ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

IN-SILICO DESIGN, SYNTHESIS AND EVALUATION OF MurB INHIBITORS AS ANTIBACTERIAL AGENT

Sanjay V. Patel[*1], Milan N. Patel
[1]Department of Pharmaceutical Chemistry, L.B.Rao Institute of Pharmaceutical Education and Research, Bethak Road, Khambhat-388620, Gujarat, India.
Email: gatu_sanju@yahoo.co.in

Received on 3-07-2011 *Accepted on 22-07-2011*

Abstract

Heterobicyclic compounds are widely distributed in nature and exhibit variety of biological activities. They may become promising candidates for exploiting more useful therapeutically active molecules. The compounds having oxazolo pyrimidinone moiety are associated with interesting wide spectrum biological activities, such as kinase inhibition, adenosine receptor antagonism, antibacterial, tumour growth inhibition and some show to inhibit the ability of ricin to inactivate the ribosomes etc. The intermediate azlactones are also useful precursors for the synthesis of amino acids, peptides, heterocycles, biosensors and antitumor or antimicrobial compounds.

In the present study, it was proposed to synthesize lead molecules of oxazolo pyrimidinone skeleton, apt for binding to the target enzyme, bacterial MurB based on the rational approach and to study their docking simulations using ArgusLab 4.0.1. Based on the above studies, an attempt was made to synthesize heterobicyclic 6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one, parent molecule from Erlenmeyer-Plochl azlactone synthesis and its derivatives. Based on it, series of parent compound analogues were designed and synthesized. The synthesized test compounds were then characterized by TLC, melting point determination, IR, ^{1}H-NMR and mass spectral studies and tested for their antibacterial activity against *S.aureus* and *E.coli*. Among all synthesized 8 test compounds, compound 7-(4-chlorophenyl)-2-(4-methoxyphenyl)-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one showed better activity against *E.coli* and compound 7-(4-

chlorophenyl)-2-methyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one showed better activity against *S.aureous* than rest of the other test compounds. The rational approach to lead discovery has prompted a better insight in developing a more specific 6,7-dihydrooxazolo[5,4-d] pyrimidin-5(4H)-one analogues as potential antibacterial agent.

Keywords: Antibacterial activity, Docking simulations, Erlenmeyer-Plochl azlactone, Heterobicyclic oxazolo pyrimidinone.

Introduction

As reviewed a decade ago by Gadebusch *et al.*[1], discoveries in the cell wall area have been made among natural products through a mixture of screens for empirical (i.e. antibacterial) and target-directed (i.e. pathway or enzyme) activity. The underlying concept of the initial targeted screens, that inhibitors of cell wall synthesis will cause the formation of spheroplasts in osmotically stabilized media, was based on work of J Lederberg.[2]

The enzymes of the peptidoglycan synthetic pathway in *Escherichia coli* have been defined as essential by conditional mutants, knockouts and, in many instances, inhibitors. The advent of the genomics era has enabled a search for broadly conserved 'underexploited' antibacterial targets-defined as those targets for which no or few antibacterial inhibitors have been described. Though long pursued, such underexploited targets exist in the peptidoglycan pathway.

Peptidoglycan biosynthesis requires more than 10 synthetic transformations each one of them requiring a specific enzyme.[3] These enzymes include MurA, MurB, MurC, MurD, MurE, MurF, MraY, MurG and the transglycosylase and transpeptidase families of enzymes. Mur proteins are highly conserved among various bacterial species, and common structural motifs can be identified. For this reason, a potential Mur inhibitor would be expected to be bactericidal and to have a wide spectrum, which validates the choice of these important bacterial enzymes as targets for the development of new inhibitors. **Figure-1** displays the biosynthetic pathway of peptidoglycan precursor UDP-*N*-acetylmuramyl pentapeptide (m-A_2pm) from UDP-*N*-glucosamine. Inhibition of any of these essential enzymes leads to loss of cell shape and integrity followed by bacterial death.[4,5,6] This can be apply in both gram-positive and gram-negative organisms. Of these enzymes

only MurA, the transglycosylases and the transpeptidases have been the targets of commercial antimicrobial agents. β-lactam antibiotics inhibit transpeptidases; vancomycin inhibits transglycosylases; fosfomycin inhibits MurA.[7] Despite the unprecedented commercial success of β-lactam and glycopeptide antibiotics, their clinical use has recently been compromised by the emergence of resistant bacterial strains. Therefore one of the attractive strategies to overcome resistance to β-lactam antibiotics and vancomycin is to find novel inhibitors of the cell wall biosynthetic enzymes other than the transpeptidases and the transglycosylases.[6]

