinson protein], 3k21 and 4wvd [Anti-Parasitic protein], 4e9u [Anti-Infective protein], 3hj3 [Anti-protozoal protein], 1jk2 and 3wx4 [Anti-Bacterial protein] were considered in antimicrobial studies of Ornidazole and its derivatives. Since all compounds fulfilled the criteria for good membrane permeability, oral bioavailability, low toxicity and the potential inhibitory activities towards all protein and their antimicrobial activity has been tested. The present work deals with the insilico *docking studies of all target proteins inhibitors with ornidazole and its derivatives. The* insilico *docking studies were carried out using AutoDock Vina 1.1.2 program. The docking energy of fluro-ornidazole with 4e9u shows binding energy -5.6 kcal/mol whereas ornidazole shows binding energy -5.3 kcal/mol. Fluro-ornidazole with 3hj3 shows binding energy -5.8 kcal/mol whereas ornidazole shows binding energy -5.1 kcal/mol. These results clearly indicate that the fluro-ornidazole has similar or higher binding sites and interactions with all the proteins compared to the standard drug ornidazole.*

Keywords: Antimicrobial action, molecular docking, molecular properties prediction.

INTRODUCTION

Approximately, out of 100,000 proteins in the human proteome, only about 500 are currently targeted out of which approximately 40,000 drugs were only approved worldwide [1]. This suggests that there is considerable scope for identification of new targets and for the design and discovery of new drugs. On the other hand, the current average failure rate for drugs in clinical trials is 81% [2]. A lack of efficacy causes 30% of these failures and concerns with toxicological and clinical safety account for another 30% [3]. Failure in the later stages of drug development is very expensive, particularly if a potential drug reaches phase II or III clinical trials before problems emerge. These issues emphasize the importance of identification of molecules that are likely to reach the market at the early stage of drug discovery. In 1991, the industry observed a failure rate of 40% due to bioavailability and pharmacokinetic issues [3] but incorporation of ADME [absorption, distribution, metabolism, and excretion] principles earlier in the drug development process to eliminate weak candidates has reduced failures for these reasons to 10% [4]. This approach

has been facilitated by development of theoretical methods for prediction of "drug-like" properties of small molecules over the last 20 years. These have ranged from the simple, but effective and widely accepted, Lipinski "rule of five" [5] to sophisticated algorithms for prediction of ADME properties [6,7]. Input to these algorithms has been facilitated by use of text-based molecular representation through the powerful SMILES approach [8].

Structure-based drug design against protein targets has been facilitated by the growing number of protein structures. However, not all these proteins are targets for drug design. The number of "druggable" proteins [those that may be targets for drugs] was first addressed in 2002 by Hopkins and Groom, [9] with an upper estimate of 1500 and more recent reviews13, 14 suggest that this number remains valid. A goal of structure-based drug design [10] is to use structures of these proteins, or homology models derived from related proteins, to identify lead compounds computationally. The main method is molecular docking [11, 12] in which an attempt is made to locate molecules in a binding site using stereo chemical and energetic considerations, with various simplifying assumptions depending on the required throughput.

Appreciation of the science of structure-based drug design requires familiarity with structural biology, thermodynamics, molecular association, stereochemistry and computational methods; while appreciation of the biopharmaceutical properties of drugs requires an understanding of cell biology, physiology, preclinical formulation, and physical organic chemistry. The subject of structure-based drug design is only properly understood in the context of an integrated approach, this is a key in commercial drug design and it is also required in teaching of drug design. Here, a computational approach using Web-based software is shown to be effective for this purpose. The most effective approach is to require to measure interactions between the ligand and the protein, which provides an understanding of contacts based on hydrogen bonding, electrostatics, and hydrophobic association. Preparation of images with coloring of key amino acids involved in the ligand interaction is also required. These activities provide a basis for modification of the ligand.

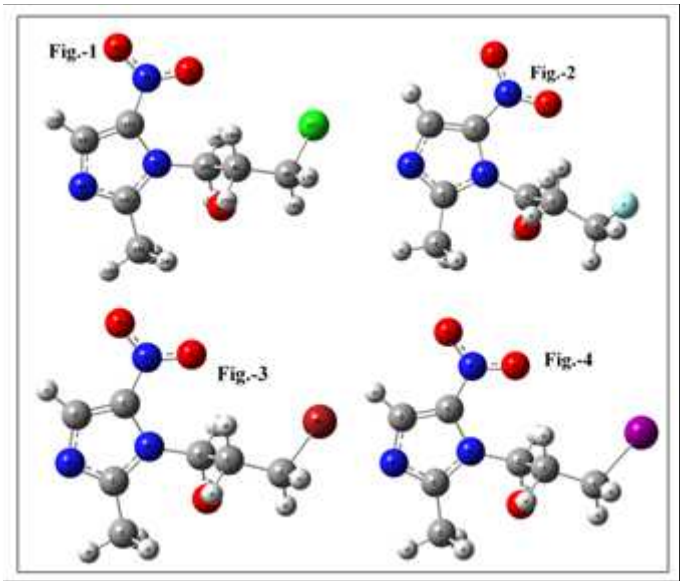

Fig. 1 – optimized Molecular structure of ornidazole (compound-I)
Fig. 2 -. optimized Molecular structure of Fluro-ornidazole (compound-I-a)
Fig. 3 - optimized Molecular structure of Bromo-ornidazole (compound-I-b)
Fig. 4- optimized Molecular structure of Iodo-ornidazole (compound-I-c)

Ornidazole, chemically known as 1-chloro-3- [2-methyl-5-nitro-1H-imidazol-1-yl] -2 proponol [Fig.1].The structure of Ornidazole is of some interest as it possesses a strong electron attracting nitro group as well as a strong electron donating OH group and has the possibilities of intramolecular as well as intermolecular hydrogen bonding. Internal rotation about a C-C bond may result in several isomeric conformations. Ornidazole is a 5-nitroimidazole derivative drug which has antimicrobial action. It is used in the treatment of protozoal infections and also in the treatment of prophylaxis of anaerobic bacterial infections.Parasitic and bacterial infections affecting the gastrointestinal tract represents a significant cause of morbidity and mortality worldwide. Nitro heterocyclic drugs have been available since the early 1960s for the treatment of anaerobic protozoa. The application of these drugs has widened and they are presently used to treat anaerobic pathogenic bacteria and protozoa. 5-nitroimidazoles are a well-established group of antiprotozoal and antibacterial agents that inhibit the growth of both anaerobic bacteria and certain anaerobic protozoa, such as Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia. Ornidazole exerts a

rapid and reversible antifertility effect in male rats [13]. Ornidazole is administered orally, vaginally, or intravenously.It has been used for amebic liver obscesses, duodenal ulcers, giardiasis, intestinal lambliasis and vaginitis [14-16]. Ornidazole has recently been used with success in patients with active chron's disease [17]. Ornidazole is one of the most frequently used antibiotics for curing Helicobacter pylori infection. Ornidazole has also been preferred for surgical prophylaxix because of its longer elimination half life and excellent penetration in to lipidic tissues versus other nitroimidazole derivatives theraphy [18-19].

