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In-Silico Drug Design Approach for Identification of Antibacterial Drug Leads for Treatment of Gastric Lymphoma

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ABSTRACT
Gastric lymphoma, lymphoma that originates in the stomach, is an uncommon condition, accounting for less than 15% of gastric malignancies and about 2% of all lymphomas. However, the stomach is a very common extra-nodal site for lymphomas (lymphomas originating somewhere else with metastasis to stomach). It is also the most common source of lymphomas in the gastrointestinal tract. Identification of anti-bacterial leads for development of drug against B-cell lymphoma which are non-carcinogenic is conducted in this study. Oxygen-insensitive NADPH nitroreductase is a potential novel target for development of drugs to alleviate symptoms of B-cell lymphoma. Oxygen-insensitive NADPH nitroreductase is selected as the target receptor.

Biopharmaceutical and Pharmacokinetic information allows us to fast up drug development by Insilico methods. This new approach is termed as Insilico drug designing; have identified 10 anti-bacterial drug leads considering oxygen-insensitive NADPH nitroreductase as the target by using well known algorithms and softwares like AutoDock vina, Hex, Q-site finder. Identified 10 drug leads with lower energies are subjected for toxicity and admet analysis to evaluate the carcinogenicity property. Among the 10 anti-bacterial compounds evaluated for toxicity analysis Benzimidazoles, Tosufloxacin, Ertapenem, Grepafloxacin, Sitafloxacin, Oxacillin, Ofloxacin, and Levofloxacin have anti-carcinogenic property. Benzimidazoles, Tosufloxacin, Ertapenem, Grepafloxacin, Sitafloxacin, Oxacillin, Ofloxacin, and Levofloxacin have E total values -733.73, -393.76, -589.69, -485.84, -527.70, -597.95, -460.85, and -460.85 respectively. These anti-bacterial compounds with lower energies and anti-carcinogenic property are the lead for further optimization and invitro screening by clinical studies for development of the drug against Gastric lymphoma.

Keywords: Lymphoma, Oxygen-insensitive, NADPH nitroreductase, AutoDock Vina, Benzimidazoles.

1. INTRODUCTION
Gastric lymphoma is the Non- Hodgkin's type of B-cell lymphoma, gastric B-cell mucosal-associated lymphoid tissue (MALT)-lymphoma, which originates in stomach. The stomach is the most frequently involved site for extranodal lymphomas, accounting for nearly two-thirds of all gastrointestinal cases. The symptoms of this lymphoma include epigastric pain, early satiety, fatigue, weight loss, haematemesis, and melaena or abdominal mass. It is widely accepted that gastric B-cell, low-grade mucosal-associated lymphoid tissue (MALT)-lymphoma is caused by infection of *Helicobacter pylori* (*H. pylori*), a microaerophilic, Gram-negative pathogen that is highly specific for the human gastric mucosa. MALT-lymphomas may engender different clinical and endoscopic patterns. Although some aspects still remain unclear, the pathogenetic cascade of gastric lymphoma has been revealed. Structured lymphatic tissue; i.e. lymphatic follicles, is lacking in normal gastric mucosa. Indeed, through the alimentary tract, lymphatic tissue is exclusively present in tonsils and Peyer's patches. However, following inflammatory processes, lymphatic follicles may appear on gastric mucosa, configuring the so-called MALT, as described by Wright in 1983[1]. Ten years later, Genta et al [2] clearly showed that the main cause of MALT onset on gastric mucosa was *H. pylori*-related gastritis because *H. pylori* is the stimulus to the acquisition of gastric MALT, however, the first step on the pathway to the development of gastric lymphoma is the acquisition of organized lymphoid tissue of MALT-type only by limited number of immunological stimuli within which subsequent genetic events may occur that result in the development of lymphoma [3].