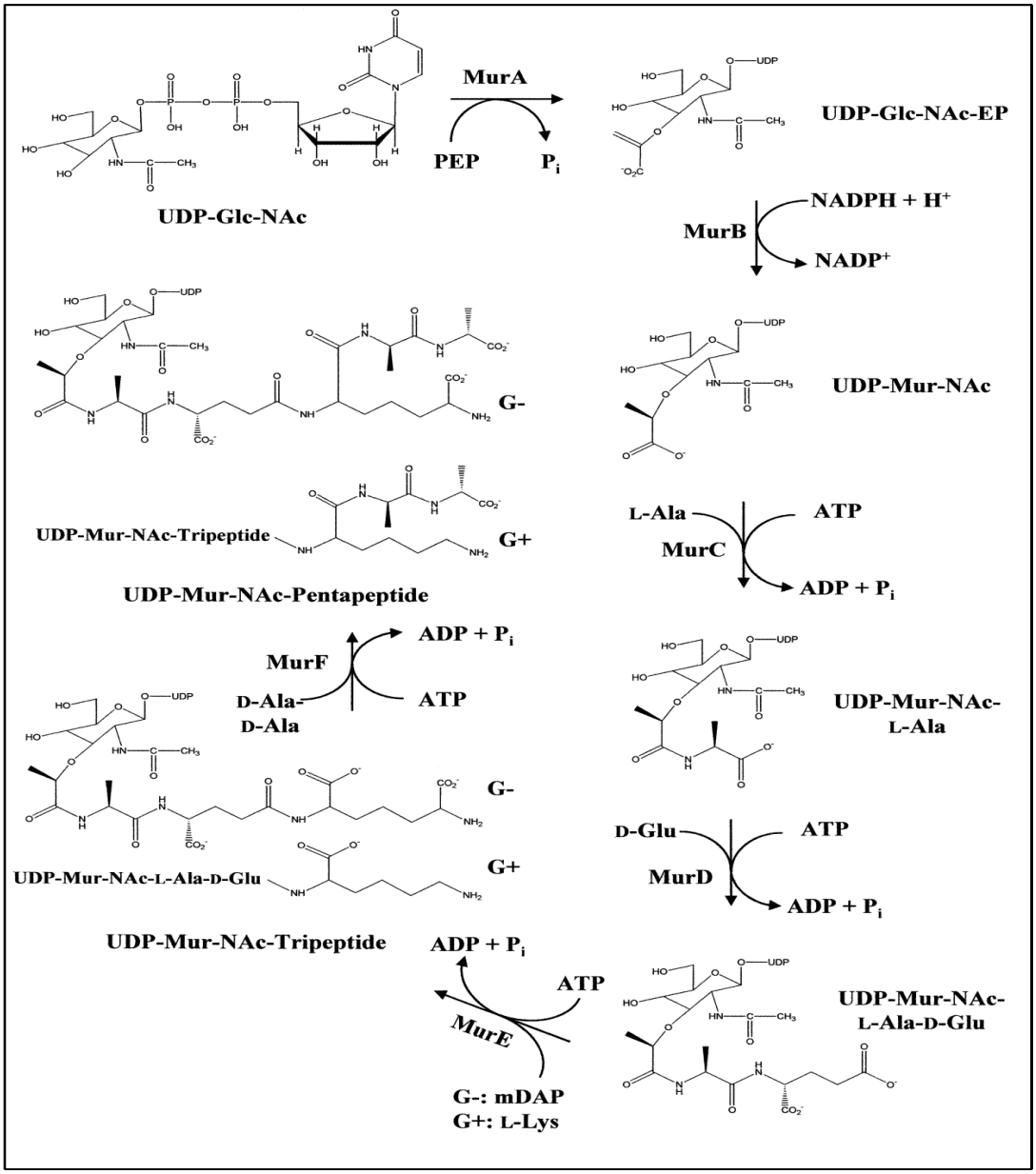


Figure 1 Biosynthetic pathway of the peptidoglycan precursor UDP-*N*-acetylmuramyl pentapeptide(m-A_2pm) from UDP-*N*-glucosamine by the sequential action of MurA to MurF enzymes in the cytoplasm of gram-

positive and gram negative bacteria. UDP-Glc-NAc, UDP-*N*-acetyl glucosamine; UDP-Glc-NAc-EP, UDP-*N*-acetyl glucosamine enolpyruvate; UDP-Mur-NAc, UDP-*N*-acetyl muramic acid; mDAP, meso-diamino pimelic acid; G+, Gram-positive bacteria; G–, Gram-negative bacteria.

Many of the inhibitors discussed here have been reviewed by El Zoeiby *et al*.[8] who present an excellent synopsis of the targets and inhibitors and assert that better understanding of the enzymology of the targets will provide insight for the design of new inhibitors. Indeed, new inhibitors have been found, but few have antibacterial activity. Equal attention must be paid to endowing inhibitors of cytoplasmic enzymes with antibacterial activity.

Peptidoglycan is an essential bacterial cell wall polymer composed of alternating units of UDP-N-acetyl glucosamine and UDP-N-acetylmuramic acid, cross-linked via short peptide chains and its role is unique to bacteria. During the biosynthesis of this polymer, the enzyme MurB carries out the reduction of enolpyruvyl uridine diphosphate N-acetyl glucosamine (EP-UNAG) to uridine diphosphate N-acetylmuramic acid (UNAM), an intermediate in the assembly of the UNAM-pentapeptide portion of its cell wall precursor. The reduction of EP-UNAG to UNAM by the MurB flavoprotein involves a sequence of two half-reactions. First, bound FAD (flavin adenine dinucleotide) is reduced by the two electron transfer from NADPH. Then, these two electrons are transferred to the C-3 of the enolpyruvyl group (Benson *et al*., 1993).

Two excellent past reviews[6,9] have covered the attractive qualities of these targets and discussed approaches for the discovery of new inhibitors. In first, on the basis of crystal structure analysis of the MurB-enoylpyruvate-UDP-N-acetylglucosamine(EP-UNAG)[10] complex, it was found that the carboxylate of the substrate interacts with residues Arg159 and Glu325 and could be responsible for transition state stabilization whereas the diphosphate moiety of the substrate interacts with residues Tyr190, Lys217, Asn233 and Glu288. Thus, templates were chosen that contained functional surrogates of the diphosphate with side-chains oriented to occupy space similar to the glucosamine and uridine moieties as potential MurB inhibitors. The 4-thiazolidinones met these criteria and a series of compounds were synthesized. In a subsequent paper, an imidazolinone moiety met the need for a heterocyclic bioisosteric replacement to eliminate the multiple diastereomers of thiazolidinone based structures. Most of the compounds described were discovered or

designed as inhibitors of MurB enzymes without a requirement for concomitant antibacterial activity.

In the present study, Docking simulations were carried out on the reported inhibitors of MurB enzyme using docking software. Based on it, series of 6,7-dihydrooxazolo[5,4-d] pyrimidin-5(4H)-one analogues were designed, synthesized, characterized and evaluated for its anti-bacterial activity against *S.aureus* and *E.coli*.