To reduce side effects of the drugs, it is of interest to develop new agents for the topical use of modified orinadazole for the long-term management of several diseases. For the researchers, the prospect of overcoming the systemic side effects of a drug, achieving an effect at a much lower dose, is very attractive. Modification of the structure of a known drug is one way to develop new drugs. The purpose of this study is to take a known medicinal drug and modify its structure by adding, deleting or changing something. After, all the information is known, such as the drug's structure, in this case orinadazole, one can make modifications. Also it is important to know how the drug functions for the treatment of the illness it targets. Orinadazole cures the illness but the treatment comes with side effects and in this study I have planned to add fluorine [F], Bromine [Br] and Iodine instead of chlorine as shown in figures [2-4] respectively. Fluorine makes it more polar so it can be more soluble and also overcome the side effects from its calculated *Silico* Physicochemical and Drug-Likeness Properties values. Once the structure is modified with fluorine, the drug may cure without any undesirable side effects.

Several protein structures which have cocrystallized fluorine-containing ligands were examined and geometrically inspected in several studies. All the fluorine-containing ligands collected from the PDB were docked within their corresponding protein binding sites, introducing the fluorine hydrogen bonding contribution improves the results of the docking experiments in terms of accuracy and ranking.

Fluorine atoms are often present in drugs and druglike molecules as proven by the relatively frequent occurrence [14.4%] in the refined MDDR database of fluorine-containing molecules [20-21]. In fact, fluorine atoms are often introduced in drug skeletons to modify pharmacokinetic properties, such as oral absorption[22] and to occupy key positions[23] where they will modulate metabolic reactions,[24]blocking metabolic routes of oxidation[25-26] . Thus, knowledge of the nonbonding behavior of covalently bonded fluorine is necessary to approach any kind of molecular modeling problem correctly, whether it is molecular recognition or pharmacokinetics and metabolism. Evidence regarding fluorine hydrogen bonding has largely been published from the 1960s,[27] and in the recent decades the hydrogen bonding acceptor capabilityof all the halogen atoms has often been investigated, in both their ionic and bonded forms. It is commonly accepted that fluorine is a stronger acceptor than the other halogens [27] but is not as strong as oxygen and nitrogen, [28-30] whereas in their ionic [31-34] and metalbonded [35] forms all the halogens act as considerable proton acceptors. The acceptor capability of halogenated compounds was quantitatively measured by Laurence and Berthelot [36] who used FT-IR technique to produce a broad scale of hydrogen bonding acceptor capability. In this scale fluorinated compounds exhibit substantially different values from those of their heavier halogen counterparts, as the latter are very weak acceptors comparable to ∏ bases.[36-37]The survey by Howard and co-workers[38]of the X-ray data stored in the Cambridge Structural Database [CSD][39] revealed short contacts of fluorine atoms to acidic hydrogen and was reinforced by theoretical calculations from which half of the binding energy of a hydrogen bond to oxygen was assigned to the fluorine. Furthermore, the large scale analysis conducted by Shimoni and Glusker[40] in the CSD revealed that acidic hydrogen prefer to bind to the stronger acceptors such as oxygen and nitrogen compared to fluorine atoms. The geometry of hydrogen bonds involving fluorine's was only investigated on small molecule contacts in the CSD[41] although the amount of deposited crystal structures on the Protein Data Bank archive[42] has increased significantly through the years [1000% of growth in the last 10 years] [43].

Ornidazole and Nitroimidazole derivatives were low molecular weight anti-microbial compounds with excellent activity against anaerobic microorganisms. For this purpose, I have reported modified orinadazole [Fluro-orinadazole] which is the low molecular weight with no bad effect in mutagenic, tumorigenic, irritating and reproductive effects compared to the standard drug ornidazole and so selected the subject of the present study.

In the light of the above findings and we undertook rational molecular design of novel antimicrobials in the class of orinadazole and its derivatives. To this, all the proposed compounds were initially submitted to Molinspiration [44], ALOGPS 2.1 [45], molsoft [46] and Osiris [47] programs to predict their molecular properties that are important for drug candidates. Subsequently, we docked all of these molecules into the active sites of the proteins that were considered in antimicrobial studies mentioned above as well as into the active site of all the proteins which is a well-recognized target for Anti-Parkinson, Anti-Parasitic, Anti-Infective, Anti-protozoal and Anti-Bacterial activities of 5-Nitroimidazole -based compounds. Finally, since all compounds fulfilled the criteria for good membrane

permeability, oral bioavailability, low toxicity and potential inhibitory activities towards 1ucf, 3k21 and 4wvd,4e9u,3hj3,1jk2 and 3wx4 proteins.

Computational Details

All of the electronic structure calculations were performed using density functional theory [DFT] as implemented in the Gaussian 09 programs [48]. Becke's three-parameter hybrid B3 functional [49] is used, along with the correlation functional of Lee–Yang–Parr [LYP] [50]. The standard 6-311++G [d, p] basis set is used for the geometric optimization. The DFT methods, especially the B3LYP hybrid DFT method, have been shown to reproduce accurate geometries [51], Ground-state optimized geometries of the molecules are calculated without any geometrical restriction, except those enforced by symmetry. The title molecules are found to be minima on their respective potential energy surfaces as revealed by the lack of imaginary frequencies. To obtain the true minimum energy for the potential energy surface scan of the title molecules as shown in fig. [5].

