JJBS

Volume 14, Number 1, March 2021
ISSN 1995-6673
Pages 75 - 82
https://doi.org/10.54319/jjbs/140110

Jordan Journal of Biological Sciences

In Silico Screening for Inhibitors Targeting 4-diphosphocytidyl-2-C-methyl-D-erythritol Kinase in *Salmonella typhimurium*

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Received Feb 28, 2020; Revised July 5, 2020; Accepted July 7, 2020

Abstract

In bacteria, enzymes of the isopreniod biosynthetic pathway can serve as molecular targets for developing new antibiotics in combating infections caused by resistant microbes. The amino acid sequence of the enzyme 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (IspE) was used to construct a tertiary structure via homology modeling, and its binding site was predicted by RaptorX and GalaxyWEB servers. The quality of the model was checked by three evaluation tools online. In Ramachandran plot, 99.3% of the residues were in the favoured region. The Z-plot of ProSA indicates that the model is in the range of X-ray experimentally solved proteins, and the overall quality by ERRAT is 90.2% so it is a reliable model for molecular docking. A total of 50 compounds from drug bank data base were docked using this model and ten experimental compounds were found to have binding affinities higher than the control, ADP (-8.0 Kcal/mol) in both iGEMDOCK and AutoDOCK Vina docking tools. The highest two compounds, bentiromide and Fluocinolone acetonide (-10.1 Kcal/mol) show probabilities of intestinal absorption but may cross the blood brain barrier. Although not inhibitory to the P-glycoproteins and cytochromes, these compounds can bind androgen and estrogen receptors. Therefore, investigating the pharmacodynamics and pharmacokinetics is essential in developing drugs. The compounds obtained in this study could be useful as lead-like agents in antibiotic design.

Keywords: Homology modeling, Molecular docking, Isopreniod biosynthesis, Molecular targets.

1. Introduction

Antibiotic resistance is a global issue that has serious consequences on life of patients since it negatively affects drug design projects. Many factors play role such as poverty, inadequate dosage, unregulated dispense of drugs, and untrusted drug suppliers (Hart and Kariuki, 1998; Okeke *et al*., 1999; WHO, 2000). In USA, it is estimated that 23000 people die of infections by resistant microbes each year in addition to increasing the cost from 6000-30000$ as compared to infections in patients with antibiotic-sensitive microbes (Cosgrove, 2006; CDC, 2019).

Due to antibiotic resistance, scientists are investigating alternative molecular targets to develop new antibiotics such as quorum sensing pathway, Shikimate biosynthesis pathway, Isoprenoid synthesis, bacterial cytoskeleton, divisome complex and antimicrobial peptides (Al-khayyat, 2019; Tanhaeian *et al*., 2020). Docking experiments were used for this purpose such as the study of Al-Khayyat, (2017) and Al-Khayyat *et al*., (2019).

Isoprenoids are a large diverse group of 50000 produced by living orgainsmsms. Isoprenoids are important in many functions like electron transport, cell signaling, production of glycoprotein, breakdown of protein and cell division (Hunter, 2007 Heuston *et al*., 2012; Zhao *et al*., 2013). Docking experiments were used for this purpose such as the studies of Al-Khayyat, (2017), Nasab *et al*., (2018) and Al-Khayyat *et al*., (2019).

Bacteria use the non-mevalonate pathway for producing isoprenoids. The enzyme 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (IspE) catalyzes the phosphorylation 4-diphosphocytidyl-2-C-methyl-D-erythritol by using ATP (Tidten-Luksch *et al*., 2012). In this study, homology modeling and molecular docking approaches were carried out on IspE to find inhibitors that may interfere with the non-mevalonate pathway as a preliminary screening method in the antimicrobial drug design process.

2. Materials and methods

2.1. Sequence retrieval, Binding site prediction and Homology modeling

Amino acid sequence of IspE of *Salmonella typhimurium* LT2 (Post *et al*., 1993) was obtained from Uniport database having ID: sp|P30753. Binding site was predicted by RaptorX maintained by Xu Group at University of Chicago. It is an online server, (Källberg *et al*., 2012), at (http://raptorx.uchicago.edu/). GalaxyWEB web server (Shin *et al*., 2014) was also used which is maintained by Computational Biology Lab., Department of Chemistry, Seoul University. This binding site prediction tool can be accessed at: (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=SITE). The three dimensional structure of the enzyme was built by RaptorX (Källberg *et al*., 2012).