Materials and Methods

Chemistry

The chemical structure and three-dimensional (3D) structures were drawn by using Structure Builder (MarvinSketch 5.2.3_2, ChemAxon). Docking studies were done using the facilities of Molecular Modeling softwares (Arguslab 4.0.1, Mark Thompson and Planaria Software LLC and Molegro Molecular Viewer 2010.2.0.0) installed on Intel Pentium-4 machine. The melting point of all compounds was determined using a melting point apparatus (DBK Precision melting point apparatus) and found to be uncorrected. The reactions were monitored by thin-layer chromatography (TLC) and the R_f values were determined using TLC plates with the solvent system (n-hexane: ethylacetate). The IR studies were done with FT-IR (DRS-8400, Shimadzu Corporation). The NMR study was carried out by the instrument NMR (Bruker Avance II 500 MHz FT-NMR, TOPSPIN 1.3 Version), and the mass spectral studies were done using LC-MS (MDS SCIEX API 2000 LC-MS/MS).

***In Silico* Approaches**

One of the most important considerations in docking simulations is the selection of appropriate docking and scoring algorithm. There are several algorithms and number of scoring functions available today for docking. Roughly, the scoring functions are categorized into knowledge based and empirical categories. In the present study, an attempt was made to work with Arguslab for the docking calculations.

The crystal structure of 39kDa of *E.coli* MurB in complex with inhibitor, (5Z)-3-(4-chlorophenyl)-4-hydroxy-5-(1-naphthylmethylene)-furan-2-(5H)-one (i.e. naphthyl tetronic acid) **(PDB code: 2Q85)** was retrieved from protein data bank of Brookhaven National Laboratory in PDB format as starting point[11] **(Figure 2)**. The crystal protein structure 2Q85 consists of total 342 amino acids and resolution value of protein is 2.51 Å was then

implemented in ArgusLab 4.0.1. All water molecules from protein structure were deleted. In protein structure missing bond orders, hybridization states, charges and angles were assigned and explicit hydrogens were added using different parameters. Then, protein structure was energy minimized by Line search method using geometry optimization method. The validation of docking algorithms **(Figure 3)** was performed by changing different docking parameters until the lowest RMSD value was obtained. The grid resolution of 0.5 Å and ArgusDock as dock engine were selected for docking simulation while other parameters were set as default, the RMSD value of best pose of ligand was obtained 1.88 Å.

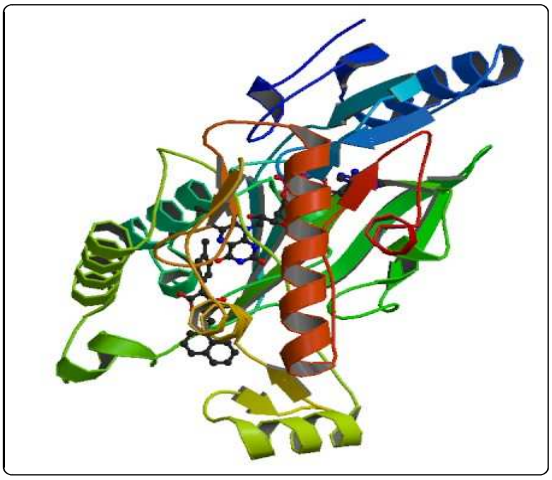


Figure 2 Flat-ribbon presentation of the crystallographic structure of ternary complex of naphthyl tetronic acid-FAD-2Q85, showing the ball and stick models of FAD and naphthyl tetronic acid as enzyme bound inhibitor.

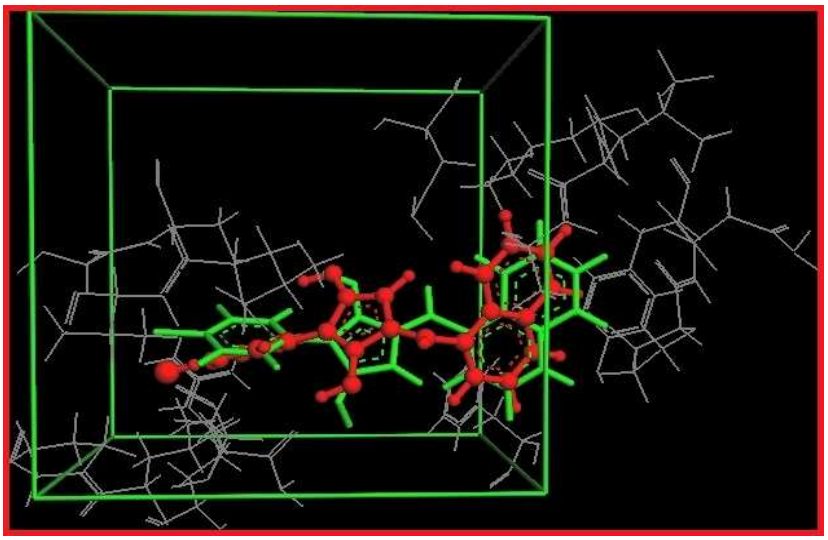


Figure-3: Validation of docking algorithm using crystal structure of *E.coli* MurB(2Q85).

All molecular docking simulations were carried out using Arguslab 4.0.1 installed on Intel Pentium-4 machine. All the ligand structures were constructed using ChemSketch 5.2.3_2 software installed on Intel Pentium-4 machine. The ligands were saved in MDL-Mol file. The ligands were then imported in MarvinView 5.2.3_2 software installed on Intel Pentium-4 machine and explicit hydrogen atoms were added and then converted to 3D conformations. The ligands were then saved in a single MDL-Mol file. The ligands were energy minimized by Line search method, using geometry optimization function in ArgusLab 4.0.1 and then lower energy conformation were selected for further studies. Different docking parameters were taken as per validation of docking algorithms **(Table 1).** All docking calculations were carried out using grid based A-Score function with a Grid resolution of 0.5 Å. The binding site on the receptor was defined as extending in X, Y and Z direction around Dock molecule with bindingsite box size of around 20 Å. MolDock optimization search algorithm with maximum of 10 runs was used through the calculation keeping all other parameters as default. Then lowest binding free energy pose of conformation was displayed and saved in .pdb format. Then .pdb file was imported into the Molegro Molecular Viewer 2010 2.0.0 for analyzing different interactions such as hydrogen bonding, hydrophobicity, electrostatic interactions, docking view etc.

