ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In silico Binding and Interaction Studies of Inflammatory Mediators in Isoniazid and Rifampicin Induced Toxicity against Vitamin B12 and Beta-Carotene.

Sherry Joseph M, Udhaya Lavinya B, Sanchi M Dhinoja, Arman Firoz, Saikat S Das, Monika N Jha, Gunaseelan D[1], and EP Sabina*.

School of Biosciences and Technology, VIT University, Vellore - 632014, Tamilnadu, India.
[1]Department of Computer Science, College of Computer Science & Information Systems, JAZAN University, JAZAN-82822-6694, Kingdom of Saudi Arabia.

ABSTRACT

Isoniazid (INH) and rifampicin (RIF) form the backbone of anti-tuberculosis treatment but their use leads to hepatotoxicity in a very small percentage of patients. We in our current study would like to screen cytokines, proteins and receptors mediating inflammation and apoptosis by *in silico* methods to predict the mechanism by which hepatotoxicity is induced. We would also like to look at the mechanism by which previously reported hepatoprotective natural compounds function against INH and RIF-induced inflammation and apoptosis. The pdb structures of chosen cytokines and other inflammatory mediators are to be retrieved from protein data bank and those of the chosen ligands are to be generated using services on Corina molecular networks. Docking studies are to be done on PatchDock online server and analyzed on PyMol molecular viewer. The results obtained would help guide further *in vitro* and *in vivo* studies to know the exact mechanism of action of both toxic and protective drugs.

Keywords: Isoniazid, rifampicin, inflammatory mediators, docking

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The treatment of *Mycobacterium tuberculosis* infections worldwide has depended on use of Isoniazid (INH) and Rifampicin (RIF) as primary drugs of choice. When treated with INH alone a transient increase in serum transaminase levels has been seen in about 10-20% of patients while serious hepatotoxicity occurs in about 1-2% of recipients. When RIF was concomitantly administered with INH it was seen that this percentage of incidence rose to about 27% indicating a synergistic effect [1,2]. It is known that INH is acetylated in the liver to form acetyl-INH which can be hydrolysed to form acetyl hydrazine which then is oxidized by CYP2E1 enzymes to form acetyldiazene and its breakdown products that are hepatotoxic [3]. RIF known to be a potent inducer of CYP2E1 can cause a synergistic increase in the production of toxic metabolites of INH [4]. It has also been proven that oxidative stress is the major mechanism by which these toxic metabolites of INH induce hepatotoxicity [5]. A growing number of natural and synthetic products have been experimented to bring down this INH and RIF induced hepatotoxicity with varying degrees of success including the likes of Kaempferol, N-acetyl cysteine, *Spirulina fusiformis* among others. *Spirulina fusiformis* is of particular interest having been shown to have antioxidant properties and to protect from INH and RIF induced hepatotoxicity. In a recent study the interaction of INH, RIF and selected active components of *Spirulina fusiformis* (β-carotene, phycocyanobilin, vitamin B12) were studied for their *in silico* interaction with Pregnane X Recpeptor and Farnesoid X Receptor [6]. Since they showed a significant level of interaction and provided a possible mechanism of action for treatment of INH and RIF induced hepatotoxicity, we in our current study proposed to further expand this effort by studying the interaction of INH,RIF, β-carotene and vitamin B12 against an expanded set of receptors. Since oxidative stress and apoptotic mechanisms played a role in INH and RIF induced hepatotoxicity we in our study assessed interaction of ligands (INH, RIF, β-carotene, vitamin B12) with selected pro-inflammatory, anti-inflammatory, pro-apoptotic and anti-apoptotic receptors and proteins. The selected receptors included Interleukins, cytokines, and apoptotic receptors. Our study focused on looking at the mechanism of action and possible biological interactions of both the antibiotics and the hepatoprotective active compounds of *Spirulina fusiformis*.

MATERIALS AND METHODS

Receptor preparation

3D structures of the receptors used in present study were retrieved from *Research Collaboratory for Structural Bioinformatics* (RCSB) protein data bank (PDB) (http://www.rcsb.org/pdb/home/home.do) and prepared for docking by removing water molecules and adding hydrogen atoms for generation of 3D structures of respective compound.