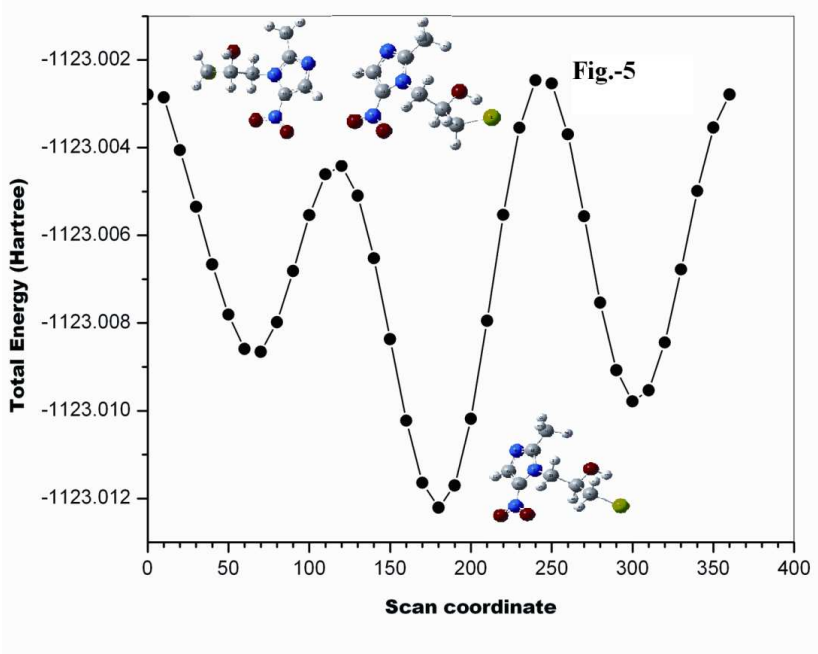

Fig. 5- Conformation analysis of ornidazole

Drug likeness and molecular property prediction:

The second part of the integrated approach is to examine the biopharmaceutical properties of the original ligand and modified ligands. The rule of five [52], the number of rotatable bonds [nrotb], and polar surface area [PSA] [53] were determined using the Molinspiration program. In these calculations log*P* [octanol/water partition coefficient] is calculated as a sum of fragment based contributions and correction factors based on the methodology developed by Molinspiration while PSA is calculated based on the methodology published by Ertl *et al*. [53] as a sum of fragment contributions O– and N– centered polar fragments. Absorption [%ABS] was calculated by equation: %ABS = 109-0.345×PSA [54] .Solubility [AlogPs] was calculated using ALOGPS 2.1 program. These kinds of results provide an excellent basis for understanding the biopharmaceutical issues associated with ligand design. These kinds of results provide an excellent basis for understanding the biopharmaceutical issues associated with ligand design. Most of the properties violate the Lipinski rules, including the very negative LogP [miLogP], and the related high numbers of hydrogen bond acceptors and donors. This provides a good basis for discussion of the likely difficulty of oral delivery of this agent, based on its probable poor absorption properties. In addition, the number of rotatable bonds [nrotb] is high, and this allows discussion of potential affinity problems and the need to build in rigidity in the molecular structure.

ALOGPS program by Tetko *et al*. [55-57] is based on the largest data base and is most thoroughly evaluated. The method was developed on the basis of neural Network ensemble analysis of 12908 organic compounds from the PHYSPROP database of the Syracuse Research Corporation. The theoretical toxicity properties [mutagenicity, tumorigenicity, irritating and reproductive effects], druglikeness and drug-score values were calculated in the Osiris Property Explorer. The prediction of the Osiris calculations is a fragment-based approach and the occurrence frequency of each fragment is determined within the collection of traded drugs and within the supposedly nondrug-like collection of commercially available chemicals [58]. Drug-likeness score predicts an overall drug-likeness score

using a MolSoft's program. Drug likeness may be defined as a complex balance of various molecular properties and structure features, which determine whether particular molecule is drug or non-drug.

DOCKING STUDIES

The docking simulations were performed using AutoDock Vina 1.1.2 program [59], Current distributions of AutoDock consist of two generations of software: AutoDock 4 and AutoDock Vina. AutoDock 4 actually consists of two main programs: autodock performs the docking of the ligand to a set of grids describing the target protein; autogrid precalculates these grids. AutoDock Vina does not require choosing atom types and pre-calculating grid maps for them. Instead, it calculates the grids internally, for the atom types that are needed [60]. AutoDock Vina achieves an approximately two orders of magnitude speed-up compared with the molecular docking software developed in AutoDock4, while also significantly improving the accuracy of the binding mode predictions. Further speed-up is achieved from parallelism, by using multithreading on multicore machines. AutoDock Vina automatically calculates the grid maps and clusters the results [59]. Models of protein binding sites were based on the structure deposited in the Protein Data Bank[61] under the PDB ID 3k21[62], 4wvd[63] , 1ucf [64] ,4e9u[65] ,3hj3[66],1kj2 [67] and 3wx4 [68] were employed. Settings for input grid boxes are given in details in Supplementary data. Default docking parameters and flexible space of 24x24x24 A3 were validated by re-docking native ligand which docked exactly in the position present in the crystal structure. Subsequently, all ligands were docked using same docking parameters. We have inspected the first 10 poses for each compound and no meaningful differences in binding mode have been noticed. Consequently, in the docking studies, we have focused only on the best ranked pose.

Pharmacophoric Mapping

Pharmacophoric mapping which involves ligand interaction patterns, hydrogen bond interaction, hydrophobic interactions was evaluated using Accelrys Discovery Studio 3.5 DS Visualizer [69].