Table-1: Different Docking Parameters used for validation of docking simulation.

Different Docking Parameters Used For Validation of Docking Simulation	
Grid Resolution=0.50 Å	Augmented Root Node=Standard
Docking Engine=ArgusDock	Maximum number poses=150
Calculation Type=DOCK	Parameter set=ascore.prm
Scoring function=AScore	Ligand=Flexible
Binding site bounding box=20×20×20 Å	Docking Precision=Regular

Design of synthesized test compounds and their docking

Molecular docking studies offer precise information for studying the interactions between ligand and protein residues and aids as a guide for the drug design. In the present study, initially different reported MurB

inhibitors were docked into 2Q85 to understand ligand-protein interactions and to analyze the active site necessary responsible for biological activity. From the docking simulation of all literature findings, it was concluded that amino acid residues viz. Tyr190, Asn233, Ala265, Ser229, Gly228, Gln288, Leu290, Leu218, Gly123, Lys217 and Glu325 are mainly involved in showing MurB inhibitory activity. In the present study, the key feature was focused on the hydrogen bond donor/acceptor and hydrophobic characteristics of the active site. Then *in-silico* docking simulation of heterobicyclic molecules 2, 7-diphenyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one and 2-methyl,7-phenyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one were carried out in showing MurB inhibitory activity. Initially, phenyl group was chosen for its steric and/or hydrophobic interaction with the protein residues. Secondly, the substitution on phenyl ring of the parent molecule was selected as the hydrogen bond acceptor or hydrogen bond donor to form bond or to impart hydrophobicity. A total of eight 6,7-dihydro- oxazolo[5,4-d]pyrimidin-5(4H)one derivatives were designed for the molecular docking study. The flexible molecular docking simulation was run for each of the designed derivatives. The best poses were then retained on the basis of MolDock Scores and Rerank Scores to predict the activity of the designed molecules.

The docking studies of 7-(4-chlorophenyl)-2-methyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one (stick-thin) in active site of *E.coli* MurB (wire frame) was carried out **(Figure 4)**. The compound was almost align with MurB bound inhibitor (i.e. naphthyl tetronic acid) and oxygen of oxazolo ring and one nitrogen(-NH) of six membered pyrimidinone ring showed single H-bonding interaction with the amino acid side chain Tyr190 and the other nitrogen (-NH) of the pyrimidinone moiety stabilized the binding recognition by hydrogen bonding with residue Gln288. The docked ligand arranged in such a conformation that showed proper and stable complex with high lipophilic interaction where the p-chloro phenyl ring was embedded within the hydrophobic active site residues. Another different synthesized derivative 7-(4-chlorophenyl)-2-(4-methoxyphenyl)-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one was docked in best binding conformation into 2Q85 **(Figure 5)** and involved similar binding residues as reported inhibitors **(Figure 6)**. The oxygen and nitrogen of oxazolo ring showed single-single van der Waals' H-bonding interaction with the amino residue

Asn233 and Tyr190 respectively. The nitrogen and oxygen of carbonyl group of pyrimidinone stabilized the binding recognition by hydrogen bonding with residues Asn233 and Ala265 respectively. The docked ligand arranged in such a conformation that showed proper and stable complex with high hydrophobic interaction where the p-chloro substituted phenyl ring was embedded within the hydrophobic active site residues viz. Ser229, Leu218.

Molecular docking was carried out for all eight derivatives of parent molecules among them compounds 7-(4-chlorophenyl)-2-(4-methoxyphenyl)-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one and 7-(4-chlorophenyl)-2-methyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one showed better binding interactions within active site of MurB enzyme (2Q85).

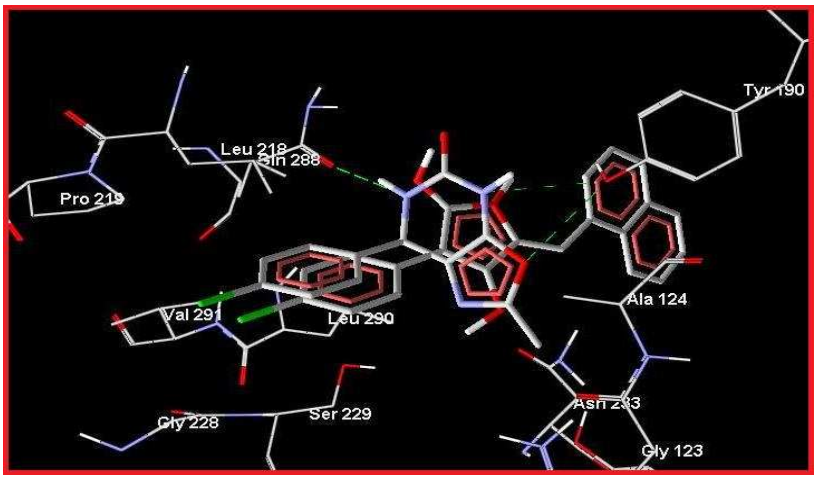


Figure 4 Docking of 7-(4-chlorophenyl)-2-methyl-6,7-dihydrooxazolo[5,4-d] pyrimidin-5-(4H)-one (stick-thin) in active site of *E.coli* MurB (wire frame) and alignment to MurB bound inhibitor-naphthyl tetronic acid.