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2.2. Quality assessment of the Model

The accuracy of the model was assessed by three online tools: Ramachandran plot analysis by RAMPAGE (Lovell *et al*., 2003). The site is maintained by Crystallography and Bioinformatics Group at Department of Biochemistry, School of Biological Sciences, University of Cambridge, available at: (http://mordred.bioc.cam.ac.uk/~rapper/rampage. php). PROSA (Wiederstein and Sippl, 2007) determines if the protein model lies within the range of experimentally determined protein folds. It can be accessed at (https://prosa.services.came.sbg.ac.at/prosa.php). The site is maintained by Center of Applied Molecular Engineering, Department of Biosciences, University of Salzburg. ERRAT (Colovos and Yeates, 1993) computes and compares the interaction of non-bound heavy atom pairs with standards statistically. It is available at (http://servicesn.mbi.ucla.edu/ERRAT/) at Molecular Biology Institute, University of California, Los Angeles.

2.3. Ligand selection, Molecular docking

The chemical structure of the control, ADP was downloaded from ZINC database (Irwin *et al*., 2012). A total of 50 experimental structures were obtained from Drugbank database (Wishart *et al*., 2008). Molecular docking was performed by AutoDock Vina (Trott and Olson,2010) designed by Oleg Trott from Molecular graphics Laboratory at the Scripps Research Institute, La Jolla, California. The Autogrid tool was employed to pre-calculate a grid. This grid has a size of 60×60 and a box center of 2.238, -1.631 and 19.862 for x, y and z respectively. Interactions between ligand and the enzyme model were visualized by LIGPLOT+ (Wallace *et al*., 1996). Results were redocked again in iGEMDOCK, a second tool by Hsu *et al*. (2011). It was developed at National Chiao Tung University, Taiwan.

2.4. Pharmacologic properties of the compounds

The pharmacokinetics and toxicity profiles (ADMET) of the compounds were predicted by admetSAR 2 (Yang, 2019). The computed parameters were: (i) human intestinal absorption (HIA), (ii) blood-brain barrier (BBB) penetration, (iii) plasma glycoprotein (P-gp) substrate and inhibition probabilities, (iv) Cytochromes (CYP P450), substrate and inhibition probabilities (v) Ames mutagenesis test (AT), (vi) Hepatotoxicity (Hep.), (viii) Androgen receptor binding (ARB), (ix) Estrogen receptor binding (ERB), and (x) thyroid receptor binding (TRB).

3. Results and Discussion

The three dimensional structure of IspE was built by RaptorX using a template of 2.01A° X-ray diffraction of ternary complex of 4-diphosophocytidyl-2-C-methyl-D-erythritol kinase, belongs to *Escherchia coli*. The model (Fig. 1) consists of 283 amino acids and contains disordered region about14%. 31% of the molecule appears as α-helices, 27% β-sheets and 41% as coils. RaptorX predicted the binding residues for ADP. These are V^{66}, N^{65}, L^{66}, I^{94}, K^{96}, M^{100}, G^{101}, G^{103}, L^{104}, G^{105}, G^{106}, G^{107} and N^{110}. GalaxyWEB server predicted that ADP binds V^{57}, V^{60}, $N^{65,}$ L^{66}, I^{67}, K^{96}, M^{100}, G^{101}, G^{105}, G^{106}, G^{107}, Ser^{108} and N^{110}.

Shan *et al*. (2011) studied the crystal structure of the enzyme in *Mycobacterium tuberculosis* and found that it is composed of two domains; ATP binding domain at the N-terminal which extends from M^{1} to G^{148}. It is composed of four β stands β1, β4-β6 and four α-helices: α1-α4 in addition to $I3_{10}$ helix. The substrate binding domain is located at C-terminal and consists of ten β-strands β2, β3, β5-β13 and six α-helices. These are α5-α10. Mutagenesis at A^{100}, G^{102}, M^{103} and A^{104} blocked ATP binding sites and mutants of Y^{28} and Y^{185} reduced the interaction of the enzyme with the substrate. Part of the substrate enter the catalytic region, where the amino acids K^{13} and D^{140} are important in catalysis. Almuqri *et al*. (2016) stated that adenine of ADP makes contact with N^{70}, L^{71} and D^{109}. The phosphate makes H-bonds with A^{100}, G^{102}, G^{105} and G^{106}. L^{56}, L^{64}, P^{98}, V^{99}, G^{101}, M^{103} and A^{104} form hydrophobic interactions.