Table 1: Receptors used for molecular docking and their functions

PDB ID	Receptor	Role/function
1MK3	BCL-2 like protein 2 (BCL-W)	Anti-apoptotic
2LPC	B-cell lymphoma extra-large protein (BCL-XL)	Anti-apoptotic
2MHS	Myeloid cell leukemia 1 protein (MCL-1)	Anti-apoptotic
1IJZ	Interleukin-13 (IL-13)	Anti-inflammatory
1IRP	Interleukin-1 (IL-1)	Anti-inflammatory
2H24	Interleukin-10 (IL10)	Anti-inflammatory
2BID	BH3 interacting domain (BID)	Pro-apoptotic
2K7W	BCl2 associated X protein (BAX)	Pro-apoptotic
2YV6	BCl2-antagonist/killer protein (BAK)	Pro-apoptotic
1ALU	Interleukin-6 (IL-6)	Pro- inflammatory
1M47	Interleukin-2 (IL-2)	Pro- inflammatory
1O7Y	Interferon-γ-inducible protein (IP-10)	Pro- inflammatory
1T4Q	Interleukin-1 BETA F101W	Pro- inflammatory
1Z92	Interleukin-2-ALPHA RECEPTOR	Pro- inflammatory

Ligand preparation

Present study included 4 ligands (Table 2) whose SMILES were obtained from Pubchem compound database (https://pubchem.ncbi.nlm.nih.gov/) and submitted on Corina molecular networks

ISSN: 0975-8585

(https://www.molecular-networks.com), an online server for generation of 3D structures of the compounds. The PDB files were downloaded further studies. Corina molecular networks server is used to produce 3D conformation from input canonical SMILES. The structures of the ligands are shown in figure 1.

Table 2: Ligands used for molecular docking

Ligand	Pubchem compound ID	Molecuar formula	Molecular weight
Isoniazid	3767	$C_6H_7N_3O$	137.14
Rifampin	5381226	$C_{43}H_{58}N_4O_{12}$	822.94
Beta-carotene	5280489	$C_{40}H_{56}$	536.87
Vitamin B12	16212801	$C_{63}H_{88}CoN_{14}O_{14}P$	1355.37

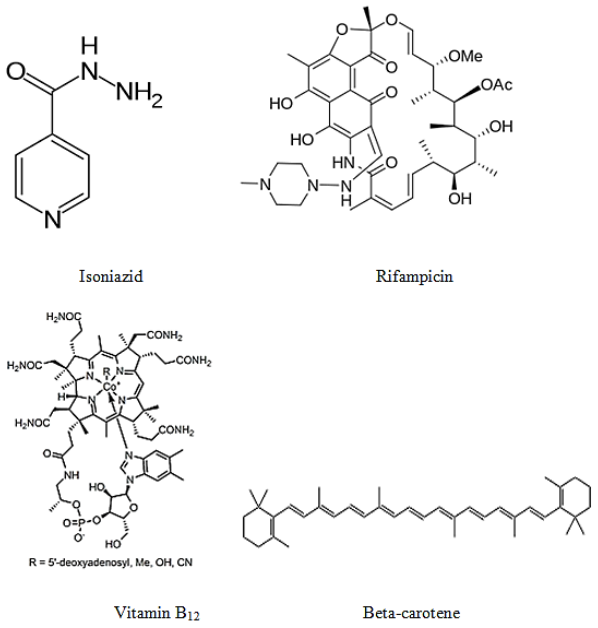

Figure 1: Structures of ligands used for molecular docking

Molecular docking

PatchDock server (http://bioinfo3d.cs.tau.ac.il/PatchDock/) was used for carrying out molecular docking experiments. It is a molecular docking tool, targeted to find docking transformations that produce good molecular shape complementarity based on shape complementarity principles [7]. The input files include the receptor protein and ligand in PDB format. The user's email address is given in order to obtain the results. *Patchdock* server provides an URL which gives the top 20 solutions in a table via e-mail.

Analysis of docked complexes

The binding patterns of the ligands with the receptor proteins in the docked complexes thus obtained were analyzed on PyMol molecular viewer (http://www.pymol.org/). The interacting residues and hydrogen bond lengths were labeled and the docked pose captured in the form of pictures in each case.

RESULTS AND DISCUSSION

The docking experiments revealed the binding and interaction patterns of isoniazid, rifampicin, vitamin B12 and beta-carotene with the chosen receptors. Table 3 represents the scores and ACE of the docked complexes. INH showed significant interaction with 1T4Q with a score of 2280 and an ACE of -155.31. The interacting residues of this complex are SER-43, LEU-62, GLU-64, LYS-65 (Figure 2a). The lengths of the four bonds were ≤ 3 Å indicating significant interaction.

