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Design, *In Silico* Studies and Molecular Docking Studies of (2E)-2-Cyano-3-Phenyl-N'-(Phthalazin-1-yl) Prop-2-enehydrazide Derivatives as Antimicrobial Agents

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ABSTRACT

The increasing number of multidrug resistant microbial infections has created a critical need for the discovery of novel antimicrobial agents. Hence, in this study, a novel series of (2E)-2-cyano-3-phenyl-N'-(phthalazin-1-yl)prop-2-enehydrazides (**4a-l**) were designed and in silico studies were carried out to predict molecular properties, ADME properties and toxicities of title compounds. Further, molecular docking studies were performed against *S. aureus* Gyrase B (PDB ID: 4URO) and *E. Coli* Mur B (PDB: 2Q85). In silico screening indicated all the derivatives obeyed Lipinski's rule of five and showed favourable ADME properties. From molecular docking studies it was observed that 3-methoxy-4-hydroxy derivative **4k** exhibited good binding affinity with binding site of *S. aureus* gyrase B with XP G score -6.362 Kcal/mol compared to that of crystal ligand novobiocin (-5.964 Kcal/mol). Compound **4a** showed good binding affinity with *E. coli* Mur B active site with docking score -6.564 Kcal/mol than that of co-crystal ligand (-6.026 Kcal/mol). From the present study, compounds **4k** and **4a** were proposed to serve as promising molecules among the designed compounds for further evaluation as antibacterial agents.

Keywords: Hydralazine, antimicrobial activity, molecular docking, *in silico* studies

INTRODUCTION

Microbial infections caused by bacteria, fungi, viruses and parasites are highly contagious, resulting in serious complications impacting public health. Antibiotic resistance with current antimicrobial drugs has posed a critical challenge in the treatment of these infections, leading to a gradual

increase in the frequency of microbial infections. These infections markedly cause mortality as well as morbidity, adversely affecting patients' health and delaying their recovery. [1,2]. Hence, discovery of new antibacterial agents is crucial to overcome the limitations of current therapies and also to address the multidrug-resistant infections. Cinnamamides and phthalazines are two privileged structural scaffolds that have been investigated in the search of novel antimicrobial agents.

Cinnamamide is a privileged structural scaffold in drug discovery. It exists in two geometric isomers *cis* and *trans* form. Cinnamamide derivatives have attracted considerable interest in pharmaceutical research due to their synthetic accessibility, low toxicity profiles, and multifunctional therapeutic potential. Several cinnamides derivatives were reported to have diverse biological activities including antimicrobial[3], antitubercular[4-6], antimalarial[7], antidiabetic[8], antiinflammatory[9], anticancer[10-12], antiviral [13-15], anticonvulsant[16], neuroprotective[17, 18], antioxidant [19] etc. Few examples of marketed drugs containing cinnamamide nucleus are Cinromide which is used as is anticonvulsant and Tranilast is an antiallergic drug [20]. In addition, a significant number of patented chemical compounds were found to contain the cinnamamide nucleus.

Phthalazine, a nitrogen-containing heterocyclic compound which is also known as benzopyridazine or benzo-orthodiazine [21]. Research on phtahlazine has revealed that introducing structural versatility improves its potential with regard to various pharmacological activities including anticancer[22], anticonvulsant[23], antihypertensive[24], antimicrobial[25] activities etc. Hydralazine, a phthalazine-containing drug, has gained more attention for the synthesis of new drugs due to its pharmacological potential. Studies have shown that hydralazinealso has potent antioxidant[26], anticancer[27], antimicrobial[28, 29], and anti-aging[30] benefits.

In view of the pharmacological potential of cinnamamide pharmacophore and hydralazine, it was planned to design a novel series of cinnamoyl hydralazine derivatives as antimicrobial agents. *In silico* studies were carried out for title compounds to predict their molecular properties. Further, molecular docking studies were also performed against *S. aureus* gyrase B (GyrB) (PDB: 4URO) and *E. coli* Mur B (PDB: 2Q85).