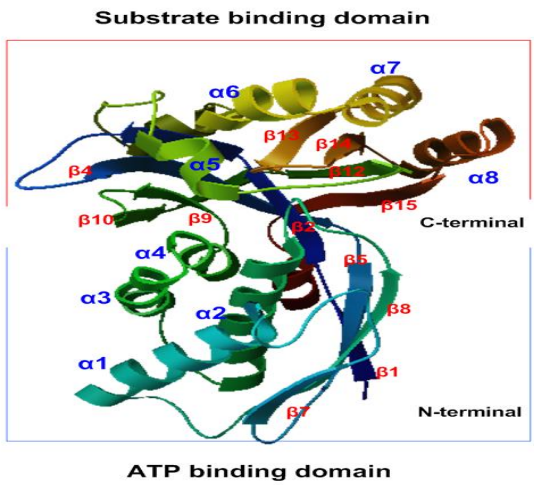


Figure 1. The three dimensional structure of IspE visualized by Python molecular viewer (Sanner, 1999).

Quality assessment was performed and found that is a reliable model. In Ramachandran plot 99.3% of the residues are within the favoured region where only E^{154} and N^{193} lie on the allowed region. It is proposed that good reliable model should have more than 90% of their residues in favored region (Laskowski *et al*., 1993). In ProSA the Z-score is -8.67 within the normal range of experimentally solved structures. In ERRAT, the overall quality of the model is 90.1% (Figs. 2 and 3).

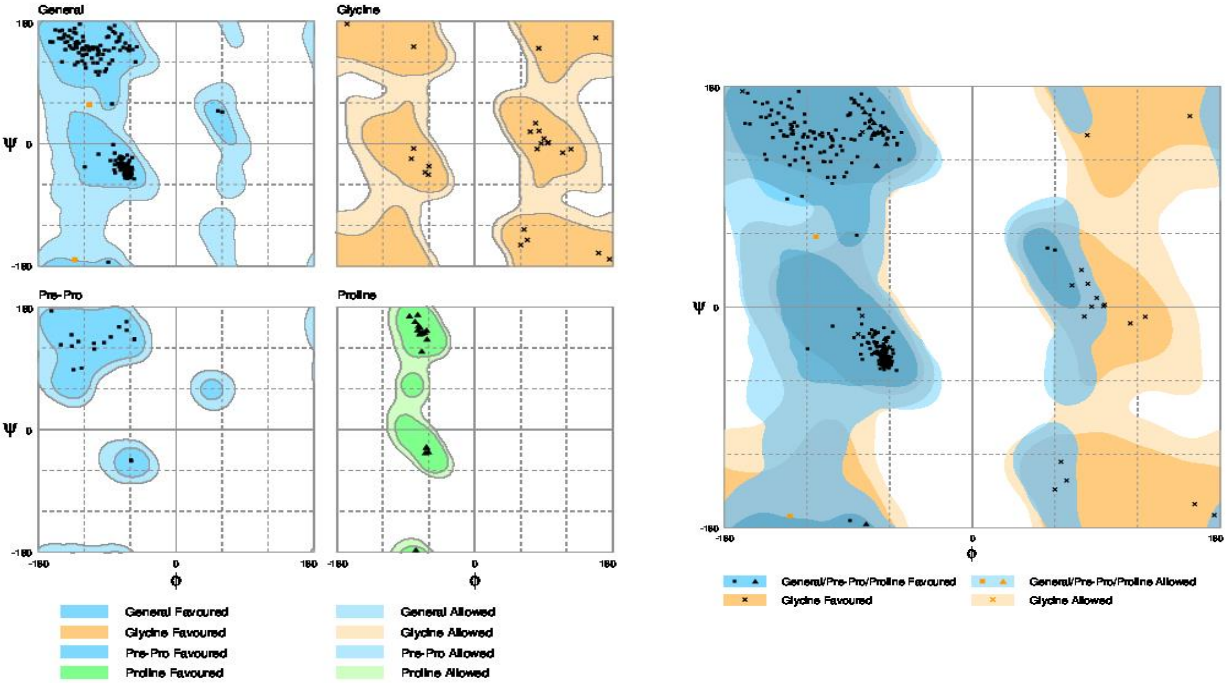
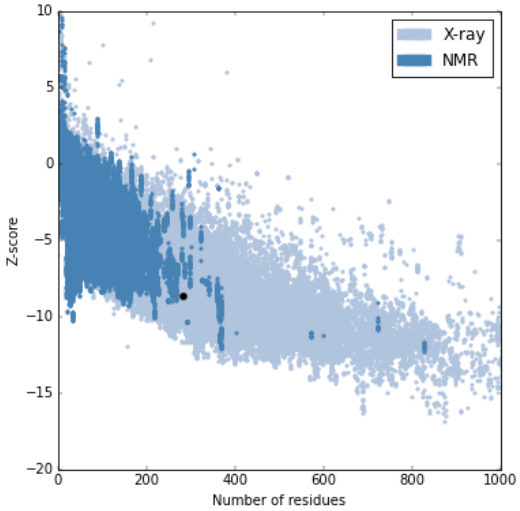
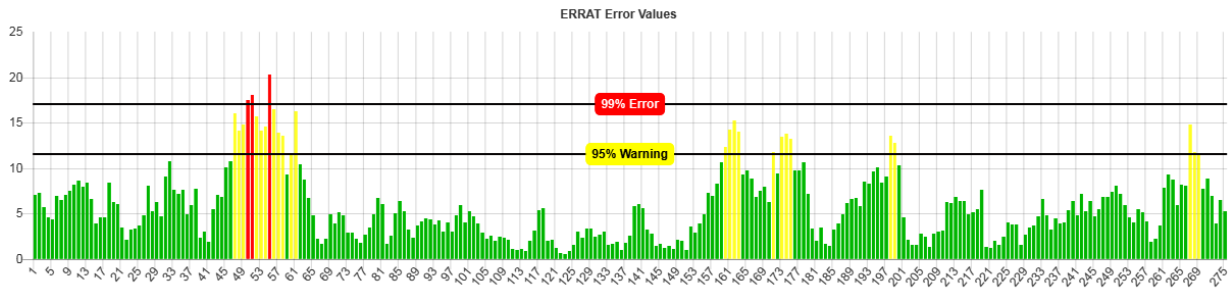