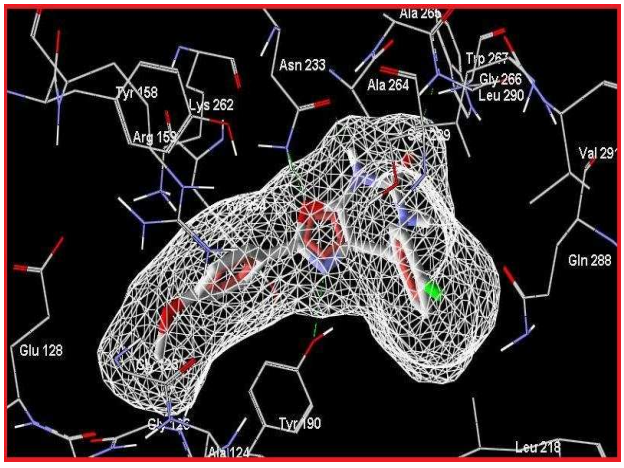


Figure 5 Best binding conformation of 7-(4-chlorophenyl)-2-(4-methoxyphenyl)-6,7-dihydrooxazolo[5,4-

d]pyrimidin-5(4H)-one (sticks) and space occupied (white wireframe around ligand) in the 2Q85 binding pocket showing residues (wireframe) involved in its recognition.

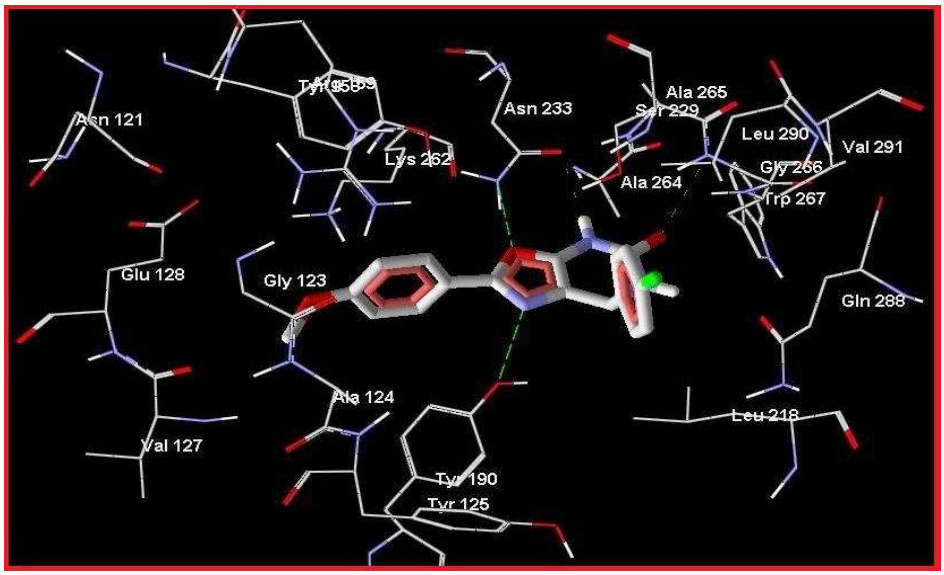


Figure 6 Binding interaction for compound 7-(4-chlorophenyl)-2-(4-methoxyphenyl)-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one(stick) and minimized in the 2Q85 binding pocket, showing residues (wireframe) involved in its recognition.

Synthesis

The chemicals and reagents used in the research work were of AR and LR grade, procured from Finar reagents, s.d.fine Chem. Ltd., Ranbaxy fine chemicals limited, Qualigens Fine Chemicals, Shital Chemical Industries and National Chemicals. The chemicals were used without further purification.

Based on the molecular docking simulations studies, it was decided to synthesize 6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one derivatives. The starting materials for the synthesis of 4-benzylidene-2-methyl/phenyl oxazole-5-one are acetyl glycine/benzoyl glycine and different arylaldehydes. The acetyl glycine/benzoyl glycine was condensed with different arylaldehydes with the addition of acetic anhydride and sodium acetate anhydrous to give 4-benzylidene-2-methyl/phenyl oxazole-5-one, the intermediate compound. This is known as Erlenmeyer-Plochl azlactone synthesis. In the next step, it was further treated with carbamide to yield oxazolo[5,4-d]pyrimidinone analogues.

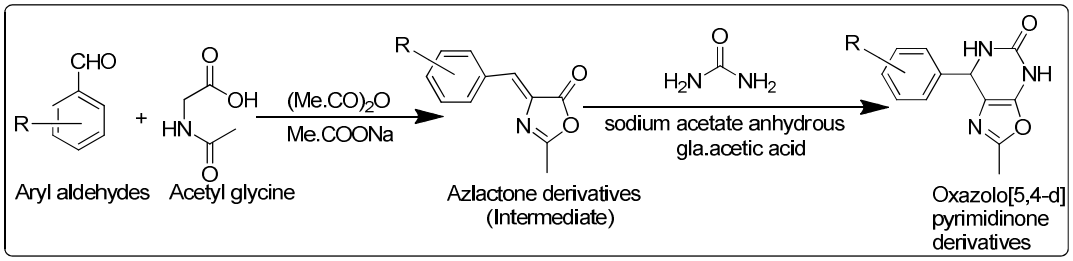
General Synthesis method

Synthesis of (Z)-4-(substituted-benzylidene)-2-methyloxazol-5(4H)-one[12] (Figure 7)

A mixture of 0.032 mol of acetyl glycine, 0.047 mol of different arylaldehydes (4-chlorobenzaldehyde, 3-nitrobenzadehyde, vanillin), 0.023 mol of sodium acetate anhydrous and 0.079 mol of acetic anhydride was warmed in a 500 ml flask (equipped with a reflux condenser) on a water bath with occasional stirring until solution was complete (10-20 minutes). Boiled the resulting solution for 1 hour, cooled and leave in a refrigerator overnight. Stirred the solid mass with 60 ml of cold water, transferred to a buchner funnel and washed well with cold water.

Synthesis of 7-substituted-phenyl-2-methyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one derivatives[13] (Figure 7)

A mixture of 0.005 mol of different (Z)-4-(substituted-benzylidene)-2-methyloxazol-5(4H)-one, 0.9 g (0.015 mol) of carbamide, 0.1 g of anhydrous sodium acetate in 2.0 ml of glacial acetic acid was refluxed for 4 hour. After monitoring the reaction on TLC, the reaction mixture was cooled and poured on ice, the product thus obtained was filtered and recrystallized from ethanol to yield product.