ISSN: 0975-8585

Table 3: Scores and atomic contact energy (ACE) of the docked complexes

	Receptor	Beta Carotene		Vitamin B12		INH		RIF	
		Score	ACE	Score	ACE	Score	ACE	Score	ACE
1	1MK3	6680	-471.27	8444	-678.23	2366	-143.99	5884	-385.44
2	2LPC	6028	-359.81	8190	-716.29	2076	-128.00	5492	-355.67
3	2MHS	6540	-357.09	7870	-693.52	2312	-97.28	5968	-386.20
4	1IJZ	5148	-398.12	6990	-707.54	2072	-118.40	4568	-375.11
5	1IRP	6026	-355.62	6910	-353.90	2372	-164.31	5650	-273.33
6	2H24	6868	-471.84	7324	-861.11	2028	-149.09	5582	-461.66
7	2BID	5148	-398.12	7484	-520.65	2116	-133.42	5542	-278.74
8	2K7W	6398	-503.99	7944	-935.63	2562	-145.25	5546	-632.21
9	2YV6	5160	-385.45	6232	-320.73	2462	-139.90	4834	-199.54
10	1ALU	5660	-384.34	5620	-406.32	1974	-96.70	5108	-287.04
11	1M47	5168	-281.80	4642	-242.06	1920	-102.30	4306	-322.68
12	1O7Y	6668	-216.07	7464	-515.82	2148	-165.47	6196	-367.83
13	1T4Q	5658	-351.77	6576	-473.58	2280	-155.31	5090	-296.69
14	1Z92	6092	-317.57	6954	-464.20	2228	-156.70	5892	-295.59

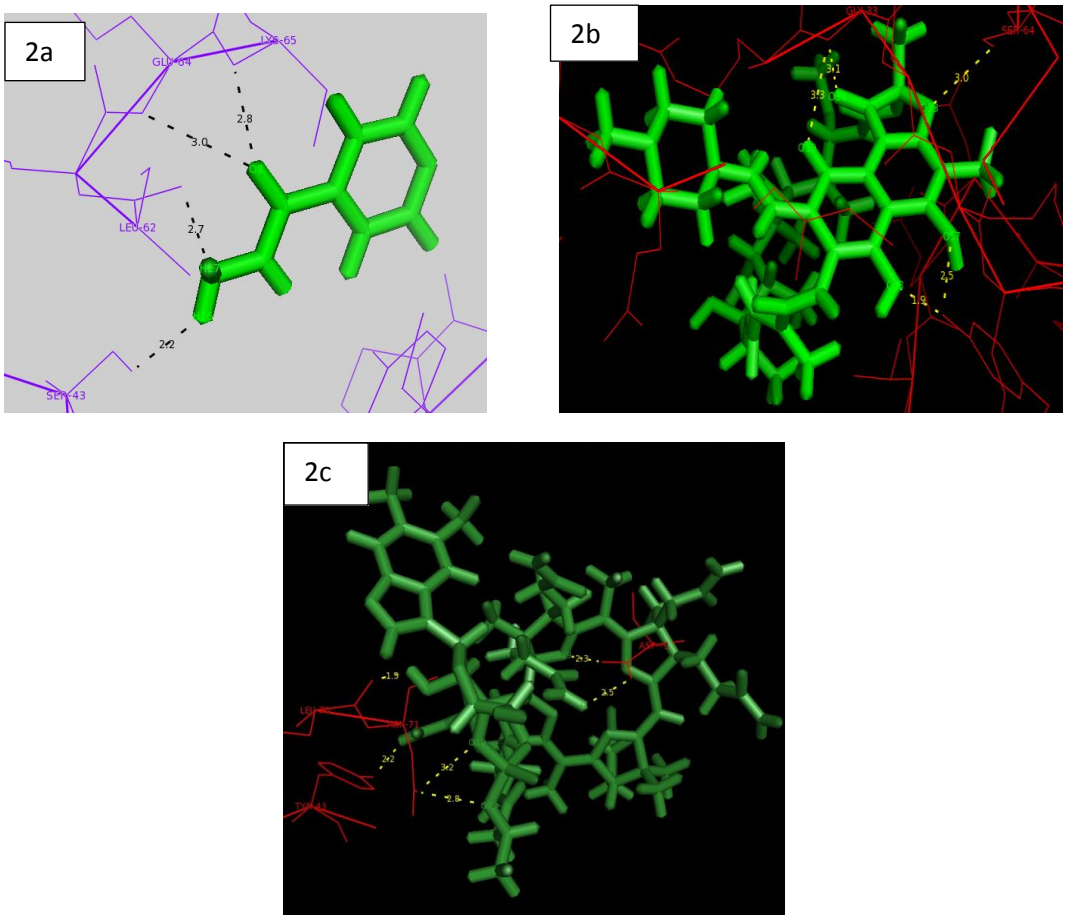

Figure 2: Docked poses of the complexes (a) 1T4Q-INH complex (b) 1Z92-RIF complex (c) 1Z92-vitamin B_{12} complex