Figure 2. Ramachandran plot of IspE as predicted by Rampage.



(A)



(B)

Figure 3. (A) Z-plot by ProSA of the model (black dot). The constructed model lies within normal range of X-ray determined experimental structures. (B) ERRAT analysis of the model. Black bars represent misfolded regions. On the vertical error axis, the two lines determine the confidence in which it is possible to reject region.

A total of 50 compounds were obtained from Drug bank database according to Lipinski rule of five which states that for a compound to be easily absorbed it should have molecular weight less than 500, hydrogen bond donors less than 5, hydrogen bond acceptors less than 10 and logP (measurement of lipophilicity) less than 5

(Lipinski *et al*., 2001). The compounds were docking by two softwares to get more accurate estimations of binding. Table (1) shows the results with the interactions presented. Table (2) shows the characteristics of the compounds. Figs. 4 and 5 show the interactions with ADP, control and the compound Fluocinolone acetonide respectively, while Fig. 6 shows the chemical structures of ligands. These ligands had their binding affinity higher than control so may be useful as lead compounds to design new antibiotics since it occupies the active site and interacts with its amino acid residues.

Table 1. Docking scores of IspE against experimental compounds with the interactions

Compounds	Binding affinity (Kcal/mol) by AutoDock Vina	Residues of the model forming:		Estimated ΔG (Kcal/mol) by iGEMDOCK
		Hydrogen bonds	Hydrophobic interactions	
ADP (control)	-8.0	K^{10},H^{26},V^{56}, G^{103}, G^{105}, T^{240}	L^{28}, Y^{25}, G^{101},T^{181} , $F^{185,}$ G^{239}	-69.8
Fluocinolone acetonide	-10.1	H^{26}, S^{108}, D^{141}	K^{10},Y^{25}, G^{139}, A^{140}, V^{156},T^{181}, F^{185}	-74.0
Bentiromide	-10.1	H^{26}, G^{107}, ,T^{181}, T^{240}	K^{10},Y^{25}, L^{28}, G^{101}, G^{105}, G^{106}, S^{108}, F^{185}	-73.2
Amsacrine	-9.7	G^{107}, S^{108},T^{181}	H^{26},Y^{25}, L^{28}, G^{105}, V^{156}, F^{185}	-74.6
Isradipine	-9.6	A^{140}, D^{141}	N^{12}, L^{28}, F^{32}, G^{139}, V^{156},T^{181}, F^{185}	-72.4
Troglitazone	-9.2	G^{103}, G^{107}, S^{108}	K^{10},Y^{25}, H^{26}, G^{105}, D^{141}, V^{156}, P^{182}, F^{185}	-80.2
Entacapone	-9.1	G^{103}, G^{105}	Y^{25}, L^{28}, G^{101}, S^{108}, T^{181}, F^{185}	-85.5
Betamethasone	-9.0	H^{26}, D^{141}	K^{10},Y^{25}, L^{28}, F^{32} ,G^{105}, S^{108}, A^{140}, V^{156}, T^{181}, F^{185}	-75.4
Diflorasone	-8.9	H^{26}, D^{141}	K^{10},Y^{25}, L^{28}, G^{105}, S^{108}, A^{140}, D^{141}, T^{181}, F^{185}	-77.7
Revatio	-8.6	A^{140}	L^{15},H^{26}, Y^{25}, G^{101}, G^{103},G^{105}, G^{106}, G^{107}, T^{181}, $F^{185,}$ G^{239}, T^{240}	-86.6
Doxazosine	-8.6	K^{10}, T^{240}	N^{65}, L^{66}, K^{96}, M^{100}, G^{101}, G^{103}, N^{110}, D^{141}, T^{181}, G^{239}	-82.6

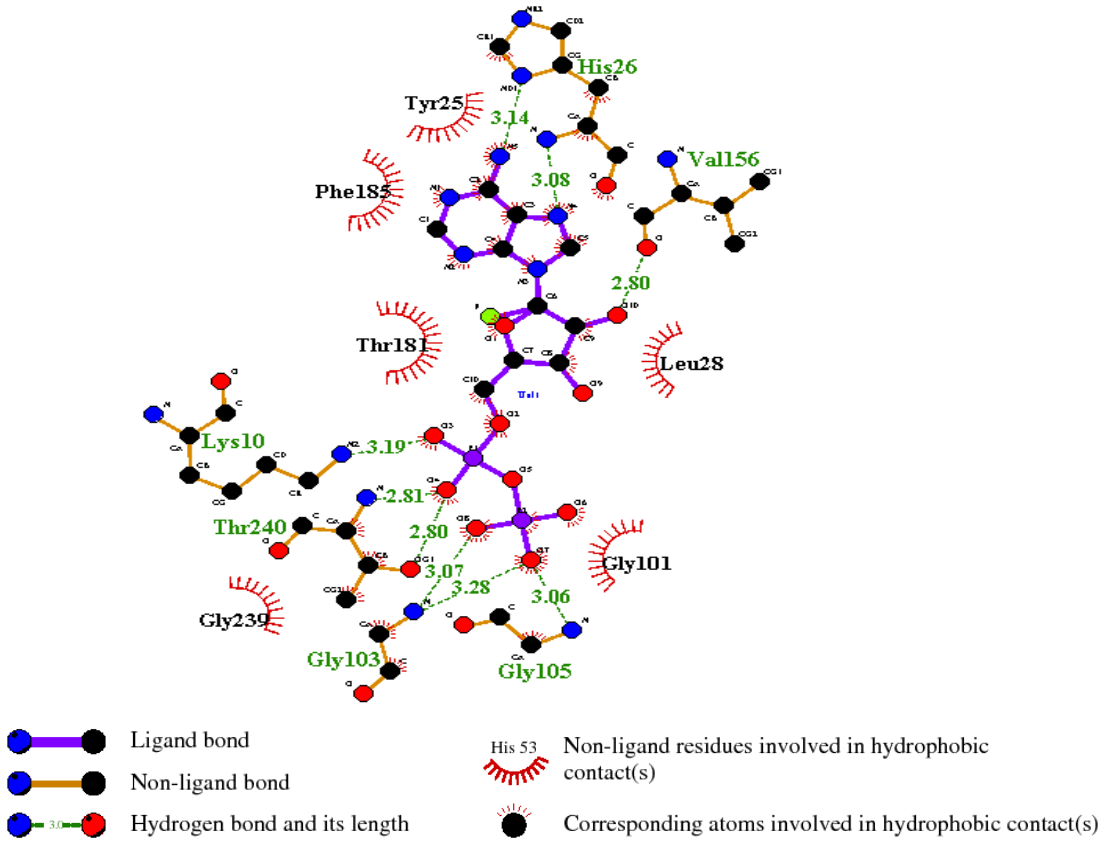


Figure 4. Docking result of ADP with the model, visualized by LIGPLOT+ (Wallace *et al*., 1996).