MATERIAL AND METHODS

In silico studies

Molecular properties, ADME and toxicity prediction

Swiss ADME was used for prediction of various physicochemical parameters of title compounds **(4a-l)** that influence molecule activity such as Molecular Weight, volume, Molecular Polar Surface Area (PSA), Hydrogen bond acceptor/donar, log P and Number of rotatable bonds were calculated for the prediction of drug- likeliness of any molecule which indicates overall potential of the compound to be a drug candidate. ADME properties of title compounds were also estimated using SwissADME webtool in which, pharmacokinetic properties including GI absorption, blood brain barrier permeability and skin permeability and structural alerts were predicted[31]. ProTox-II was used to predict the toxicity of designed compounds, in which oral toxicity, organ toxicity, carcinogenicity, mutagenicity were predicted [32].

Molecular docking studies

Ligand preparation

Ligand (**4a-l**) structures were drawn using chemsketch and prepared using Epik module of Schrödinger, such as ligand's stereochemical nature enhancement and protonation state enhancement, developing tautomers and ligand energy was minimized by using force field OPLS_3 at pH 7.0±2.0[33].

Protein preparation

S. aureus DNA GyrB (PDB ID: 4URO) and *E. Coli* MurB (PDB: 2Q85) were considered in this study to determine the binding interaction modes and binding affinities of the title compounds. 4URO represents the N-terminal domain of the *S. aureus* GyrB protein, a key component of bacterial DNA gyrase. 4URO is known to bind to and be inhibited by novobiocin, a coumarin antibiotic that targets the ATPase domain of gyrase. 2Q85 represents the *E. coli* MurB bound to naphthyl tetronic acid inhibitor (**973):** ((5z)-3-(4-chlorophenyl)-4-hydroxy-5-(1 naphthylmethylene)furan-2(5h)-one). This inhibitor binds to MurB's active site, by H- bonds, to prevent its catalytic function. The target crystal structures were imported to Maestro and protein preparation

was done using protein preparation wizard. Protein preparation includes hydrogen atoms addition, bond order and formal charge correction, atomic clash removal, alteration of protein tautomeric and ionization states. Hydroxyl and thiol groups of protein were reoriented to optimize the H-bonding network. Protein structures were optimized at neutral pH and energy was minimized by OPLS_3 force field for all atoms. A receptor grid was generated and undesirable water molecules were removed around inhibitor binding site of selected targets using Glide v7.1 and Protein preparation wizard respectively [34].

Molecular docking

The binding affinity between the selected targets and title compounds was determined by GLIDE-XP docking. The prepared ligands (**4a-l**) were docked into the grid box generated in selected target proteins using Monte Carlo-based simulated algorithm minimization method. (Gscore (Glide Score) was used to represent binding affinity[34].

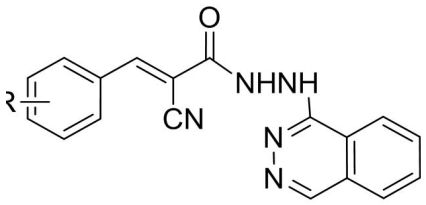
RESULTS AND DISCUSSION

***In silico* studies**

Molecular properties, ADME and toxicity prediction

All the molecular properties of the designed derivatives **(4a-l)** were predicted by using SwissADME web-based tool. The results suggested that all the derivatives obeyed Lipinski rule of five, which is estimated from the molecular properties such as molecular weight, H-bond acceptors and donors indicating their good oral bioavailability. Molecular properties of the title compounds was observed to be in the range of miLogP: 1.63-3.72; TPSA: 90-131; MW: 315.33-405.41; HBA:4-7; HBD:2-4; nviol:0-1; roB: 5-8. (Table 1). The results

Table 1: Prediction of molecular properties of title compounds



4a-l

General structure of title compounds

Compound	R	Mol. wt	HBA	HBD	Nrtb	TPSA (oA)	Log Po/w
4a	H	315.33	4	2	5	90.7	2.29
4b	4-Cl	349.77	4	2	5	90.7	2.76
4c	4-Nitro	360.32	6	2	6	136.52	1.85
4d	4-OCH_3	345.35	5	2	6	99.93	2.35
4e	3,4-$(OCH_3)_2$	375.38	6	2	7	109.16	2.42
4f	3,4,5-$(OCH_3)_3$	405.41	7	2	8	118.39	2.00
4g	2,4,5-$(OCH_3)_3$	405.41	7	2	8	118.39	2.44
4h	4-OH	331.33	5	3	5	110.93	1.81
4i	3,4-$(OH)_2$	347.32	6	4	5	131.16	1.63
4j	3-OH	331.10	5	3	5	110.93	1.97
4k	3-OH, 4-OCH_3	361.35	6	3	6	120.16	2.08
4l	4-C_6H_5	405.41	4	2	6	90.70	3.72

