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Original Article

BIOLOGICAL AND DOCKING STUDIES OF NOVEL AROYLHYDRAZONES

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ABSTRACT

Objective: Novel aroylhydrazone schiff bases were synthesized and were screened for their biological activities.

Methods: Using HCl as a catalyst, all the compounds were synthesized at room temperature and were characterized by IR and NMR techniques. The synthesized Schiff bases were screened for antibacterial, antifungal activities. *In silico* molecular docking, method was performed to study their anti-tuberculosis activity against enoyl acyl carrier protein reductase (InhA) from *Mycobacterium tuberculosis* (PDB id: 2NSD).

Results: Compound P1 showed good antibacterial activity against gram positive (*S. aureus)* and gram negative (*E. coli*) bacterial strains and compound J1 showed good antifungal activity against *A. niger*. Molecular docking results reveal that compound B1 made two numbers of electrostatic interactions with 2NSD with more negative C docker interaction value. This indicated that the compound B1 was more active with minimum binding potential which is comparable with that of standard compound isoniazid.

Conclusion: Aroylhydrazones having good biologically activities compared to that of standards were prepared.

Keywords: 2,4,5-trifluorobenzaldehyde, DPPH, Tuberculosis, Molecular docking, InhA

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INTRODUCTION

Tuberculosis (TB) "19th-century illness" could be a high communicable killer disease. In 2014, 9.6 million individuals fell sick with TB and 1.5 million died from the malady. Over ninety-fifth of TB deaths occur in low and middle-income countries, and it's among the highest five causes of death for women aged fifteen to forty-four. TB could be a leading killer of HIV-positive people: in 2015, one in three HIV deaths was because of TB. Globally in 2014, associate calculable 480000 individuals developed multidrug-resistant TB (MDR-TB). TB epidemic by 2030 is among the health targets of the recently adopted sustainable development goals [1].

Therefore, there's associate pressing got to develop new medicine against this world's most difficult public ill health. Drug discovery and development could be a complicated, time intense and a rich method [2]. It becomes still more expensive when the safety, efficacy and other issues are raised. *In silico* drug design, computational approach plays a significant role in all stages of drug development from the initial lead design to final stage clinical development [3]. Docking software is a valuable tool in pharmacy and medicine as most drugs are small molecules designed to interact with biologically relevant target proteins (receptors) in order to act on the biological pathway they are involved in.

The full therapeutic possibilities of hydrazides were realized after the discovery of isonicotinic acid hydrazide (INH) drugs which are in clinical practice for more than 50 y [4]. Due to the presence of azomethine group (>C=N-NH-CO) hydrazones are considered as a special group of compounds and their industrial applications are vast [5]. Hydrazones are used as antimicrobial, antioxidant, anti-tuberculosis, anticancer, antimalarial anti-inflammatory, antiplatelet diseases due to their interesting chemical and structural properties [6-12].

Keeping the various biological applications of hydrazones in mind and in continuation of our earlier research [13], in the present study we reported novel biologically active hydrazone shiff bases using 2,4,5-trifluorobenzaldehyde as a source of the carbonyl compound. The novelty of the present work resided in it's synthesize and molecular docking analysis. Drug discovery and development is a complex, lengthy and costly process, entrenched with a high degree of uncertainty that a drug will actually succeed. It becomes still more expensive when the safety, efficacy and other issues are raised. For faster development, nowadays almost every multi-national drug company and Contract Research Organization (CRO) involved in drug discovery has adopted computational methodology in different stages of the design process.

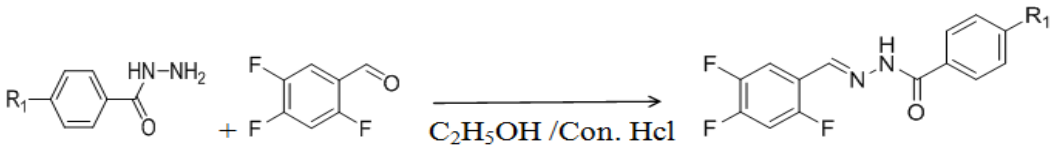
Many computational methods complement one another and may be combined to help rationalize the drug discovery process [14]. In this research, we attempted to find the anti-tubercular nature of synthesized compounds by virtual screening method. Virtual screening uses computational docking method and is used to predict the binding position and orientation of small molecules with active sites of pathogens. The crystal structure of pathogen available in Protein Data Bank could be used for docking studies.