Table 2. Molecular properties of the best selected compounds obtained from Drug bank database

Compounds	Database ID	Mass (g/mol)	LogP[1]	HBD[2]	HBA[3]
Fluocinolone acetonide	DB00591	452.48	2.47	2	6
Bentiromide	DB00522	404.41	2.99	4	5
Amsacrine	DB00276	393.45	4.66	2	5
Isradipine	DB00270	371.14	3.00	1	5
Troglitazone	DB00197	441.54	4.16	2	5
Entacapone	DB00494	305.28	2.50	2	6
Betamethasone	DB00443	392.46	1.93	3	5
Diflorasone	DB00223	410.45	1.91	3	5
Revatio	DB00203	474.57	2.35	1	8
Doxazosine	DB00590	451.47	2.53	1	9

LogP: the logarithm of the partition coefficient in an octanol/water system, HBD: hydrogen bond donors, HBA: hydrogen bond acceptors.

Docking programs uses different algorithms to measure scores. The scoring function of AutoDock Vina (Trott and Olson, 2010) is:

$$c=\sum_{i<j} f t_i t_j (r_{ij}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where the summation is: overall of the pairs of atoms that can move relative to each other, normally excluding 1–4 interactions, which means atoms separated by 3 consecutive covalent bonds. Each atom i is assigned a type t_i, and a symmetric set of interaction functions ft_it_j of the interatomic distance r_{ij} must be defined. This value represents the sum of intermolecular and intramolecular contributions:

$$c = c_{\text{inter}} + c_{\text{intra}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

iGEMDOCK is a virtual screening software environment using post screening analysis with pharmacological interactions. iGEMDOCK gives interactive interfaces to prepare both the binding site of a given target and the ligands that are docked into the binding site by GEMDOCK docking tool. After that, iGEMDOCK will make protein-compound interactions of electrostatic (E), hydrogen-bonding (H), and van der Waals (V) energies (Yang and Chen, 2004) and the pharmacological scoring function is:

$$E_{\text{pharma}} = E_{\text{GEMDOCK}} + E(\text{E})_{\text{pharma}} + 2E(\text{H})_{\text{pharma}} + 0.5\, E(\text{V})_{\text{pharma}} \ldots\ldots(3)$$

