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DESIGN, SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 2-MERCAPTOBENZIMIDAZOLE DERIVATIVES

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ABSTRACT

Dihydrofolate reductase (DHFR) is the important target for antimicrobial drugs belonging to the class of antimetabolites as the enzyme plays important role in the de novo purine synthesis. Since 2-mercaptobenzimidazole(2MBI) shares structural similarity with purine nucleotides, We here report the *in silico* screening to obtain best fit molecules as DHFR inhibitors, synthesis of some 'best fit' 2MBI derivatives (Mannich bases) and their *in vitro* antimicrobial assay using paper disc method. The structures of these molecules were elucidated by Infrared. These compounds were then subjected for *in vitro* antimicrobial activity against gram +ve and gram -ve bacteria using Ciprofloxacin as standard at concentrations of 50ug/ml, 100ug/ml and 200ug/ml. Some of the compounds show satisfactory activity at 200ug/ml.

INTRODUCTION

The current interest in the development of new antimicrobial agents can be partially ascribed both to the increasing emergence of bacterial resistance to antibiotic therapy and to newly emerging pathogens. Despite advances in antibacterial therapy, many problems remain to be solved for most available antimicrobial drugs and hence requirement of newer drugs (Sahu, 2006).

Even most of the drugs exert their pharmacological reactions depend only upon their successful binding to their receptor's active site inside the body thus either mimicking or mitigating the effect of natural ligand's binding to the receptor. Hence the understanding of mode of binding of ligands to their receptors will be crucial in successful design of more efficient drugs. Experimental methods to identify these binding modes are more expensive and time consuming. The computational process of searching for a ligand that is able to fit both geometrically and energetically the binding site of a protein is called molecular docking (Teodoro *et al*., 2000).

Over the past few decades, Mannich bases of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their wide gamut of biological activities ranging from antibacterial, antifungal, anticancer, antiparkinson to anticonvulsant and anti–HIV. Many effective antimicrobial agents show a heterocyclic moiety within their structure and, in particular, that substituted Benzimidazole, Benzoxazole (Sarhan *et al*., 2006)

EXPERIMENTAL

Selection of Amines

The primary and the secondary amines for mannich reaction were scrutinized on basis of availability and cost. Applying the Scheme of synthesis the structure of various derivatives were drawn. (ChemDraw 8.0) (Table 1)

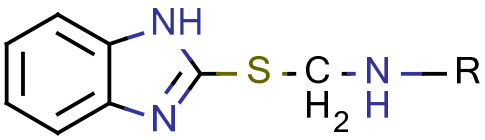
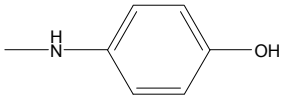
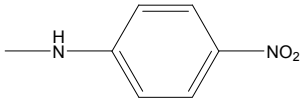
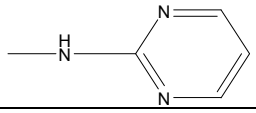
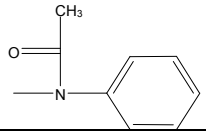
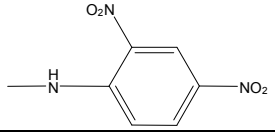
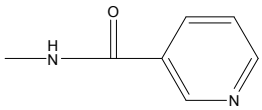


Table 1
Selection of various amines and their codes

Sr. no	Code	R =
1	PAP	
2	PNA	
3	AMP	
4	ACN	
5	DHH	
6	NCT	

Molecular modeling

Molecular docking was performed for 2-MBI analogues using the GLIDE® integrated Maestro® 8.0 interface. The structure for each compound drawn using ChemDraw 8.0 was as saved as mol file. The high-resolution X-ray structure of S.aureus DHFR complex with inhibitor (RCSB PDB id code 1DRF) was imported into GLIDE module and the docking simulations were carried out with cofactor NADPH to explore the binding interactions of 2-MBI Mannich bases (Maestro 8.0, 2007). Trimethoprim was included in the docking run. The low energy conformations of these ligands were generated by LigPrep module.