MATERIALS AND METHODS

All anhydrous solvents and reagents of AR grade were obtained from commercial suppliers and used without any further purification unless otherwise noted. All the reactions were carried out at room temperature. Melting points were determined by open capillary and are uncorrected. FT-IR spectral measurements were recorded using Perkin Elmer Spectrum-1 FT-IR spectrometer in 4000-400 cm^{-1}. NMR was recorded with Bruker NMR spectrometers operating at 400 MHz for ^{1}H and 100 MHz for $^{13}CNMR$. DMSO-d_6 was used as a solvent and TMS as an internal reference.

Synthesis

To the ethanolic solution of benzo hydrazide (0.1 mol), 2,4,5-trifluorobenzaldehyde (0.1 mol) and few drops of con HCl were added. The reaction mixture was stirred well at room temperature for 30 min. Insoluble solid gradually generated was filtered and washed with petroleum ether(40-60%) and dried in vacuum desicator. The crude solid was recrystallised from absolute ethanol. Similar reaction and purification procedures were applied to prepare all the compounds.



R1 =-H,-Cl,-OMe

Scheme 1: General procedure for the synthesis of aroylhydrazone schiff bases

Determination of antimicrobial activity

The antimicrobial activities of synthesized compounds were screened against gram-positive bacteria (*Staphylococcus aureus*), gram-negative (*Escherichia coli*) and fungi (*Aspergillus niger*) strains by agar well diffusion method [15] at a concentration level of 100µg/ml. The microorganisms used were obtained, identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur. Nutrient agar was used as culture media and DMSO was used as a solvent. Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/ml) was placed on agar plate seeded with the appropriate test organism in triplicates. The Petri plates were incubated at 37 °C for 24 h for bacterial strains and 48 h for fungi. After incubation, the plates were observed for the zone of inhibition. The Antimicrobial activities of synthesized compound were compared with Erythromycin and Gentamycin as standard. Each sample was tested in triplicate.

Molecular docking studies

Molecular docking studies were performed using accelrys discovery studio software program. The target protein enoyl acyl carrier protein reductase (InhA) from *Mycobacterium tuberculosis* (PDB ID-2NSD) was downloaded from protein data bank and the active site was chosen. It was processed by the addition of hydrogen, assigning the bond order, identifying overlaps, creating zero-order bonds to metals and creating disulphide bonds. The co-factors, unwanted water molecules and chains were deleted. Then energy minimization was done followed by grid generation. The designed ligands were saved as mol format and were examined so as to generate the best pose by analyzing the binding interactions. The scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site A of 2NSD.

RESULTS AND DISCUSSION

Aroylhydrazide derivatives were synthesized via condensation reaction by Schiff base route. All the products were immiscible in polar solvents and soluble with DMSO and DMF. The postulated structures of the newly synthesized compounds (table 1) were in good agreement with their FT-IR, ^{1}H NMR and ^{13}C NMR spectral data.

(E)-N'-(2,4,5-triflorobenzylidene)benzohydrazide (B1) was derived from benzohydrazide and 2,4,5-triflorobenzaldehyde (1:1) Yield: 96%. FT-IR: (ν in cm^{-1}) 3409(NH), 3069(Ar-CH), 2848(Ali-CH), 1645(CO). ^{1}H NMR δ in ppm (400 MHz, DMSO-d$_6$): 12.1(s, 1H, enolic NH), 8.7(s, 1H, CH=N), 7.9-7.5(m, 7H, Ar-H). ^{13}C NMR δ in ppm (100 MHz, DMSO-d$_6$) 163(CO), 138(C=N), 133, 132, 128, 127, 119, 113, 107, 106(Ar-C).

(E)-4-chloro-N'-((2,4,5-triflorobenzylidene)benzohydrazide (P1) was derived from 4-chloro benzohydrazide and 2,4,5-trifloro-benzaldehyde. (1:1) Yield: 97%. FT-IR: (ν in cm^{-1}) 3475(NH), 3059(Ar-CH), 2850(Ali-CH), 1616(CO).^{1}H NMR δ in ppm (400 MHz, DMSO-d$_6$): 11.9(s, 1H, enolic NH), 8.6(s, 1H, CH=N), 7.9(d, 2H, o-ArCl), 7.8(s, 1H, o-ArF), 7.7(s, 1H, m-ArF), 7.0(d, 2H, m-ArCl).^{13}C NMR δ in ppm (100 MHz, DMSO-d$_6$) 162(CO), 157(C=N), 154, 152, 148, 145, 144, 137, 129, 124, 119, 113, 107, 106(Ar-C).