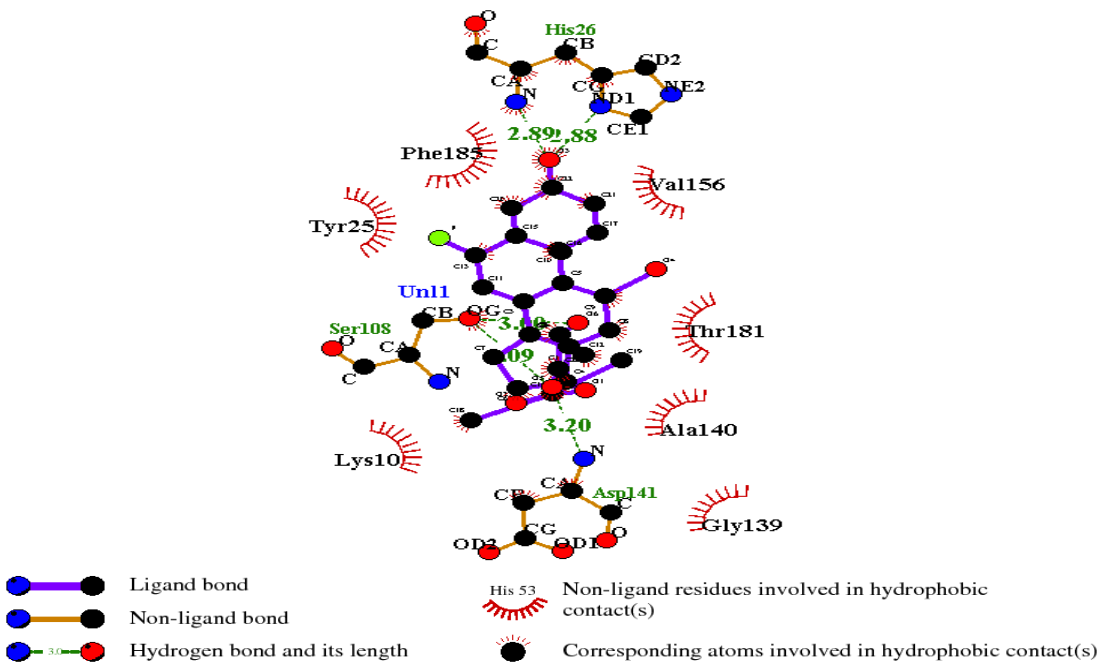


Figure 5. Docking result of Fluocinolone acetotonide with the model, visualized by LIGPLOT+ (Wallace *et al*., 1996).

Previous approaches exploited cytidine or phosphate –sugar moieties of the substrate then non substrate-like inhibitors were used in screening for lead compounds (Tidten-Luksch *et al*., 2012; Tang *et al*., 2011). By Molecular docking, Almuqri *et al*. (2016) identified five novel compounds that may act as inhibitors of IspE in *M. tuberculosis* acting on ATP binding site. These were downloaded from ZINC database and designated as ZINC33113258, ZINC21040510, ZINC33113258, ZINC89917226 and ZINC1471760. In the study of Hirsch *et al*. (2008), compounds were investigated as inhibitors of IspE by structure-designed approaches. The compounds occupied the cytidine binding pocket (i.e. substrate active site) of this enzyme and resulted in lower micromolar activities.