*Mol. Wt: molecular weight, HBA: No. of H-bond acceptors; HBD: No. of H-bond donors; TPSA: Topological Polar Surface Area; Nrtb: No. of rotatable bonds, Log Po/w:octanol-water coefficient

Table 2: Prediction of pharmacokinetic properties of title compounds by Swiss ADME

Compound	GI	BBB	Pgp	Cytochrome P450 inhibition				
			substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
4a	High	No	No	Yes	No	Yes	No	No
4b				Yes	Yes	Yes		No
4c				Yes	No	Yes		No
4d				Yes	No	Yes		No
4e				Yes	No	Yes		Yes
4f				No	No	Yes		Yes
4g				No	No	Yes		Yes
4h				Yes	No	No		No
4i				Yes	No	No		No
4j				Yes	No	No		No
4k				Yes	No	Yes		No
4l				Yes	Yes	Yes		No

*GI absorption: Gastrointestinal absorption, BBB permeant: Blood brain barrier permeation, Pgp substrate: P-glycoprotein substrate

Table 3: Prediction of toxicity of title compounds using ProTOX-II

Compound	Mutagenicity	Cardiotoxicity	Hepatotoxicity
4a	Inactive	Inactive	Active
4b	Inactive	Inactive	Active
4c	Active	Inactive	Active
4d	Active	Inactive	Active
4e	Active	Inactive	Active
4f	Active	Inactive	Active
4g	Active	Inactive	Active
4h	Inactive	Inactive	Active
4i	Active	Inactive	Active
4j	Active	Inactive	Active
4k	Active	Inactive	Active
4l	Inactive	Inactive	Active

of in silico data indicates that, the title compounds may have the potential to become a lead compound. Prediction of ADME properties of title compounds by SwissADME indicated that the title compounds may exhibit high GI absorption, moderate skin permeability and bioavailability score 0.55. None of the derivatives exhibited P-glycoprotein inhibition and BBB permeation, and few of the title compounds were found to be Cytochrome P450 enzyme inhibitors (Table 2). Toxicity studies by ProTOX-II revealed that all the title compounds are inactive for cardiotoxicity, and active for hepatotoxicity, and few compounds were found to be inactive for mutagenicity (Table 3).

Molecular Docking

Ligand preparation

Title compounds were prepared using Ligprep with Epik module of schrodinger.

Protein preparation

S. aureus DNA GyrB (PDB: 4URO) in

Table 4: Docking results of *S. aureus* GyrB with title compounds (4a-l)

Compound	R	XP G scores (Kcal/mol)	Interacting amino acids
4a	H	-5.496	-
4b	4-Cl	-5.042	-
4c	4-Nitro	-4.526	-
4d	4-OCH_3	-4.198	Arg 144
4e	3,4-$(OCH_3)_2$	-5.319	-
4f	3,4,5-$(OCH_3)_3$	-4.408	Arg 144
4g	2,4,5-$(OCH_3)_3$	-4.391	-
4h	4-OH	-4.816	-
4i	3,4-$(OH)_2$	-5.153	Asp 81, Gly 85
4j	3-OH	-4.261	-
4k	3-OH, 4-OCH_3	-6.362	Gly 85, Asn 54, Arg 84
4l	4-C_6H_5	-4.386	-
Standard	Novobiocin	-5.964	Gln 91, Gln 92

Table 5: Docking results of E. coli Mur B with title compounds (4a-l)