RESULTS AND DISCUSSION

Molecular properties such as membrane permeability, hydrophobicity and bioavailability are associated with some basic molecular descriptors such as log *P* [partition coefficient], log S [solubility], molecular weight, number of hydrogen bond acceptors and donors in a molecule. The application of guidelines linked to the concept of drug-likeness has gained wide acceptance as an approach to reduce attrition in drug discovery and development [70]. The knowledge of oral bioavailability, membrane permeability and toxicity for a series of drug-like molecules is a fundamental step for the optimization of their design, synthesis and biological applications. One of the major prerequisites for the selection of orally bioavailable drug candidates is the prediction of their absorption properties [71]. It is generally accepted that some of the physicochemical descriptors of the drug molecules can be useful for predicting drug absorption. Lipinski *et al*. [52] proposed "the rule of five" according to which orally active drugs should have [a] a molecular weight under 500 Daltons, [b] limited lipophilicity [expressed by log$P \leq 5$], [c] a maximum number of 5 hydrogen bond donors [expressed as the sum of OH and NH groups], and [d] a maximum number of 10 hydrogen bond acceptors [expressed as the sum of Nand O atoms]. Molecules violating more than one of these rules are not expected to be viable drug candidates. In addition to the molecular properties discussed by Lipinski, other parameters have been applied as a computational filter for membrane permeability and oralbioavailability. Among recently published articles, a few should be mentioned. Zhao *et al*. reported [55] that the absorption of a drug is usually very low if the calculated solubility is <0.0001 mg/L. The solubility parameter, log S, is another important parameter for determining drug likeness. The absorption of a compound is considerably influenced by its solubility. Generally, high log S values correspond to good absorption. Over 80% of the marketed drugs have log S >-4, which corresponds to the solubility of 0.1 mmol/l [72]. Further, in a large study based on over 1100 drugs candidates, Veber *et al*. [73] proposed that the descriptors molecular flexibility [rotatable bond count $\leq$ 10] as well as polar surface area [PSA $\leq$ 140 A2] or total hydrogen bound count [ΣCad $\leq$ 12 H-bond donors and acceptors] correlate with oral bioavailability in rat. These findings are in agreement with those presented by Refsgaard *et al*. [71]. Also, Raevsky *et al*. [74] showed that the best descriptor of human intestinal absorption of 32 passive transported compounds is the sum of H-bond donors and acceptors [ΣCad] which should be $\leq$ 8. Palm *et al*. [75] found that PSA was the best descriptor to differentiate poorly absorbed drugs at an early stage of the drug discovery process and drugs that are completely absorbed had PSA $\leq$ 60 A^2 while drugs that are < 10% absorbed had PSA $\leq$140 A^2. Molecular polar surface area [PSA] is a very useful parameter for the prediction of drug transport properties. It is used to estimate the percentage of absorption. PSA and volume are inversely proportional to %ABS. Number of rotatable bonds dictates the conformational changes of molecules under study, which consequently decides binding of receptors or channels. They should be $\leq$10 to have good oral bioavailability.

Table 1. *In Silico* Physicochemical Properties of Proposed ornidazole and its derivatives. Important for Membrane Permeability and Oral Bioavailability*

Compound	MW	logP	logS	HBD	HBA	NROTB	AlogPs	**ΣCad**	PSA	%ABS	Viol.
Rule											
Lipinski [18]	**<500**	**≤ 5**		**<5**	**<10**						0
Zhao [21]							**>0.0001**				0
Veber [48]						**≤10**		**≤ 12**	**≤ 140**		0
Raevsky [49]								**≤ 8**			0
Palm [50]									**≤ 60**		17
Ornidazole Compound-I 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitro-1*H*imidazole	219	0.12	-1.82	1	4	4	0.37	5	83.9	80	0
Fluro- Ornidazole Compound-I-a (X=F) 1-(3- Fluro -2-hydroxypropyl)-2-methyl-5-nitro-1*H*imidazole	203	-0.18	-1.61	1	4	4	-0.30	5	83.9	80	0
Bromo- Ornidazole Compound-I-b (X=Br) 1-(3- Bromo -2-hydroxypropyl)-2-methyl-5-nitro-1*H*imidazole	213	0.26	-2.06	1	4	4	0.76	5	83.9	80	0
Iodine- Ornidazole Compound-I-c I(X=I) 1-(3-Iodo-2-hydroxypropyl)-2-methyl-5-nitro-1*H*imidazole	311.08	0.53	-2.24	1	4	4	0.87	5	83.9	80	0

**MW- molecular weight; logP, logarithm of compound partition; HBD, number of hydrogen bond donors; HBA, number of hydrogen bond acceptors; NROTB, number of rotatable bonds; AlogPs, solubility; ΣCad, total hydrogen bound count; PSA, polar surface area in A; %ABS, percentage of absorption; viol, violations*

The results disclosed in Table **1** show that, except parameter proposed by Palm [75], all candidate compounds satisfy predictions of good membrane permeability and oral bioavailability and, thus, might be considered as drug-like molecules. In Table-**1**, the calculated absorption percentage [%ABS] is also presented. As seen, all candidate compounds exhibited a great %ABS ranging from 80. Subsequently, we have used the Osiris program for calculating the fragment based drug-likeness of the proposed compounds also comparing them with ornidazole, Chloramphenicol and fluconazole the standard drugs. A positive drug likeness value [0.1-10] points out that a molecule contains predominantly fragments which are frequently present in commercial drugs. As seen in Table-**2**, although studied compounds present low or even negative drug-likeness value, all of them with the exception of compound **I-b** [-3.69] have still better drug-likeness values than chloramphenicol [-4.61]. The Osiris calculations allowed us also to predict the overall toxicity of the candidate drugs as it may point to the presence of some fragments generally responsible for the irritant, mutagenic, tumorigenic, or reproductive effects in these molecules. The Osiris study revealed that all compounds with the exception of I-**a present** a high *in silico* toxicity risk profile. In this study we also verified the drug-score, which combines drug-likeness, lipophilicity, solubility, molecular weight, and toxicity risks in one value and this may be used to judge the compound's overall potential to qualify for a drug. As seen from results collected in Table-**2**, the target compounds showed moderate to good drug-score [0.25-0.82] that revealed their potential as safe lead compounds. Interestingly, the Fluro- ornidazole [81%] Compound has better drug-score value than Ornidazole [26%].

The values of log *P* ranged from -0.18 to 0.53 for all designed molecules, while the values of log S were between -1.61 and -2.24. Both of these set of values are well within the accepted ranges for drug like molecules, as described. The polar surface areas [PSA] of all 3 molecules are less than 84 $Å^2$ [well below the "drug like" value of 140 $Å^2$]. The percentage of absorption [% ABS] calculated was found to be greater than 80 for all the molecules. They are small in size [molecular weights are less than 332 g/mol] also. All designed molecules have rotatable bonds 4, 4 H-bond acceptors, and 1H-bond donors. Compounds I-a, I-c showed positive drug-likeness values, ranging from 0.39 to 0.61 and compound I-b gave negative value for drug-likeness -3.69. Osiris calculations for 15000 nondrug like chemicals and 3300 marketed drugs found that about 80% of the marketed drugs had positive values of the drug likeness parameter, while almost all the nondrug like chemicals had negative values [78]. A positive value of drug likeness indicates that the molecule consists mostly of building blocks [fragments] that are commonly found in marketed drugs.