Compound Code	-R
U1	4-OH, 3-OCH_3
U2	3-NO_2
U3	4-Cl

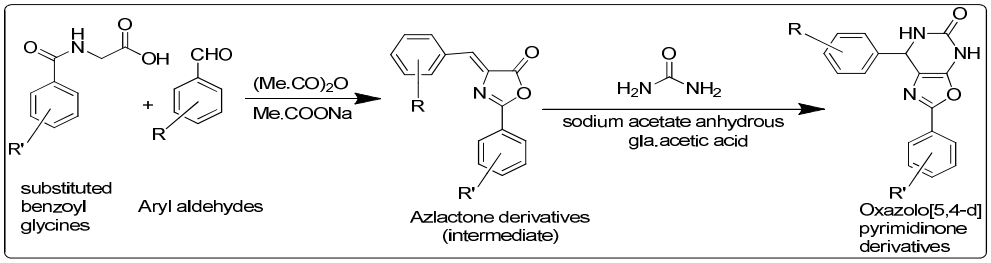
Figure-7: General Scheme of Synthesis for 7-substituted-phenyl-2-methyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one derivatives.

Synthesis of (Z)-4-(substituted-benzylidine-2-phenyloxazol-5(4H)-one derivatives[12] **(Figure 8)**

0.02 M of different arylaldehydes (benzaldehyde, 4-chlorobenzaldehyde, 4-methoxy-benzadehyde), 0.02 M of substituted(4-methyl, 4- methoxy)/unsubstituted benzoyl glycine, 0.06 M of acetic anhydride and 0.02 M of sodium acetate anhydrous were taken in conical flask and heated with constant shaking on an electric plate. As soon as the mixture got liquefied, transfer the flask on water bath and heated upto 2 hours. Then small amount of ethanol was added slowly, allowed the mixture to stand overnight. Filtered, washed with ice-cold ethanol in two portions and then with boiling water and dried.

Synthesis of 7-substituted-phenyl-2-phenyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one derivatives[13] **(Figure 8)**

A mixture of 0.005 mol of different (Z)-4-(substituted-benzylidine-2-phenyloxazol-5(4H)-one derivatives, 0.015 mol of carbamide, 0.1 g of anhydrous sodium acetate in 2.0 ml of glacial acetic acid was refluxed for 4 hour. After monitoring the reaction on TLC, the reaction mixture was cooled and poured on ice, the product thus obtained was filtered and recrystallized from carbon tetrachloride to yield product.



Compound Code	-R	-R’
U11	4-OCH_3	4-OCH_3
U12	4-Cl	4-OCH_3
U13	4-OCH_3	4-CH_3
U14	4-Cl	4-CH_3
U16	H	H

Figure-8: General Scheme of Synthesis for 7-substituted-phenyl-2-substituted-phenyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one derivatives.

Spectral Studies

Compound U3 (7-(4-chlorophenyl)-2-methyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one)

The yield was 71.33%, melting point 184-188°C, and R_f value 0.48. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2^{o} amide 3246.31, NH (b) amide 1639.55, C=O (str) 1725.38, C=C (str) aromatic 1525.74, 1491.99, 1405.19, CH (str) aromatic 3081.39. MASS m/e ratio: 263.8 $[M]^{+}$, 221.7 $[M\text{-}CHNO]^{+}$, 152.0 $[M\text{-}C_6H_4Cl]^{+}$

^1H NMR (500 MHz, DMSO-d6): 9.516 (s, 1H, NH), 7.458-7.630 (d, 4H, Ar-H), 6.212 (s, 1H, NH), 7.199 (s, 1H, CH), 1.988 (s, 3H, CH_3).

Compound U8 (Z)-4-(4-chlorobenzylidene)-2-methyloxazol-5(4H)-one (Intermediate of U3)

The yield was 69.16%, melting point 158-160°C, and R_f value 0.61. Spectroscopic analysis showed IR (KBr) cm^{-1}: C=O (str) 1790-1770, C=C (str) aromatic about 1500 and 1400 cm^{-1}, CH (str) aromatic 3090-3000. MASS m/e ratio: 221.7 $[M]^{+}$, 193.9 $[M\text{-}CO]^{+}$, 152.0 $[M\text{-}CO, C_7H_5Cl]^{+}$

Compound U16 (2, 7-diphenyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one)

Table 2 MolDock Scores and Rerank Scores of various 6,7-dihydro-oxazolo[5,4-d] pyrimidin-5-(4H)-one

Ligand(Code)	MolDock Score	Rerank Score
U1	-77.629	-62.835
U2	-67.325	-56.308
U3	-67.016	-53.220
U11	-103.497	-75.196
U12	-100.430	-85.349
U13	-105.106	-88.053
U14	-85.267	-72.974
U16	-82.494	-60.066

derivatives.

The yield was 64.29%, the melting point 194-196°C, and the R_f value 0.45. Spectroscopic analysis showed IR (KBr) cm^{-1}: amide C=O (str) 1724.42, NH (str) 2^{o} amide 3247.27, NH (b) amide 1654.01, C=O (str) 1697.41,

C=C (str) aromatic 1555.64, 1491.02, 1450.52, CH (str) aromatic 3064.03. MASS m/e ratio: 291.90 $[M]^+$, 263.9 $[M\text{-}CO]^+$, 250.0 $[M\text{-}CHNO]^+$, 134.7 $[M\text{-}C_8H_7NO]^+$

^{1}H NMR (500 MHz, DMSO-d6): 9.516 (s, 1H, NH), 7.458-7.630 (d, 4H, Ar-H), 6.212 (s, 1H, NH), 7.199 (s, 1H, CH), 1.988 (s, 3H, CH_3).