Compound	R	XP G scores (Kcal/mol)	Interacting amino acids
4a	H	-6.564	Arg 159, Glu 325
4b	4-Cl	-6.414	Arg 159, Glu 325
4c	4-Nitro	-6.099	Lys 217, Arg 159, Glu 325, Gln 288
4d	4-OCH_3	-6.046	Arg 159, Glu 325
4e	3,4-$(OCH_3)_2$	-6.263	Arg 159, Glu 325
4f	3,4,5-$(OCH_3)_3$	-5.822	Gly 123, Lys 262, Tyr 190
4g	2,4,5-$(OCH_3)_3$	-5.681	-
4h	4-OH	-6.500	Arg 159, Glu 325
4i	3,4-$(OH)_2$	-6.396	Tyr 125, Arg 159, Glu 325
4j	3-OH	-5.814	-
4k	3-OH, 4-OCH_3	-5.690	-
4l	4-C_6H_5	-5.456	-
Standard	Compound 973	-6.026	Gly123, Ser229

complex with novobiocin and *E. coli* MurB in complex with inhibitor 973 (PDB: 2Q85) were selected for molecular docking studies as they feature a known inhibitor binding domain which is crucial for determining the antibacterial activity of the title compounds 35,36. Inhibitor binding residues were defined around grid generated within the selected target. S. aureus GyrB and novobiocin complex binding site comprises residues such as Ser 55, Asp 57, Glu 58, Gly 85, Asn 54, Asp 81, Arg 144, Arg 84, Arg 200, Asp 89, Pro 87, Ile 102, Ile 86, Ser 128 within the 4 A0 surrounding co-crystallized inhibitor novobiocin. *E. coli* MurB and co-crystal ligand 973 complex binding site comprises residues such as Tyr 190, Leu 218, Ser 229, Asn 233, Pro 252, Tyr 254, Lys 262, Leu 263, Ala 264, gln 288, Leu 290, Val 291 within the 4 A^0 surrounding co-crystal ligand 973. The binding site residues of selected targets were defined using PDBsum web server.

Molecular docking

Antibacterial activity of compounds 4a-l was determined by performing molecular docking studies to predict their ability in inhibiting *S. aureus* GyrB enzyme. S. aureus GyrB is a subunit of the type II topoisomerase enzyme responsible to introduce negative supercoils into DNA, which is important for DNA replication, transcription and other important

cellular processes 37. Among all the docked complexes, **4k** possesses greater binding affinity against *S. aureus* with XP Gscore -6.362 Kcal/mol of than that of crystal ligand novobiocin (-5.964 Kcal/mol) (Table 4). Analysis of docking interaction had revealed that hydroxyl substitution on cinnamoyl moiety of **4k** formed one H-bond interaction with Asn 54 of *S. aureus* GyrB and two intermolecular H-bond interactions with active site residue Gly 85, one Pi-cation interaction with Arg 84 residues of S. aureus Gyr B (Fig 1). Co-crystal ligand Novobiocin exhibited two H-bond interactions with Gln 91 and Gln 92 residues of *S. aureus* Gyr B (Fig 2). From molecular docking studies, it was observed that compounds substituted with electron donating hydroxyl and methoxy groups on cinnamoyl moiety exhibited interactions with key amino acid residues in the binding site of *S. aureus* GyrB enzyme.

Title compounds were subjected to molecular docking with *E. coli* Mur B protein which plays a crucial role in biosynthesis of peptidoglycan, which is a vital component of the cell wall of bacteria.38 Among all the docked complexes, *4a* possesses better binding affinity with XP Gscore of -6.564 Kcal/mol (Table 5) than that of crystal ligand (973) -6.026 Kcal/mol. Compound **4a** exhibited H-bond interactions with Arg 159 and Glu 325 amino acid residues of *E. coli* Mur B (Fig 3). Compound 973 formed two hydrogen bond interactions with Gly