Scoring function

GLIDE module uses force field based MCSA minimization with free energy scoring. This Produces G-Scores and Emodel of binding scores. The scores are mentioned in Table **2.**

Validation of the docking protocol

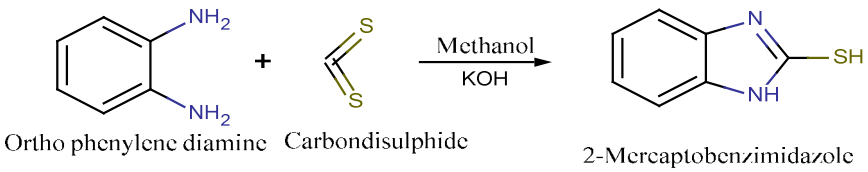
Validation of the docking protocol was carried out by correlation coefficient method and by pose regeneration of the same ligand structure as seen in the crystallized PDB.

Table 2
G-scores, Emodel and receptor contacts

Compound	G-score	E model	Receptor Contacts
PAP	-8.17	-69.5	ILE 05, HOH 715,VAL 08
PNA	-6.66	-61.5	ILE 60,PHE 34,HOH 647,PHE 31
AMP	-6.91	-57.8	ILE 07,VAL 115
ACN	-7.75	-68.7	VAL 115
DHH	-6.91	-75.9	HOH 715,HOH 679,PRO 61,HOH 647,LEU 67
NCT	-8.04	-62.4	PHE 34,VAL 08,ALA 09
Trimethoprim (reference)	-8.51	-70.7	HOH 715,VAL 115,ILE 60, GLY 116

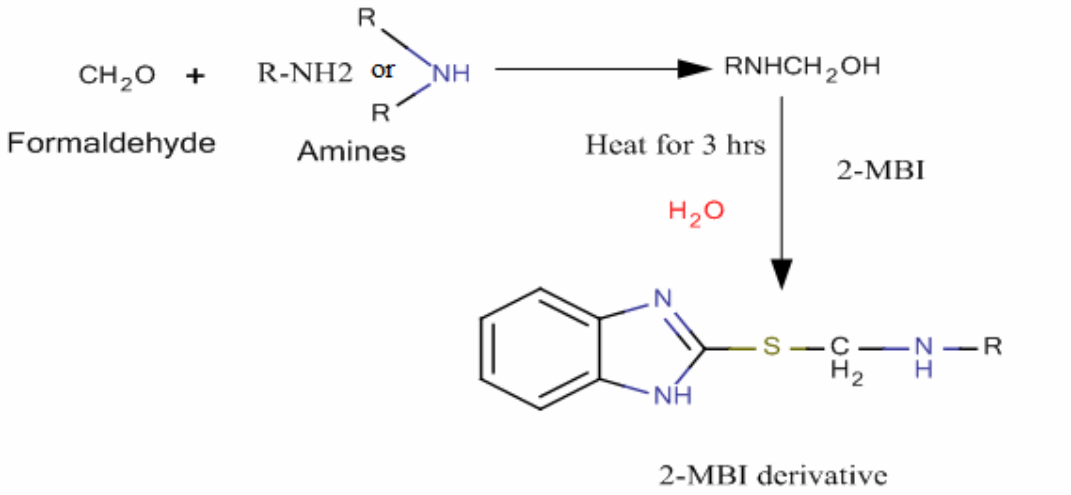
Synthesis of 2-MBI:

O-phenylenediamine & carbon disulphide reacts in the presence of aqueous methanolic KOH to form 2-mercaptobenzimidozole. One of the amino group of O-phenylenediamine is alkylated with CS_2 to from the mercapto derivative which on further removal of H_2S gas gets cyclizes to form the benzimidazole nucleus substituted at second position as 2-mercaptobenimidozole (Paul and Mae-ling,1999).



Synthesis of 2-MBI derivatives:

The nucleophilic addition of amine to the carbon of formaldehyde followed by condensation of the Mannich base on reaction with 2-mercaptobenzimidazole gives the final product (Mannich and Krosche, 1912).