Table 2. Drug-Likeness Properties of Target Compounds

Compound	Toxicity Risksa[a]				Drug-Likeness	Drug score%
	M[b]	T[C]	I[d]	R[e]		
Ornidazole I	(0.8) ±	(0.6) +	(1.0) -	(0.6) +	1.82	26
Fluro- Ornidazole Compound I-a(X=F)	(1.0) -	(1.0) -	(1.0) -	(1.0) -	0.61	81
Bromo- Ornidazole Compound I-b(X=Br)	(0.6) +	(1.0) -	(0.6) +	(0.6) +	-3.69	11
Iodine- Ornidazole Compound I-c(X=I)	(0.6) +	(0.6) +	(1.0) -	(1.0) -	0.385	27
fluconazole	-	-	-	±	-1.13	46
chloramphenicol	+	+	-	-	-4.61	10

[a]Ranked according to: (-) no bad effect, (±) medium bad effect, (+) bad effect.
[b]M,mutagenic effect;[c]T, tumorigenic effect; [d]I, irritating effect; [e]R, reproductive effect.

Drug-likeness model score (a combined effect of physicochemical properties, pharmacokinetics and pharmacodynamics of a compound and is represented by a numerical value) was computed by Molsoft (Molsoft 2007) software for the four molecules under study. As shown in the fig. 6 & 6A the dotted line graph indicates non drug-like behavior and those fall under thickest line graph are considered as drug-like. Maximum drug likeness values and drug scores were found to be 61 and 81% respectively for the compound Fluro-ornidazole (I-a). Next to it, compound I-c was predicted to have drug likeness value 0.39 and drug scores 27%. Compound I-b having Bromine [Br] substitution gave negative drug likeness value and low drug score 11% [<50%]. Hence I-b and I-c could not be treated as potential candidates even though they complied with the Lipinski's rule. It could be observed from the results that amongst compounds I-b and I-c, the drug likeness decreased. On the basis of drug likeness model score, compound I-a is a predicted as potential therapeutic candidates and can be selected for synthesis.

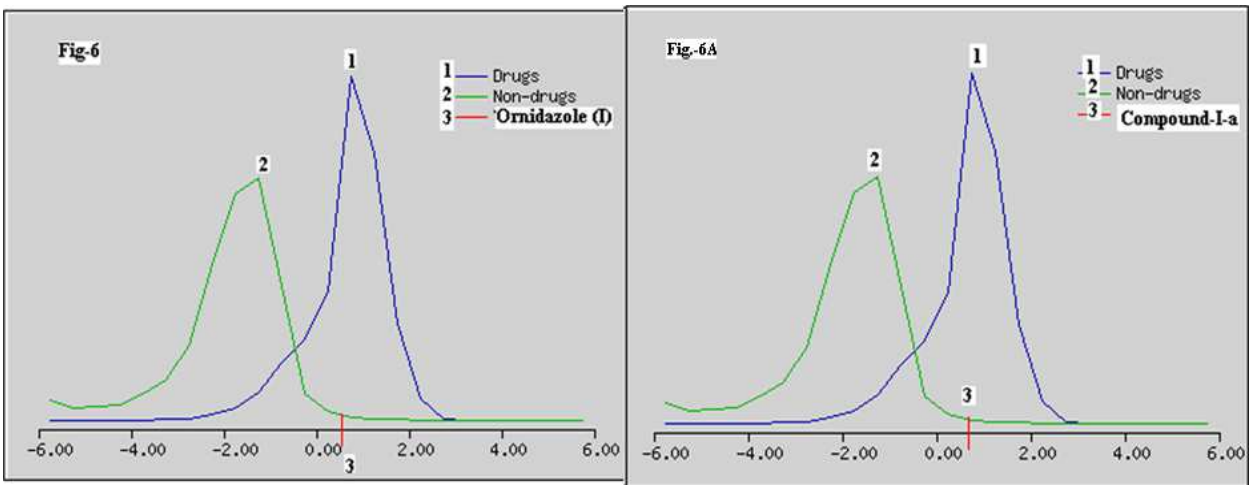

Fig. 6 & 6A- Drug likeness model score graph

DOCKING STUDIES

As it was mentioned in the introduction, the interactions of the proposed compounds with the active sites of the proteins namely1ucf [Anti-Parkinson protein], 3k21 and 4wvd [Anti-Parasitic protein], 4e9u [Anti-Infective protein], 3hj3 [Anti-protozoal protein], 1jk2 and 3wx4 [Anti-Bacterial protein] were analyzed. The docking simulations were performed using AutoDock Vina 1.1.2 program. The binding Gibbs free energies ΔGb corresponding to the best poses of the ligands from **I** to **I-c** within the binding pocket of target proteins are displayed in Table-**3**.As indicated by the negative value of the Gibbs free energy, both ornidazole and its derivatives demonstrate potential inhibitory action against all the proteins. Important to note, compound **I-a** shows more favorable binding affinity to the binding site of 4e9u, 3hj3 and1kj2 than the native ligand. The docked pose of all proteins with fluro-ornidazole and ornidazole are shown in figure .7. Since Fluro-ornidazole compound [I-a] fulfilled the criteria for good membrane permeability, oral bioavailability, low toxicity, and potential inhibitory activities towards all the proteins compared to the standard drug orinadazole.

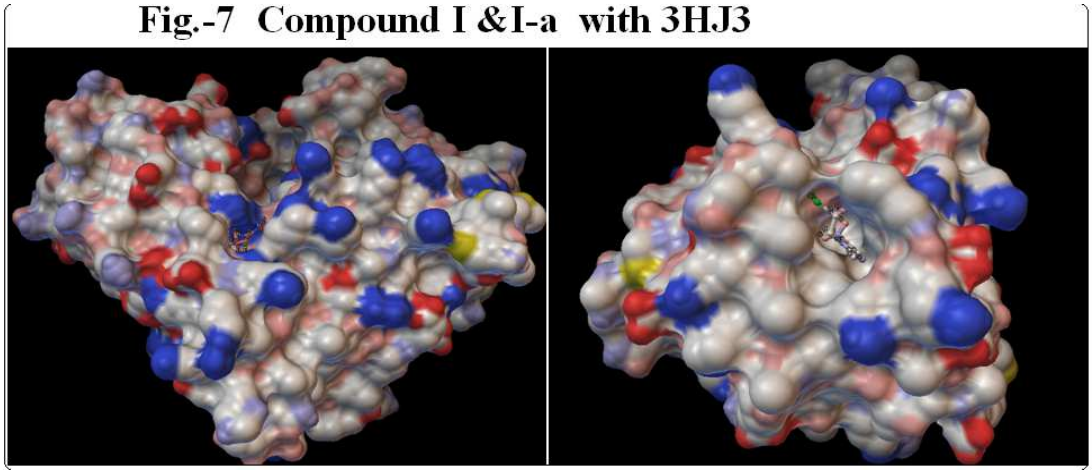

Fig .7 - Molecular surface representation of Target protein with compound I & I-a (shown in stick) docked in the active site