Gastric MALT lymphoma represents the first lymphoma where an antigen driven pathogenesis was documented together with the unexpectedly effective antibacterial treatment option, leading to the complete modification of its management [4, 5]. Clinical studies have taken this discovery further and shown that patients with early low grade gastric

MALT lymphoma treated with anti-Helicobacter therapy can show regression of their tumours. It is now generally accepted that eradication of *H. pylori* is a central component of the management of MALT lymphoma. Metronidazole (Mtz) [1-(2-hydroxyethyl)-2-methyl-5-nitroi-midazole] is a key component of combination therapies that are widely used against *Helicobacter pylori* (6). Metronidazole is considered a prodrug whose uptake and activation requires intracellular reduction, resulting in the production of cytotoxic short-lived radicals and other reactive species (7). 5-Nitroimidazole is activated via interactions with redox systems capable of reducing the low-potential (-415 mV) nitro group in position 5 of the imidazole ring (7).This property makes metronidazole effective against organisms in a low-intracellular-redox state, such as anaerobic bacteria and protozoa, as well as some microaerophiles, such as Campylobacter spp and *Helicobacter pylori*(8). The frequent use of metronidazole has resulted in increased resistance to the antibiotic by *H. pylori*. The emergence of resistant isolates that do not respond to the drug fostered an interest in understanding the primary causes of resistance to metronidazole in this bacterium. Extensive investigations of *H. pylori* established that the main causes of metronidazole resistance are mutations in the gene rdxA or frxA (9, 10, 11, &12). Success of antimicrobial regimens for *H. pylori* eradication depends on patient compliance and lack of antimicrobial resistance. Metronidazole (Mtz) containing regimens have been shown to limit effectiveness because of increasing prevalence of resistance to this drug. A high prevalence (>90%) of Mtz resistance in *H. pylori* has been reported especially in developing countries [13]. Mtz resistance may be mediated through an inability of Mtz-resistant strains to remove oxygen from the site of Mtz reduction, thereby preventing Mtz activation. This has been attributed to a mutation on the rdxA genes resulting in strains of the organism with defective oxygen insensitive NADPH nitroreductase a enzyme coded by rdxA gene proposed to reduce Mtz to active metabolites that are toxic to bacterium[14]. Infection by Mtz resistant strains is an important factor leading to treatment failure; subjecting all H. pylori clinical isolates to susceptibility testing most especially to Mtz is recommended. If not possible, a program to survey the prevalence of resistance should be implemented in a given area or population. This increasing emergence of antimicrobial resistance in *H. pylori* treatment posses serious public health problems and is therefore necessary that new drug regimens be examined. Hence the enzyme coded by the gene rdxA i.e. oxygen insensitive NADPH nitroreductase 3QDL is selected as target protein to design a novel antibacterial agent that facilitate treatment in future [15].

2. METHODS

Collection of Compounds

The biochemical compounds found in the natural sources were collected through a literature survey [16, 17, & 18]. These compounds were screened using Lipinski's rule of 5. The structure of molecules that concurred with the Lipinski rule was downloaded in the SDF format from the chemical database, PubChem, the 3D structure of the ligands is viewed by Swiss PDB Viewer in PDB format (as shown in Figure.1&2). A total of 100 molecules were collected.

Energy Minimization

The screened biochemical compounds were energy minimized using Marvin Sketch, a java based chemical editor for drawing chemical structures, queries and reactions. 10 conformers for each molecule were obtained and the one with the least energy was selected. Subsequently, these energy minimized molecules were used as ligands for docking against the selected receptor molecule.

Selection of the Receptor

In accordance to the literature studies and the research carried out, oxygen insensitive NADPH nitroreductase with PDB ID 3QDL, an enzyme coded by rdxA gene in *Helicobacter pylori*, was selected as the target molecule. The target is coded by the rdxA gene, it reduces the anti-bacterial Metronidazole to reactive metabolite that is toxic to the bacterium, but the rdxA gene undergoes mutation in resistant *H. pylori* strains and fails to produce the oxygen

insensitive NADPH nitroreductase and hence Mtz will be ineffective in resistant strains. Its structure was retrieved from Protein Data Bank (PDB), 3QDL being the PDB ID for the protein molecule.