The IR spectra were recorded on a Spectrum RX-1 Perkin Elmier FT-IR spectrometer in potassium bromide discs. Merck GF254 pre-coated silica plates were used for TLC. The reaction was monitored by TLC columned over silica gel 120 column and acetone: ethyl Acetate (1:1) solvent system. The compounds were recrystallized using appropriate organic solvent. The melting points are uncorrected and taken on digital melting point apparatus. The physical data of these compounds is mentioned in Table 3

Table 3
Physical constants and yield of the derivatives.

Sr. no	Comp. Code	Yield (%)	M. P (^{o}C)	Rf. value
1	PAP	35	172.2-173.8	0.85
2	PNA	64	115.2-116.8	0.87
3	AMP	60	117.5-119.5	0.90
4	ACN	80	131-132.5	0.83
5	DHH	73	130.4-131.9	0.90
6	NCT	82	154.5-156	0.77

Table 4
IR spectra of the derivatives.

Code	IR cm^{-1}
PAP	NH(3280,3101), Ar-OH(3657), C_6H_5(876,803), C-N(1368), C=N(1509)
PNA	NH(3356), C_6H_5 (740,839), C-N(1310), C=N(1625), -CH2(1328),N=O(1599)
AMP	NH(3154), C_6H_5(739,802), C-N(1340),C=N(1585), -CH2(1463)
ACN	NH(3158,3449), C_6H_5(736),C=O(1662), C-N(1345), C=N(1598), -CH_2(1464)
DHH	NH(3307), C_6H_5(661,740,830),C-N(1337), C=N(1615), -CH_2(1443) ,N=O(1511)
NCT	NH(3151,3269,C_6H_5(650,737,807),C=O(1622),C-N(1345),C=N(1681),-CH_2(1463)

Invitro Antimicrobial Activity:

The Antimicrobial activity was performed on E.coli ATCC3750 and B. Subtilis 6633, Haffkine Institute, Mumbai using the paper disc method. This method is based on the diffusion of an antibiotic from a paper disc through the solidified agar layer of a Petri dish or a plate used for study. Growth of inoculated microorganism is inhibited entirely in a circular area 'zone', around cylinder or a paper containing a solution of the antibiotic. The diameter of the zone was calculated and the diameter of the disc was subtracted to give the diameter of inhibition for each derivative as compared to standard i.e ciprofloxacin (Gaud RS and Gupta, 2006). The derivatives were at concentrations of 50ug/ml, 100ug/ml and 200ug/ml (Table 5)

Table 5
Diameter of Zone of inhibition (mm)

Compound Code	B. subtilis (gram +ve) strain: 6633			E. coli (gram -ve) strain: ATCC3750		
	50μg/ml	100μg/ml	200μg/ml	50μg/ml	100μg/ml	200μg/ml
PAP	4	9	14	--	8	9
PNA	5	7	9	3	7	8
AMP	5	11	16	8	9	14
ACN	3	3	4	--	7	10
DHH	4	10	13	5	9	16
NCT	--	--	4	7	10	10
Ciprofloxacin	10	14	18	8	10	19

RESULTS AND DISCUSSION

The main success of the QSAR method is the possibility to estimate the characteristics of new chemical compounds without the need to synthesize and test them. So, in order to predict the biological activity of novel 2-MBI derivatives, we have docked them on DHFR receptor with help of Schrodinger software. The compounds exhibited satisfactory receptor interaction (Table 2)

The compounds were synthesized using mannich reaction exhibited the absence of thiol group peak in IR. Other characteristic groups were found in each compound.

T h e antibacterial activity revealed that NCT did not possess significant activity against B.subtilis, while PAP and ACN exhibited no activity against E.coli at lower concentration (50ug/ml). The most potent activities were observed with AMP and DHH as compared activities were observed with AMP and DHH as compared with standard ciprofloxacin at a concentration of 200ug/ml (Table 5)

CONCLUSION

Various derivatives of 2MBI were synthesized using Mannich reaction exhibited moderate to good antimicrobial activity .Since the docking score were comparable with that of the standard i.e. Trimethoprim, the compounds may be acting by DHFR inhibition.

ACKNOWLEDGEMENT

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