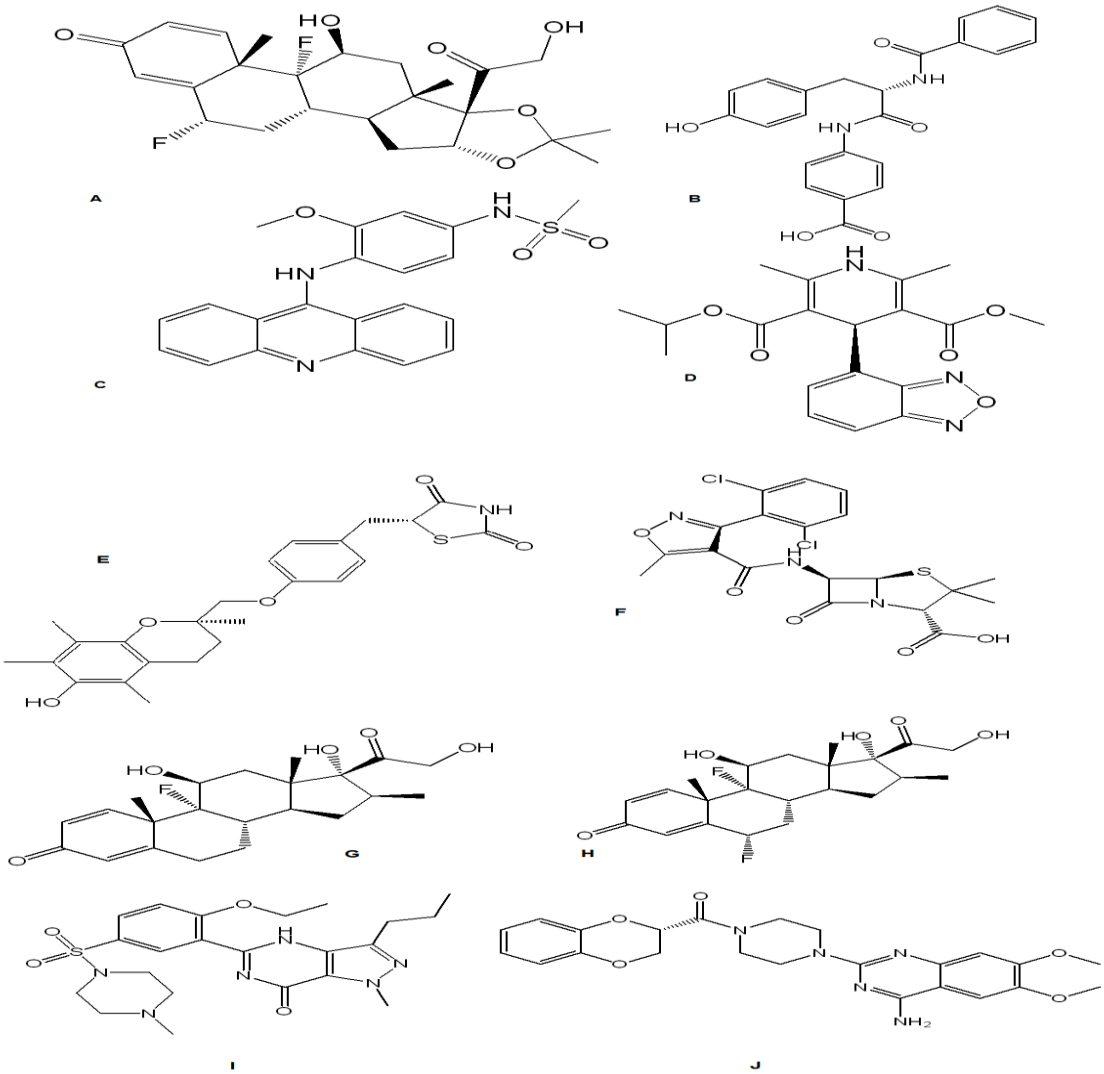


Figure 6. Chemical structures of ligands: A: Fluocinolone acetonide, B: Bentiromide, C: Amsacrine, D: Isradipine, E: Troglitazone, F: Entacapone, G: Betamethasone, H: Diflorasone, I:Revatio, J: Doxazosine.

Pharmacokinetics and dynamics were studied using AdmetSAR version 2 and results are shown in Table (3). All compounds can be absorbed from intestine but all except entacapone may cross blood brain barrier. This is preferred when the drug has to work centrally in brain but side effects can occur if the drug is required to act peripherally (van de Waterbeemd and Gifford, 2003).

Five ligands were shown to be inhibitors of P-glycoproteins. P-glycoproteins are membrane transporters functioning to pump drugs out of cells; hence, any drug that inhibit P-glycoprotein may exhibit drug interactions with other drugs co-administered (Finch and Pillans, 2014). Isradipine, revatio, amsacrine and entacapone are inhibitory to cytochromes. Different classes of cytochromes are responsible for oxidative metabolism of drugs, so their inhibition will accumulate the drugs inside human body resulting in toxicity (Zuber *et al*., 2002).

Table 3. Pharmacokinetics and pharmacodynamics of the compounds

Compounds	HIA	BBB	P-gp		CYP 3A4	CYP 2C9	CYP 2D6	CYP 3A4	CYP 2C9
			Sub.[1]	Inh.[2]	Sub.	Sub.	Sub.	Inh.	Inh.
