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FINAL REPORT

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In current project, the in-silico drug designing was studied in which I have discovered some new potent antituberculosis agents which can be anti TB drugs on further validation. In the present study, I have targeted the one enzyme, cyclopropane-fatty-acyl-phospholipid synthase 2 enzymes (3HEM protein) which are found to play an important role in the metabolism of Mycobacterium Tuberculosis. The 3D-structures of protein were obtained from the protein database and were analysed by using bioinformatics tools. The digital files of enzymes were procured from PDB database. The obtained file had the 3D enzyme structure complex with the ligand.

The next step was to introduce chemical compound (drug) having better chemical binding energies than the existing ligands. I designed chemical derivatives by using N-Phenylpyrazine-2-carbaxamide based compound. These compounds and subsequent substituted derivatives were designed virtually on 3D molecule design software and geometries were optimized by using Molecular Mechanics. The global and local minimum energies were obtained that gave us the most preferred conformations of all the derivatives in space. In this way, we have designed the libraries of all the substituent of the three base compounds.

The following one class of compounds were selected for docking study,

a) N-Phenylpyrazine-2-carbaxamide
b) 5-Chloro-N-(4-chlorophynyl)pyrazine-2-carboxamide
c) N-(3,4-dichlorophenyl)pyrazine-2-carboxamide
d) N-(4-chlorophenyl)pyrazine-2-carboxamide
e) 5-tert-butyl-N-(4-chlorophenyl)pyrazine-2-carboxamide
f) Natural inhibitor
g) N-(2-nitrophenyl)pyrazine-2-carboxamide
h) N-(4-nitrophenyl)pyrazine-2-carboxamide

These compounds are docked in the binding site of enzymes by using rigid ligand-rigid receptor docking methods. In the rigid ligand-rigid receptor docking, the results were encouraging and show binding energy in kcal/mol lower than natural inhibitor.

It is clear that the compounds selected are having better (lower) binding energy than natural inhibitor. Therefore the results shows that the binding energy values are favourable enough and could be better substitution alternative to the original ligands. It is possible to further get better values for activity of these molecules by using various mathematical and statistical calculations, but it is out of scope of this project.

Thus, the molecules which are designed in-silico can be potent anti-tuberculosis agents in the future.

Table 1.1: The binding energy values of N-Phenylpyrazine-2-carbaxamide based molecules with selected enzyme.

N-Phenylpyrazine-2-carbaxamide based molecules	Cyclopropane-fatty-acyl-phospholipid synthase 2 enzymes (3HEM protein)
	ΔG Kcal/mol. Minimum
Molecule no.1	-9.1634
Molecule no.2	-9.3775
Molecule no.3	-9.0016
Molecule no.4	-8.4074
Molecule no.5	-9.9826
Molecule no.6 Natural Inhibitor	-11.307
Molecule no.7	-12.8654
Molecule no.8	-12.1622

Also the synthesis path of these molecules is also validated since the parent base compounds are natural products or their derivatives; hence they can be either isolated from nature or can be synthesized in laboratory.

We finally decided to synthesize two molecules and checked for their biological activities using in-vitro techniques. It is reported that the synthesized molecules are active against the Mycobacterium Tuberculosis.

Synthesis and in-vitro analysis of molecules :

I have synthesized two compounds 1) N-(2-nitrophenyl)pyrazine-2-carboxamide and 2) N-(4-nitrophenyl)pyrazine-2-carboxamide, on the basis of binding energy with 3HEM protein and in-vitro analysis, Compounds shows at initial single point screening, compounds are found active @ 64 µg/ml. Synthesised compounds were tested at lower concentration against M. tuberculosis H37Rv to determine the MIC testing by micro dilution assay/ REMA method. The MIC determination is as follows.

MIC determination :

Starting concentration : Below 64 µg/ml i.e. 32 µg/ml

Screen points: 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml, 1 µg/ml, 0.5 µg/ml

Research paper have been published in the International journal :

1) Preliminary Phytochemicals Investigation and TLC Analysis of Ficus racemosa Leaves.

2) Phytochemicals investigation and TLC profiling of Cyamopsis tetragonolobal Seeds(Fabaceae)- pre family

3) Effect of Concentration of Substituted Pyrazoline on Acoustic parameters.

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