Docking of Receptor with Ligand

The energy minimized ligands and the selected target protein was subjected to docking using AutoDock Vina and compounds (approx. 10 to 15) with the least binding affinity were sorted out. These compounds were docked with the target protein using HEX and free energy of each ligand was recorded (Table.1). The binding site of all ligands was located using Q-site finder (Table.2). The interaction of the ligands Benzimidazoles and Oxacillin with the target protein oxygen-insensitive NADPH nitroreductase (3QDL) is visualized by Swiss PDB Viewer (Figure 3 & 4). The site common to maximum number of compounds was put forward as the binding site of our lead compound.

Toxicity and ADME Analysis

Toxicity analysis was carried out for the ligand molecules using PreADMET. Human health effects such as carcinogenicity and mutagenecity were evaluated and the molecules which passed the toxicity test were selected for further analysis. Further Absorption, Distribution of the compounds i.e. ADME predictions was performed using PreADMET. The absorption and distribution parameters like Human intestinal absorption (HIA %), In vitro Caco-2 cell permeability (nm/sec), and In vivo blood-brain barrier penetration (C.brain/C.blood) were calculated for the compounds which passed toxicity analysis (Table.3).

3. RESULT AND DISCUSSION

The main objective of the research was to identify the anti-bacterial leads from chemical compound database to treat the gastric lymphoma. The drug Metronidazole which is already present in the market for the treatment of gastric lymphoma has been ineffective due to the development of resistance against the drug by the *Helicobacter pylori*. Naturally the development of the MALT in gastric mucosa is by limited number of immunological stimuli, later by occurrence of genetic events in gastric MALT lead to the development of the gastric lymphoma. But when the micro-organism *Helicobacter pylori* causes gastritis it creates the stimulus for the development of gastric MALT which is an abnormal development and this leads to gastric lymphoma. The drug metronidazole which attacks *H. pylori* it is reduced by the oxygen-insensitive NADPH nitroreductase which will be coded by rdxA gene of the bacterial genome and then the Mtz will be activated to short-lived radicals and reactive cytotoxic species which will lead to death of bacteria. But due to mutations in rdxA gene the oxygen-insensitive NADPH nitroreductase will not be produced and this leads to ineffectiveness and subsequent development of resistance against the drug. In this study leads against *Helicobacter pylori* considering the oxygen-insensitive NADPH nitroreductase (3QDL) as target protein for the development of novel drug was conducted. In this regard a library of anti-bacterial ligand of 159 compounds was created from literature survey an assemblage of 100 compounds was obtained by Lipinski's screening. The 100 ligands obtained were subjected for docking and toxicity analysis and finally 8 anti-carcinogenic leads with lower energy were obtained (Table.1&3). They are Benzimidazoles, Tosufloxacin, Ertapenem, Grepafloxacin, Sitafloxacin, Oxacillin, Ofloxacin, and Levofloxacin. By active site analysis it was observed that amino acids His, Val, Cys, Pro, Trp were present at active sites 6 and 8 which were the prominent binding sites of the ligands on receptor 3QDL (Table.2). The commercially available drug for treating the gastric lymphoma was taken as the reference molecule, it was docked against target 3QDL using Hex and AutoDock Vina, and it bound to the target at active site 1 with E total 164.50. Benzimidazoles showed highest docking score with E-total -733.73 followed by Tosufloxacin, Ertapenem, Grepafloxacin, Sitafloxacin, Oxacillin, Ofloxacin, and Levofloxacin with E-total values -393.76, -589.69, -485.84, -527.70, -597.95, -460.85, and -460.85 respectively. These are best leads compounds for gastric lymphoma treatment. Among 8 compounds identified Benzimidazoles and Oxacillin showed best results with highest E total values. The Hex docking results i.e. E total values of the Metronidazole and 8 lead compounds are compared as shown in below graph (Figure.5).

Metronidazole has the E total score of -164.50 which is very low compared to the leads identified.