Table 3. Binding Free Energy (ΔG_b) Corresponding to the Best Docking Poses of Compounds in the Active Site of Target proteins

Ligands	. Binding Energy ΔGb (kcal/mol)						
	Anti-Parasitic protein		Anti-Parkinson protein	Anti-Infective protein	Anti-protozoal protein	Anti-Bacterial protein	
	3K21	4WVD	1UCF	4E9U	3HJ3	1KJ2	3WX4
Ornidazole I(X=Cl)	-5.4	-5.0	-4.8	-5.3	-5.1	-5.1	-5.0
Fluro- Ornidazole Compound I-a(X=F)	**-5.2**	-4.9	-4.8	**-5.6**	**-5.8**	**-5.2**	-4.8
Bromo- Ornidazole Compound I-b(X=Br)	-5.3	-5.1	-4.2	-5.2	-5.3	-4.7	-4.9
Iodo- Ornidazole Compound I-c(X=I)	-5.6	-5.1	-4.7	-5.3	-5.4	-5.1	-4.9

Hydrophobic Effect

Protein-ligand complexes may be stabilized by the so-called hydrophobic effect. In protein-ligand interactions, this effect often leads to the stacking of aromatic residues against the ligands. Two variations of the hydrophobic effect are discussed in literature: the ‘‘classical’’ hydrophobic effect and the ‘‘non-classical’’ hydrophobic effect [77]. The classical hydrophobic effect results mainly from highly favorable entropy of formation. Here, small hydrophobic solutes induce an ordering of the water molecules at the solvent-surface interface. In the case of the ‘‘nonclassical’’ hydrophobic effect, the complex formation is mainly enthalpy driven due to favorable interactions between the solute molecules forming the complex as well as favorable interactions between the solvent molecules. Hydrophobic effect of protein-ligand interactions of the compounds orinadazole and fluro-orinadazole are shown in fig. 8.

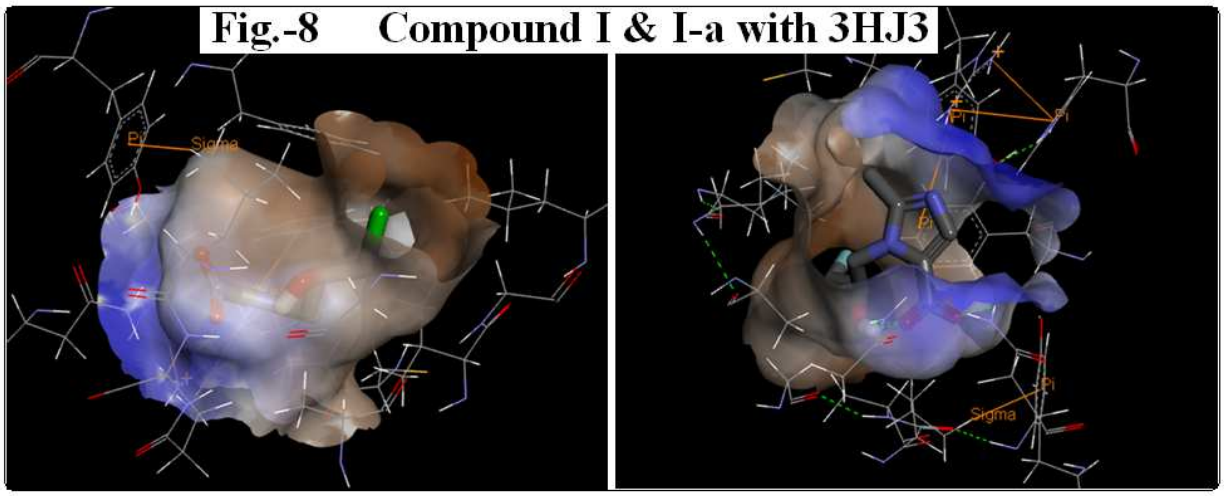

Fig. 8 - Hydrophobic effect representation of Target protein with compound I & I-a. The hydrophobic intensities of the binding site ranges from -3.00 [least hydrophobic area - blue shade] to 3.00 [highly hydrophobic area –brown shade]

Hydrogen Bonds

Hydrogen bonds may be established between polar hydrogen atoms and lone pairs of hydrogen bond acceptors. Due to the large number of hydrogen bond donors and acceptors present in carbohydrates, they tend to form hydrogen bonds when in complex with a protein. Here, both binding partners compete with water molecules for the hydrogen bonds. As a result, the overall enthalpic gain from a hydrogen bond formed between carbohydrate and protein may be small. Although carbohydrates often displace all water molecules in the binding site, in a number of cases conserved water molecules are observed in the binding site. These water molecules mediate protein-carbohydrate interactions, especially if no or only few direct hydrogen bonds are established [78-79]. However, water molecules may also help in stabilizing oligosaccharide conformations [80]. Although some approaches exist for taking into account the solvent molecules in the binding site [81], calculating water mediated hydrogen bonds of the carbohydrate with itself is still very difficult.

The interaction of amino acids, hydrogen bonding and 2-D diagrams depicting interactions of different target proteins with the Fluro- Ornidazole [I-a] and Ornidazole [I] drug molecule is given in Fig.9 and this clearly demonstrated the binding positions of the ligand with the protein target. In the 2-D diagrams, Residues circled in green participate in van der Waals interaction with the ligand while residues in pink forms electrostatic interactions. H-bond and alkyl-π interactions are shown by green and light pink dotted lines, respectively. Hydrogen bonding pocket is shown for clarity. Analysis of the receptor/ligand complex models generated after successful docking of the fluro-ornidazole and ornidazole were based on the parameters such as hydrogen bonds distance, amino acid interactions, binding energy and orientation of the docked compound with the active site. As a general rule, in most of the potent therapeutic agent, both hydrogen bond and hydrophobic interactions between the compound and the active sites of the receptor have been found to be responsible for mediating the biological activity.