Fluocinolone acetonide	+	+	+	-	+	-	-	-	-
Bentiromide	+	+	-	-	+	-	-	-	-
Amsacrine	+	+	-	+	+	-	-	-	-
Isradipine	+	+	-	+	+	-	-	+	+
Troglitazone	+	+	-	+	+	-	-	-	-
Entacapone	+	-	+	-	+	-	-	-	-
Betamethasone	+	+	+	-	+	-	-	-	-
Diflorasone	+	+	+	-	+	-	-	-	-
Revatio	+	+	+	+	+	+	-	+	-
Doxazosine	+	+	+	+	+	-	-	-	-

[1]Sub.: substrate, [2] Inh.: inhbition

Table 3. Continued

Compounds	CYP 2C19 Inh.	CYP 2D6 Inh.	CYP 1A2 Inh.	AT	Hep.	ERB	ARB	TRB
Fluocinolone acetonide	-	-	-	+	-	+	+	+
Bentiromide	-	-	-	-	+	+	+	-
Amsacrine	-	-	+	+	+	+	+	+
Isradipine	+	-	+	-	+	+	-	+
Troglitazone	-	-	-	-	+	+	+	+
Entacapone	-	-	+	-	+	-	-	+
Betamethasone	-	-	-	-	-	+	+	+
Diflorasone	-	-	-	-	-	+	+	+
Revatio	-	-	-	-	+	+	+	+
Doxazosine	-	-	-	-	+	+	-	+

4. Conclusion

This enzyme, IspE is an excellent target for drug design approaches since its inhibition may affect isoprenoid biosynthesis in bacteria. The study of pharmacokinetics is essential before considering these experimental structures as lead compounds due to side effects. Chemical modification may improve their binding to ATP binding site and may also reduce their adverse effects.

Acknowledgment

The author is very grateful to the University of Mosul/ College of Education for Pure Sciences for their provided facilities which helped to improve the quality of this work

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