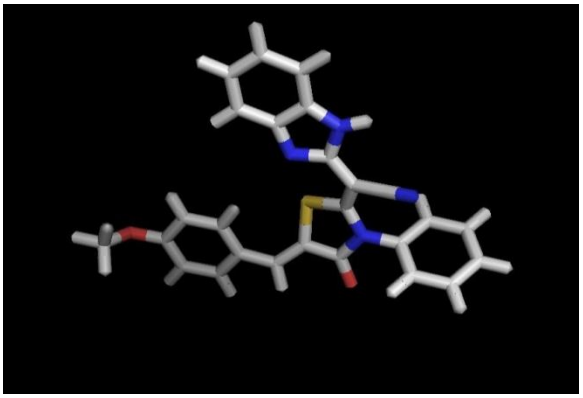

Figure 1: 3D structure of Benzimidazoles

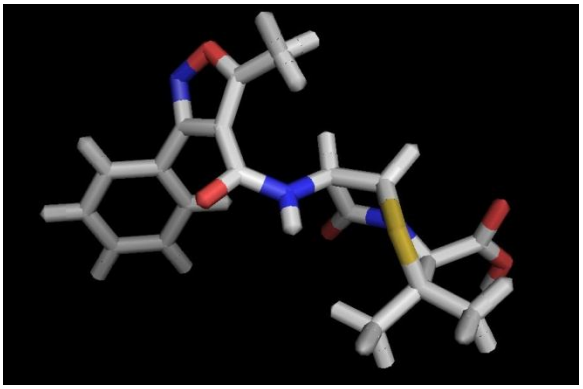

Figure 2: 3D Structure of Oxacillin

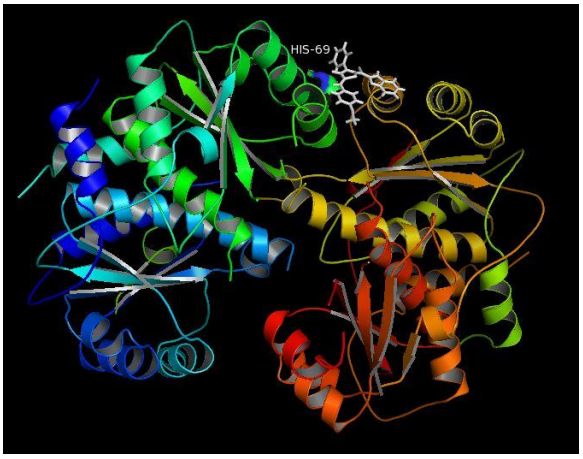

Figure 3: Interaction of Benzimidazoles with target protein 3QDL at HIS-69 active site

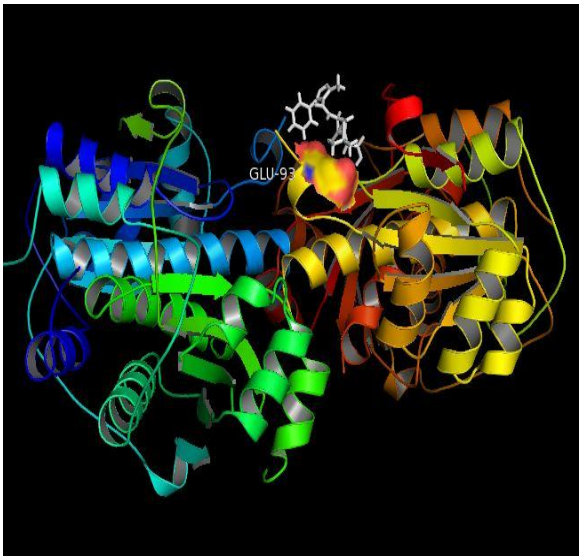

Figure 4: Interaction of Oxacillin with target protein 3QDL at GLU-93 active site