Compound U15 ((4E)-4-Benzylidine-2-phenyl-1,3-oxazol-5(4H)-one)

(Intermediate of U16)

The yield was 72.04%, melting point 164-166°C, and R_f value 0.41. Spectroscopic analysis showed IR (KBr) cm^{-1}: cyclic ketone C=O (str) at 1793.86 and 1770.71, C=C (str) aromatic at 1554 and 1449.15 cm^{-1}, CH (str) aromatic 3064.99.

MASS m/e ratio: 250.0 $[M+1]^+$, 144.2 $[M\text{-}C_7H_5O]^+$, 105.2 $[M\text{-}C_9H_6NO]^+$

^{1}H NMR (500 MHz, DMSO-d6): 8.312-8.328 (d, 2H, Ar-H), 8.135-8.152 (d, 2H, Ar-H), 7.725-7.739 (t, 1H, Ar-H), 7.636-7.667 (t, 2H, Ar-H), 7.523-7.566 (t, 3H, Ar-H).

Anti-bacterial activity[14,15]

Antimicrobial activity was determined by the agar diffusion method. All nine synthesized test compounds were tested against two species of bacteria, namely, *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative). Stock solutions of synthesized test compounds and standard drug were prepared in DMSO. Streptomycin was used as a standard. Nutrient agar medium (peptic digest of animal tissue 5 gms/litre, beef extract 1.50 gms/litre, yeast extract 1.50 gms/litre, sodium chloride 5 gms/litre and agar 15 gms/litre in distilled water at a pH 7.4 ± 0.2 at 25˚C, sterilized by autoclave at 15 lb pressure at 121˚C for 15 min) was used for the agar diffusion method. The petri-dishes were thoroughly washed and sterilized in a hot air oven at 160˚C for one hour. The inoculum was added to the medium, which was poured into sterile petri-dishes for solidifying. Wells (bores) were made in the medium using a 10mm diameter sterile borer after solidification. To the respective bores, 0.1 ml of the test and standard solutions was added. A control bore containing only DMSO was maintained in each plate. The petri-dishes were kept at room temperature for 30 min for diffusion to take place, and then incubated at 37˚C C for 24 hrs. The zone of inhibition was observed and measured using a

scale.

Results and discussion

The designing of a theoretical molecule before arriving at the NCE is a promising approach. This can be achieved by the rational approach to new drug discovery. In the present study, before arriving at a potential molecule of 6,7-dihydro-oxazolo[5,4-d]pyrimidin-5(4H)-one, docking analysis was performed. This aids in arriving at a theoretical prototype of 6,7-dihydro-oxazolo[5,4-d]pyrimidin-5(4H)-one which has fairly optimum binding with its complimentary site on its receptor MurB. Here, docking has generated an insight into developing an ideal MurB inhibitor from 6,7-dihydro-oxazolo[5,4-d]pyrimidin-5(4H)-one class. Thus in the present study, an approach of designing 6,7-dihydro-oxazolo[5,4-d]pyrimidin-5(4H)-one by docking simulations analysis followed by the synthesis, characterization and biological evaluation was carried out.

Molecular docking was carried out for all eight synthesized test derivatives of 6,7-dihydro-oxazolo[5,4-d]pyrimidin-5(4H)-one, among them 7-(4-chlorophenyl)-2-(4-methoxyphenyl)-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one and 7-(4-chloro phenyl)-2-methyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one showed better binding interactions within active site of MurB enzyme (2Q85). The starting materials for the synthesis of 4-benzylidene-2-methyl/phenyl oxazole-5-one were acetyl glycine/benzoyl glycine and different arylaldehydes. The acetyl glycine/benzoyl glycine was condensed with different arylaldehydes with the addition of acetic anhydride and sodium acetate anhydrous to give 4-benzylidene-2-methyl/phenyl oxazole-5-one, the intermediate compound. All compounds were purified by recrystallization from methanol, which gave needle shaped crystals. In the next step, it was further treated with carbamide to yield 6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one analogues and purified by either carbon tetrachloride or methanol. The test compounds were characterized by melting point determination and thin layer chromatography and structure was established by IR, ^{1}H-NMR and mass spectral studies The yields of all final test compounds were in the range of 40-88%. Out of the eight test compounds synthesized and tested for their antibacterial activity **(Table-3)**, compound 7-(4-chloro phenyl)-2-(4-methoxy phenyl)-6,7-dihydro oxazolo [5,4-d] pyrimidin-5(4H)-one showed activity better than rest of the other test compounds against *E.coli* and compound 7-(4-chlorophenyl)-

2-methyl-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one showed activity better than rest of the other test compounds against *S.aureous.* On the basis of biological activity results, it could be easily understood that the introduction of electron withdrawing groups -NO_2 and –Cl in phenyl ring at the 7^{th} position to the heterobicyclic frame work enhanced antibacterial activities. However, their activity was not comparable with that of the standard streptomycin **(Figure 9)**. Further, from the docking simulation studies, it was found that compound 7-(4-methoxyphenyl)-2-(p-tolyl)-6,7-dihydrooxazolo[5,4-d]pyrimidin-5(4H)-one showed very high Rerank Score against the target enzyme MurB, however, in reality when tested against the organisms it did not generate any useful data.

Table-3: Zone of inhibition (mm) of the synthesized test compounds.

Compound code	**Conc.(µg/ml)**	**Zone of inhibition diameter (mm)**	
		S.aureus* *(gram positive)	***E.coli* *(gram negative)***
U1	500	13	12
	1000	15	14
	1500	17	17
	2000	20	19
U2	500	--	17
	1000	11	19
	1500	14	20
	2000	16	24
U3	500	14	17
	1000	16	20
	1500	17	22
	2000	19	25
U11	500	16	--
	1000	17	14
	1500	19	16
	2000	21	19
U12	500	19	--
	1000	20	13
	1500	22	15

	2000	25	19
U13	500	16	13
	1000	17	15
	1500	19	18
	2000	22	20
U14	500	18	14
	1000	19	15
	1500	22	17
	2000	24	19
U16	500	15	--
	1000	17	12
	1500	20	15
	2000	22	16
Streptomycin	100	16	17
	200	18	19
	300	19	21
	400	21	22

Degree of activity is measured by the zone of inhibition (mm), (--) No inhibition (resistant, not sensitive).