Table-4 Interaction of amino acids, hydrogen bonding distance of different target proteins with the Fluro- Ornidazole[I-a] and Ornidazole [I] drug molecule

Protein	Compound	Hydrogen Bond		Distance Å	Pi Interaction Pair		Distance Å
		Donor atom	Acceptor atom		Donor atom	Acceptor atom	
3K21	Orinadazole (I)	A:ASN27:ND2	O2	2.8	Nitroimidazole ring	A:ARG167:NH1:B	5.13
		A:ARG170:NE	O4	3.05	Nitroimidazole ring	A:ARG167:NH2:B	5.16
		A:ARG170:NH1	O3	2.97			
		A:ARG170:NH2:B	O3	2.87			
	I-a	A:ARG170:NH1	O2	2.95			
		A:ARG167:NH1	O2	2.87			
		A:ARG167:NH2:B	O3	2.93			
		A:ARG170:NH2:B	O2	3.01			
		A:HIS20:ND1	O3	3.04			
		A:ARG170:NH1:B	O2	3.21			
		A:ARG167:NH1	O3	3.2			
1UCF	Orinadazole (I)	A:TYR67:HN	O2	2.09			
	I-a	H15	O3	2.38			
4WVD	Orinadazole (I)	A:SER355:HG	O3	2.25			
		A:SER355:HG	O4	2.36			
	I-a	H 15	A:ILE357:O	2.00			
		H15	A:ARG353:O	2.61			
4E9U	Orinadazole (I)	A:GLN165:HE22	O3	2.35	A:TYR41	nitroimidazole ring	5.16
		A:ASN168:HD21	O4	2.46			
		O3	H15	2.29			
	I-a	A:GLN165:HE22	O3	2.37	nitroimidazole ring	A:TYR41	5.16
		A:ASN168:HD21	O4	2.45			
		H15	O3	2.18			
3HJ3	Orinadazole (I)	O3	H15	2.19			
		H15	A:GLU294:OE2	2.36			
		A:HIS403:HE2	O2	2.07			
	I-a	A:HIS348:HE2	O3	2.2			
		A:THR303:HN	O2	2.02			
1KJ2	Orinadazole (I)	A:TYR67:N	O3	2.92			
	I-a	A:TYR67:N	O3	2.85			
3WX4	Orinadazole (I)	A:LYS20:HZ2	O3	1.8			
		A:LYS20:HZ2	O4	1.88	Nitroimidazole ring	A:LYS23:NZ	4.6
		A:LYS20:HZ3	O3	2.3			
		A:LYS23:HZ2	O4	1.92			
		A:GLU94:OE1	H15	2.29			
	I-a	H15	A:TYR52:O	2.19	A:HIS30	Nitroimidazole ring	5.10
		A:ASN34:HD22	N6	2.23			

As shown in Table-3 , Fluro- Ornidazole shows binding energy -5.2 kcal/mol and standard drug Ornidazole -5.4 kcal/mol with 3k21. Comprehensively shown in Figure- 9, the compound Fiuro-ornidazole (I-a) demonstrates van der Waals interactions with GLU23, ILE19 and electrostatic interactions with ARG167 & 170, HIS20. Compound I-a is a hydrogen bond donor from electrostatic residues ARG167 &170, HIS20 at a distances 2.87 Å, 2.93 Å, 3.2 Å,

2.95 Å, 3.01 Å, 3.21 Å and 3.04 Å respectively. whereas in compound -I demonstrates only the electrostatic interactions with TYR28, ASN27 & 24, HIS20, ARG170 and hydrogen bond donor from electrostatic residues ASN27 and ARG170 at a distances 2.8 Å, 3.05 Å, 2.97 Å and 2.87 Å . The arene-arene [π- π] interactions are established between compound –I and ARG167.The Fluro- Ornidazole [I-a] shows binding energy -5.6kcal/mol and Ornidazole[I] -5.3 kcal/mol with 4e9u, the compound I-a demonstrates van der Waals interactions with VAL137, PHE22, LEU141, CYS44, ALA134, HIS18 and electrostatic interactions with TYR41 & 248, ARG45, LEU164, GLN165, ASN168 and GLY161. Compound I-a is a hydrogen bond donor from electrostatic residues ASN168 and GLN165. whereas in compound-I demonstrates van der Waals interactions with ALA134, GLY138, PHE22, LEU141, VAL137, CYS44, HIS18 and electrostatic interactions with TYR248, GLN165, ASN168, GLY161, ARG45, LEU164. Compound- I is a hydrogen bond donor from electrostatic residues ASN168 and GLN165 at a distances 2.5 Å, 2.4 Å respectively. The arene-arene [π- π] interactions are established between compound –I and TYR41.

The Fluro- Ornidazole [I-a] shows binding energy -5.8 kcal/mol and standard drug Ornidazole[I] -5.1 kcal/mol with 3hj3, , the compound I-a demonstrates van der Waals interactions with HIS307, THR355, TYR360, PHE344 and electrostatic interactions with GLY301, LYS300, HIS348, ASP302, MET356, ASN304, THR303 and TYR353. Compound I-a is a hydrogen bond donor from electrostatic main chain residue THR303 and side chain residue HIS348 at a distances 2.02 Å, 2.2 Å respectively. Whereas in compound -I demonstrates van der Waals interactions with TYR342, PHE298, ASP426, GLY430, PHE433 and electrostatic interactions with HIS403, GLU294, ILE315, TRP316, ASN319 & 434, CYS402. Compound- I is a hydrogen bond donor from electrostatic residues HIS403 and GLU294 at a distances 2.07 Å, 2.36 Å respectively. The Fluro- Ornidazole [I-a] showes binding energy -5.2kcal/mol and Ornidazole[I] -5.1 kcal/mol with 1kj2, the compound I-a demonstrates van der Waals interactions with ARG98, GLU94, PRO66, LYS62 and electrostatic interactions with TYR67, GLY65, GLN95, ILE91and GLU90. Compound I-a is a hydrogen bond donor from electrostatic main chain residue TYR67 at a distance 2.85 Å. whereas in compound -I demonstrates van der Waals interactions with GLU94, ARG98, GLY65, PRO66 and electrostatic interactions with TYR67, GLN95, ILE91, GLU90, LYS62. Compound- I is a hydrogen bond donor from electrostatic main chain residues TYR67 at a distance 2.92 Å.