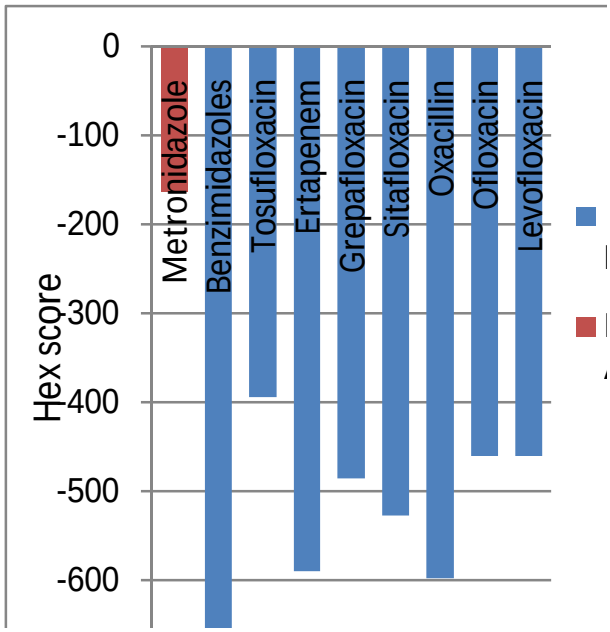

Figure 5: Comparison of the Hex-score of the Leads identified and that of Drug available in market

Table 1: Affinity and Free energy of Ligands

Rank	Name of the compound	Docking Score	E total
1	Benzimidazoles	-9.6	-733.73
2	Tosufloxacin	-8.9	-393.76
3	Ertapenem	-8.4	-589.69
4	Grepafloxacin	-8.3	-485.84
5	Sitafloxacin	-8.2	-527.70
6	Oxacillin	-8.1	-597.95
7	Ofloxacin	-8.0	-460.85
8	Levofloxacin	-7.8	-460.85

Table 2: Binding sites of leads to Target protein

Rank	Name of Lead	Active Site Bound To	Binding site residues
1	Benzimidazoles	8	3963 CB HIS C 53 3987 CB VAL C 55 4290 CB CYS C 87
2	Tosufloxacin	6	528 O PRO A 51 533 CA TRP A 52 548 N HIS A 53
3	Ertapenem	6	528 O PRO A 51 533 CA TRP A 52 548 N HIS A 53
4	Grepafloxacin	8	3963 CB HIS C 53 3987 CB VAL C 55 4290 CB CYS C 87
5	Sitafloxacin	10	1301 CA PRO A 166 1307 N LEU A 167 1316 N LYS A 168
6	Oxacillin	8	3963 CB HIS C 53 3987 CB VAL C 55 4290 CB CYS C 87
7	Ofloxacin	6	528 O PRO A 51 533 CA TRP A 52 548 N HIS A 53
8	Levofloxacin	6	528 O PRO A 51 533 CA TRP A 52 548 N HIS A 53

Table 3: Toxicity analysis

	Name of the compound	Ames test	Carcinogenicity	
			Mouse	Rat
1	Benzimidazoles	Mutagen	Negative	Negative
2	Tosufloxacin	Mutagen	Negative	Negative
3	Ertapenem	Mutagen	Negative	Negative
4	Grepafloxacin	Mutagen	Negative	Negative
5	Sitafloxacin	Mutagen	Negative	Negative
6	Oxacillin	Mutagen	Negative	Negative
7	Ofloxacin	Mutagen	Negative	Negative
8	Levofloxacin	Mutagen	Negative	Negative

4. CONCLUSION

By In Silico Drug designing 8 anti-bacterial compounds with anti-carcinogenecity property viz. Benzimidazoles, Tosufloxacin, Ertapenem, Grepafloxacin, Sitafloxacin, Oxacillin, Ofloxacin, and Levofloxacin are been identified as the lead compounds for further optimization of drug against the gastric lymphoma. Among the 8 compounds Benzimidazoles and Oxacillin gave better results. Most of the physical and chemical properties, such as hydrogen bond-acceptor, hydrogen bond-donor, heavy atom count etc. of the drug Metronidazole and the lead compound Oxacillin were analogous.