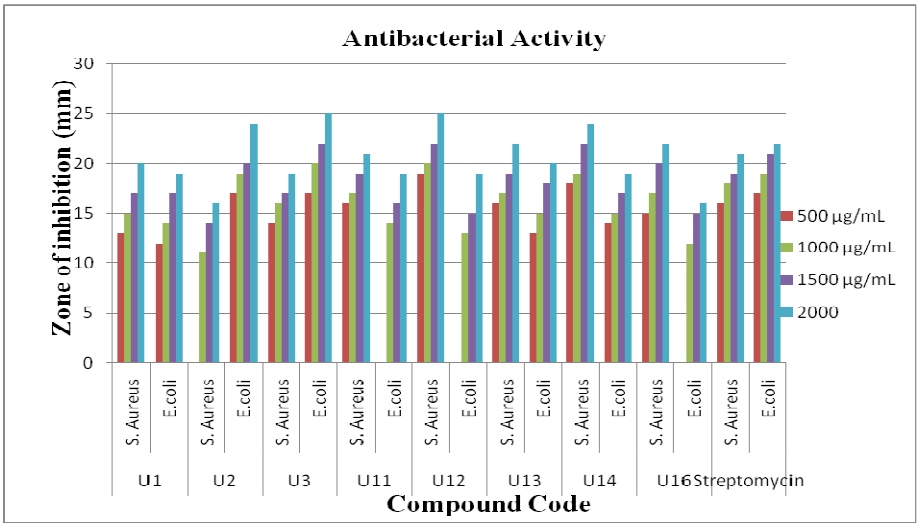


Figure 9 Comparison of zone of inhibition of synthesized test compounds and standard drug (Note: Concentration of streptomycin was considered at 100,200,300 and 400 µg/mL instead of 500, 1000, 1500 and 2000 respectively).

Conclusion

The rational approach to lead discovery has prompted a better insight in developing a more specific 6,7-dihydro-oxazolo[5,4-d]pyrimidin-5(4H)-one, as potential antibacterial agent. The data showing docking have definitely generated useful parameters needed for an ideal bacterial MurB inhibitor. The rational approach to lead discovery, presents a direct correlation between design molecules and the synthesized test compounds to generate functional activities of test compounds in reality. This approach has also paved the way for generating more useful 6,7-dihydro-oxazolo[5,4-d]pyrimidin-5(4H)-one analogues in future studies as bacterial MurB inhibitors.

Acknowledgements

The authors acknowledge the support of Dr. N. R. Tawari and Mr. Sagar S. Patel for encouragement throughout this work and helpful discussions. The authors are also wish to thank members of the microbiology section for their support and members of CSMCRI, Bhavnagar for spectral measurements.

References

1. Gadebusch H. H, Stapley E. O, Zimmerman S. B, “The discovery of cell wall active antibacterial antibiotics”, *Crit Rev Biotechnol*, 1992, 12, pp.225-243.
2. Lederberg J, “Bacterial protoplasts induced by penicillin”, *Proc Natl Acad Sci*, USA, 1956, 42, pp.574-577.
3. Stevens D. A, Ichinomiya M, Koshi Y, Horiuchi H, “Escape of *Candida* from Caspofungin inhibition at concentrations above the MIC (paradoxical effect) Accomplished by increased cell wall chitin : evidence for β-1,6-glucan synthesis inhibition by caspofungin", *Antimicrobial Agents and Chemotherapy*, 2006, 50, pp.3160–3161.
4. Isono K, Uramoto M, Kusakabe H, Kimura K, Izaki K, Nelson C. C, McCloskey J. A. J, *Antibiotics*, 1985, 38, pp.1617.
5. Kimura K, Ikeda Y, Kagami S, Yoshihara M. J, *Antibiotics*, 1998, 51, pp.1099.
6. Green D. W, “The bacterial cell wall as a source of antibacterial targets”, *Expert Opin. Ther. Targets*, 2002, 6, pp.1.

7. Russell A. D, Chopra I, "In Understanding Antibacterial Action and Resistance", Ellis Horwood, UK, Chapter 2, 1990, pp.39.
8. El Zoeiby A, Sanschagrin F, Levesque R, "Structure and function of the Mur enzymes: development of novel inhibitors", *Mol Microbiol*, 2003, 47, pp.1-12.
9. Wong K. K, Pompliano D. L, "Peptidoglycan biosynthesis: unexploited targets within a familiar pathway", In Resolving the Antibiotic Paradox, Edited by Rosen B. P and Mobashery S, New York, Kluwer Academic/Plenum Publishers, 1998, pp.197-217.
10. Mengin-Lecreulx D, Flouret B and Heijenoort J.V, "Cytoplasmic steps of peptidoglycan synthesis in *Escherichia coli"*, *J. Bacteriol*, 1982, 151, pp.1109-1117.
11. http://www.pdb.org/pdb/explore/explore.do?structureId=2Q85
12. Furniss B. S, Hannaford A. J, Tatchell A. R, Vogel's Text book of Practical Organic chemistry, 5th edition, Longman scientific & Technical, 2005, pp.1155-56.
13. Dabholkar V.V, Ahmed S.S, "Synthesis of oxazolo [4,5-d] pyrimidines", *Indian Journal of Heterocyclic Chemistry*, 2010, 20, pp.171-172.
14. Pelczar M. J., Chan ECS, Kreig N. R, In Microbiology, 5th edition, Tata McGraw Hill Publishing Company Limited, New Delhi, 1993, pp.536.
15. Indian Pharmacopoeia, 2007, 1, 50.

Corresponding Author:
Sanjay V. Patel*
Email: *gatu_sanju@yahoo.co.in*