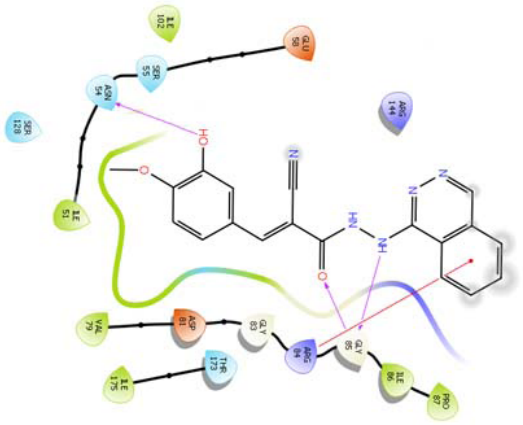


Fig. 1. Docking interactions of *S. aureus* GyrB with best docked compound 4k

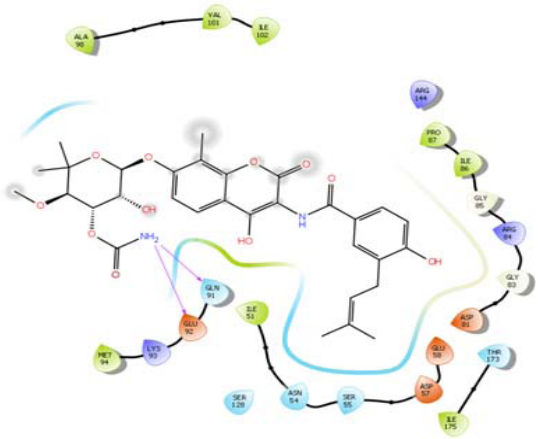


Fig. 2. Docking interactions of *S. aureus* GyrB with novobiocin

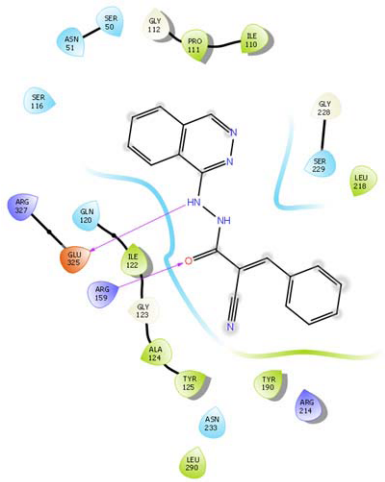


Fig. 3. Docking interactions of *E. coli* Mur B with compound 4a

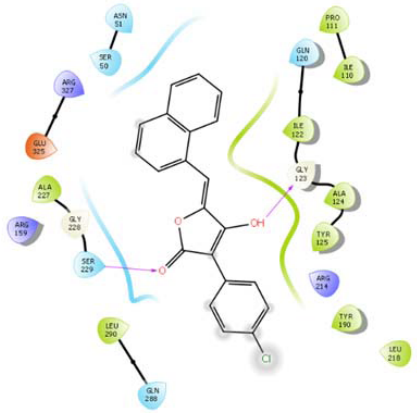


Fig. 4. Molecular docking interactions of *E. coli* Mur B with co-crystal ligand (compound 973)

123 and Ser 229 residues of *E. coli* Mur B (Fig 4). Molecular docking studies suggested that most of the compounds in the series exhibited key interactions in the binding site of *E. coli* Mur B. Particularly unsubstituted derivative, para substitution with electron withdrawing chloro group and electron donating hydroxyl groups showed good binding affinity with the active site of *E. coli* Mur B.

CONCLUSION

In present study, design. *in silico* investigation and molecular docking studies were carried out for novel (2E)-2-cyano-3-phenyl-N'-(phthalazin-1-yl)prop-2-enehydrazides for their antibacterial activity **(4a-l)**. Molecular properties, ADME properties and toxicities were predicted by Swiss ADME and ProTOX-II respectively. Further, molecular docking studies were performed against *S. aureus* GyrB (PDB ID: 4URO) and *E. coli* Mur B ((PDB ID: 2Q85). All the derivatives obeyed Lipinski rule of five, indicating drug-likeness of title compounds. Pharmacokinetic properties prediction by Swiss ADME revealed that all the compounds may exhibit good GI absorption. Among all the designed compounds, **4k** possesses greater binding affinity with S. aureus (-6.362 Kcal/mol) and compound 4a possesses better binding affinity with *E. coli* (-6.564 Kcal/mol) compared to respective standards. The results of *in silico* studies indicates that, the title compounds may have the potential to become a lead compound. Further studies needed for optimization of the antimicrobial properties of the title compounds.

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Conflict of Interest

The authors confirm that there is no conflict of interest related to the manuscript.

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