5. REFERENCES

1. Isaacson P, Wright D H. 1983. **Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma**. *Cancer*. 52: 1410-1416
2. Genta R M, Hamner H W, Graham D Y. 1993. **Gastric lymphoid follicles in *Helicobacter pylori* infection: frequency, distribution, and response to triple therapy.** *Hum Pathol*. 24: 577-583
3. Owen R L, Jones A L. 1974. **Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles.** *Gastroenterology*. 66:189-203
4. Hussell T, Isaacson P G, Crabtree J E et al. 1993. **The response of cells from low-grade B-cell gastric lymphomas of mucosa-associated lymphoid tissue to *Helicobacter pylori.*** *Lancet*. 342:571–4.
5. Wotherspoon A C, Doglioni C, Diss T C et al. 1993. **Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori.*** *Lancet.* 342:575–577
6. Malfertheiner, P., Megraud F., O'Morain, C. 1997. **Current European concepts in the management of *Helicobacter pylori* infection.** *The Maastricht consensus report*. Gut 41:8–13
7. Scarpignato, C. 2004. **Towards the ideal regimen for *Helicobacter pylori* eradication: the search continues**. *Dig. Liver Dis.* 36 :243–247
8. Land, K. M., and P. J. Johnson.1999**. Molecular basis of metronidazole resistance in pathogenic bacteria and protozoa.** *Drug Resist. Updat*.2:289–294
9. Debets-Ossenkopp, Y. J., R. G. J. Pot, D. J. Van Westerloo, A. Goodwin, C. M. J. E. Vandenbroucke-Grauls, D. Berg, P. S. Hoffman, and J. G. Kusters.1999. **Insertion of mini-IS605 and deletion of adjacent sequences in the nitroreductase (rdxA) gene cause metronidazole resistance in *Helicobacter pylori* NCTC11637.** *Antimicrob. Agents Chemother*.43:2657–2662
10. Goodwin, A., D. Kersulyte, G. Sisson, S. J. O. Veldhuyzen van Zanten, D. E. Berg, and P. S. Hoffman.1998. **Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (rdxA) that encodes an oxygen-insenstive NADPH nitroreductase.** *Mol. Microbiol.* 28:383–393
11. Kwon, D. H., F. A. K. El-Zaatari, M. Kato, M. S. Osato, R. Reddy, Y. Yamaoka, and D. Y. Graham. 2000. **Analysis ofrdxA and involvement of additional genes encoding NAD(P)H flavin oxidoreductase (FrxA) and fer-rodoxin-like protein (FdxB) in metronidazole resistance in *Helicobacter pylori*.** *Antimicrobial Agents Chemother.* 44:2133–2142
12. Kwon, D. H., M. Kato, F. A. K. El-Zaatari, M. S. Osato, and D. Y. Graham. 2000. **Frame-shift mutations in NAD(P)H flavin oxidoreductase encoding gene (frxA) from metronidazole resistant *Helicobacter pylori* ATCC43505 and its involvement in metronidazole resistance.** *FEMS Microbiol. Lett.* 118:197–202
13. Alarcon T, Domingo D, Lo´pez-Brea M. 1999. **Antibiotic resistance problems with *Helicobacter pylori*:** Review article. *Int. Journal. Antimicrob. Agent.* 12: 19-26
14. Jeong, J Y, Mukhopadhyay AK, Akada JK, ailidiene D, Hoffman P, Berg DE. 2001. **Roles of FrxA and RdxA nitroreductases of *Helicobacter pylori* in susceptibility and resistance to metronidazole.** *Bacteriol.* 183: 5155-5162.
15. PETER J. JENKS, Institute of Infections and Immunity, University Hospital, Nottingham,

NG7 2UH, UK. 2000. ***Helicobacter pylori*: towards new therapeutic targets.** *J. ed. Microbiol*. 50: 485-488

16. Arthur R. English, James A. Retsema, Arthur E. Girard, John E. Lynch and Wayne E. Barth. **A Beta-Lactamase Inhibitor That Extends the Antibacterial Spectrum of Beta-Lactams: Initial Bacteriological Characterization.** *CP*. 45: 899
17. Christian Apfel, David W. Banner, Daniel Bur, Michel Dietz, Takahiro Hirata, Christian Hubschwerlen, Hans Locher, Malcolm G. P. 2000. **Wolfgang Pirson, Gérard Rosse, and Jean-Luc Specklin Preclinical Research, F. Hoffmann-La Roche Ltd., CH-4070 Basle, Switzerland, Hydroxamic Acid Derivatives as Potent Peptide Deformylase Inhibitors and Antibacterial Agents.** *J. Med. Chem.* 43 (12): 2324–2331