RIF showed significant interaction with 1Z92 with a score of 5892 and an ACE of -295.59. The docked pose of 1Z92-RIF complex shows 5 hydrogen bond interactions of lengths ≤ 3.3 Å (Figure 2b). 1Z92 was also found to have significant interaction with Vitamin B12 with a score of 6954 and an ACE of -464.20. 1Z92-vitamin B12 complex showed 6 hydrogen bond interactions of bond lengths ≤ 3.2 Å (Figure 2c). 2H24-beta-carotene complex had a score of 6868 and an ACE of -471.84. Beta-carotene showed no hydrogen bond interaction with any of the receptors but instead we found significant geometric fit of this ligand in the

receptors and hence scoring in PatchDock being based on shape complementarity principles it gave a score of 6680, 6668 and 6540 with 1MK3, 1O7Y and 2MHS respectively.

Drugs, toxins and their toxic metabolites induce cell death and damage through varied mechanisms in which induction of the immune component and apoptotic pathways are also included [8,9]. Our docking experiments have shown that INH and RIF could possibly interact with receptors and cytokines involved in apoptosis and inflammatory mechanisms. This is consistent with the *in vivo* results. Pal et al had shown in their *in vivo* experiments that carotenoids have a significant hepatoprotective role at certain doses [10].

This could possibly be due to its suppression of inflammatory and apoptotic mechanisms by interacting with the above mentioned receptors through their geometric fit. It is also known that RIF induces the production of anti-RIF antibodies that could cause the downstream induction of inflammatory and apoptotic pathways [11]. This could add to the interactions that RIF has with apoptotic and inflammatory receptors and cytokines. Vitamin B12 has been shown to have anti-nociceptive and anti-inflammatory properties in a study done by Hosseinzadeh *et al* (2012) on mice models [12]. This is consistent with the results of our docking studies. Its possible strong binding to the anti-inflammatory and anti-apoptotic compounds could lead to their activation resulting in its protective mechanism against the drug induced toxicity.

CONCLUSION

Drug discovery is a fierce, lengthy and an incorporative strive. Drug discovery is mostly described as a linear, succeeding process followed by *in vitro* and *in vivo* studies to find out if such compounds gratify certain criteria. *In silico* technique can help in recognizing drug targets using bioinformatics tools.

REFERENCES

[1] Lee SS, Buters JT, Pineau T, Fernandez-Salguero P, Gonzalez FJ. J Biol Chem 1996; 271: 12063–12067.
[2] Vuilleumier N, Rossier MF, Chiappe A, Degoumois F, Dayer P, Mermillod B, Nicod L, Desmeules J, Hochstrasser D. Eur J Clin Pharmacol 2006; 62: 423-429.
[3] Tafazoli S, Mashregi M, O'Brien PJ. Toxicol Appl Pharmacol 2008; 229: 94-101.
[4] Tasduq SA, Kaiser P, Sharma SC, Johri RK. Hepatol Res 2007; 37: 845-853.
[5] Cederbaum AI. J Gastroenterol Hepatol 2006; 21: S22-25.
[6] Martin SJ, Baskaran UL, Vedi M, Sabina EP. Toxicol Mech Methods. 2014; 24(8): 584-592.
[7] Dina SD, Yuval I, Ruth N, Haim JW. Nucleic Acids Res 2005; 33(2): 363-367.
[8] Corcoran GB, Fix L, Jones DP, Moslen MT, Nicotera P, Oberhammer FA, Buttyan R. Toxicol Appl Pharmacol 1994; 128(2): 169-181.
[9] Williams GM, Latropoulus MJ. Toxicol Pathol 2002; 30(1): 41-53.
[10] Pal R, Rana S, Vaiphei K, Singh K. Trop Gastroenterol 2008; 29(3): 153-159.
[11] Bassi L, di Berardino L, Perna G, Silvestre LG. Am Rev Respir Dis 1976; 114: 1189–1190.
[12] Hosseinzadeh H, Moallem SA, Moshiri M, Sarnavazi MS, Etemad L. Arzneimittelforschung 2012; 62(7): 324-329.