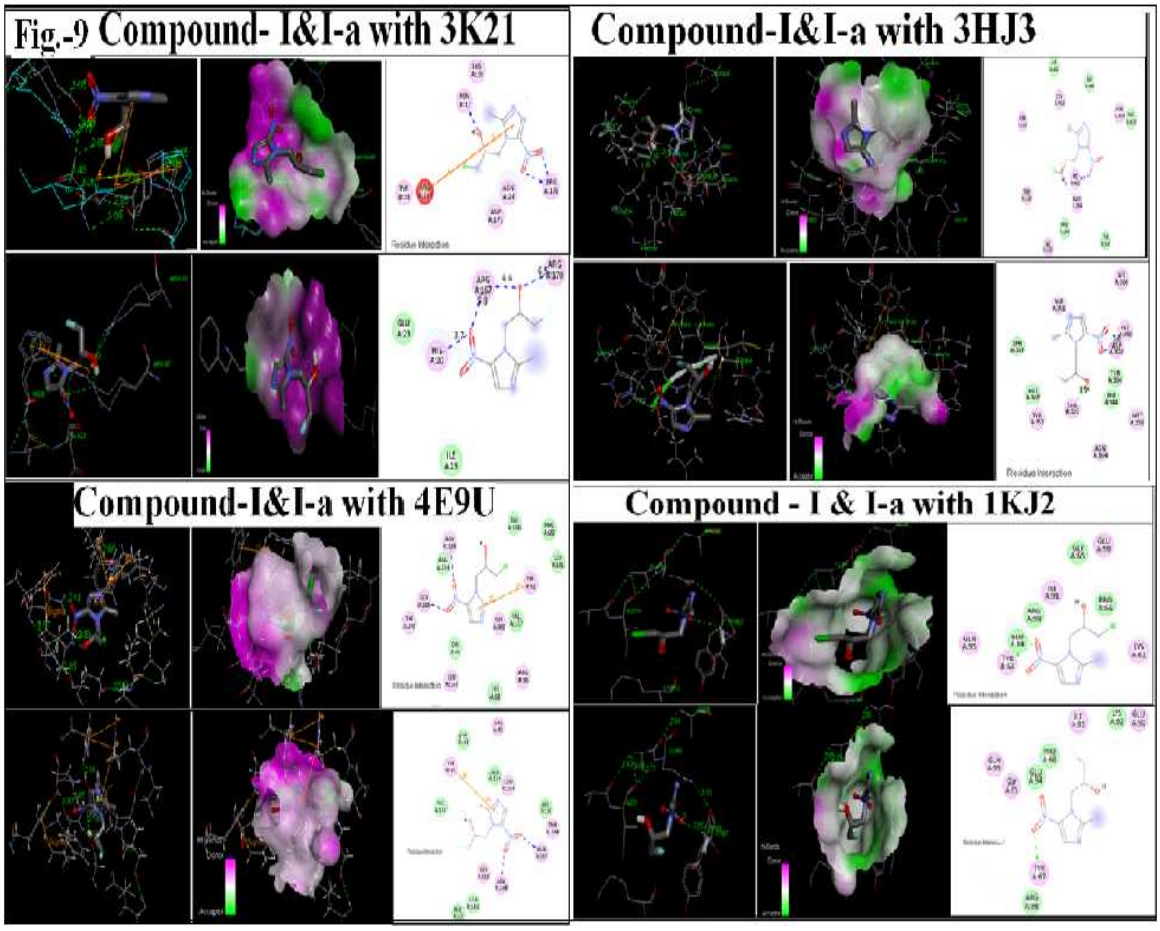

Fig. 9- The interaction of amino acids, hydrogen bonding and 2-D diagrams depicting interactions of different target proteins with the Fluro- Ornidazole[I-a] and Ornidazole [I] drug molecule

Nitroimidazoles are generally considered mutagenic chemicals. The nitrogen group present in nitroimidazole derivatives is considered responsible for the mutagenicity of these compounds. Further, more results obtained confirm that the nitro NO_2 moiety is engaged in a hydrogen bond with the amino hydrogen of ARG167, ARG170, ASN27, HIS20, TYR67, SER355, GLN165, HIS348, THR303 and LYS20. The calculated hydrogen bonding distance of different target with the Fluro- Ornidazole and Ornidazole is shown in Figure .9. Hydrogen bond interaction decides the nature and properties of biomolecules. Interactions of molecules with target proteins are absolutely crucial to various bioactivities, which in turn depend on the structural features of the molecules, including their hydrogen-bonding abilities. Most potent antibacterial and antifungal activity exhibited by the compound might be due to the presence of the electron withdrawing substituent NO2group at the 5th position in Nitroimidazole. The molecular docking analysis explains the binding energy of Fluro- Ornidazole drug molecule with different protein target. The hydrogen bonding distances between the drug molecule and the amino acids of the protein binding site is a clear manifestation of the bioactivity of the molecule. Molecular docking studies of Fluro- Ornidazole with 1ucf, 3k21 and 4wvd, 4e9u, 3hj3, 1jk2 and 3wx4 exhibited binding interactions and warrants further studies for the development of potent 1ucf, 3k21 and 4wvd , 4e9u, 3hj3, 1jk2 and 3wx4, inhibitors for the treatment of Parkinson, Parasitic, Infective, protozoal and Bacterial diseases.

CONCLUSION

As seen from the results, it is interesting to note that the Fluro- Ornidazole [81%] compound has a better drug-score value than Ornidazole [26%]. The Ornidazole and Nitroimidazole derivatives were low molecular weight anti-microbial compounds with excellent activity against anaerobic microorganisms. Even though the results of the Physicochemical and Drug-Likeness Properties of the new Compound I-a [Fluro-orinadazole] shows low molecular weight, it has no bad effect in mutagenic, tumorigenic , irritating and reproductive effects compared to the standard drug ornidazole.The results of the molecular docking study clearly demonstrates that the Fluro- Ornidazole and standard drug Ornidazole has inhibitory activity against 1ucf, 3k21 and 4wvd ,4e9u,3hj3,1jk2 and 3wx4 inhibitors for the treatment of Parkinson ,Parasitic ,Infective ,Protozoal and Bacterial diseases. These results clearly indicate that the Fluro- Ornidazole has similar binding sites and interactions with all the proteins as that of the standard drug Ornidazole. This *insilico* studies by Fluro- Ornidazole clearly showed the inhibition of all the above proteins. Further investigations on the compound Fluro- Ornidazole and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of Parkinson, Parasitic, and Infective, protozoal and bacterial diseases. On the basis of drug likeness model score and molecular docking study, the compound Fluro-Ornidazole is predicted as potential therapeutic candidates and can be selected for synthesis.

Moreover, this compound is an effective drug which is relatively safe and produces only minimal side effects. No doubt the availability of computational and in silico approaches is the landmarks in drug discovery process. The virtual screening and the docking analysis have provided a remarkable approach for accessing the lead molecules to be used as drug ligands and to understand the protein–ligand binding affinity.

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