(E)-4-methoxy-*N'*-(*(2,4,5-triflorobenzylidene*)benzohydrazide (J1) was derived from 4-methoxy benzohydrazide and 2,4,5-triflorobenzaldehyde (1:1) Yield: 98%. FT-IR: (ν in cm-1) 3474(NH), 3059(Ar-CH), 2849(Ali-CH), 1617(CO). 1H NMR δ in ppm (400 MHz, DMSO-d6): 11.9(s, 1H, enolic NH), 8.6(s, 1H, CH=N), 7.9(d, 2H, o-ArOMe), 7.8(s, 1H, o-ArF), 7.7(s, 1H, m-ArF), 7.1(d, 2H, m-ArOMe). 13C NMR δ in ppm (100 MHz, DMSO-d6) 162(CO), 157(C=N), 154, 152, 151, 148, 145, 137, 129, 119, 113, 107, 106(Ar-C), 55(OMe).

Antimicrobial activity

The antimicrobial activities of synthesized compounds were given in table 1. All the compounds show better activity against *S. aureus and A. niger.* At the same time, activity against *E. coli* was found to be less for all the three compounds. In short, P1 shows comparatively greater anti-bacterial activity and J1 shows good anti-fungal activity.

Table 1: Antimicrobial activities of aroylhydrazones

Compound id	Zone of inhibhition (mm)		
	S. aureus	***E. coli***	***A. niger***
B1	12±0.81	11±0.19	11±0.13
J1	22±0.41	17±0.25	13±0.16
P1	18±0.62	14±0.32	16±0.14
Erythromycin	28±0.31	23±0.09	25±0.14
Gentamycin	16±0.37	22±0.22	14±0.21

While comparing the presence of substituents in the aromatic ring of hydrazides, the presence of electron donating group enhanced the antimicrobial activities whereas the presence of electron withdrawing group significantly reduced the activities [16].

Molecular docking

In order to find a suitable inhibitor for *Mycobacterium tuberculosis,* InhA docking study was carried out. InhA catalyzes the reduction of long-chain *trans*-2-enoyl-ACP in the type II fatty acid biosynthesis pathway of *M. tuberculosis*. Inhibition of InhA disrupts the biosynthesis of the mycolic acids that are central constituents of the mycobacterial cell wall. The docking scores were shown in table 2.

The types and number of interactions between the synthesized aroylhydrazones and *Mycobacterium tuberculosis* InhA were shown in fig. 1-4. The more negative value of Cdocker interaction energy indicates the good binding potential or the more anti-tuberculosis activity against *Mycobacterium tuberculosis* InhA protein.

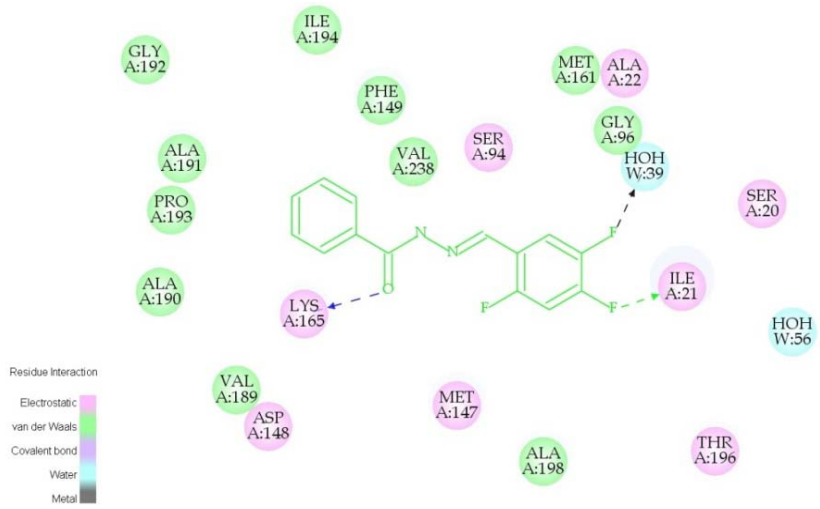


Fig. 1: Binding configuration of B1 with 2NSD

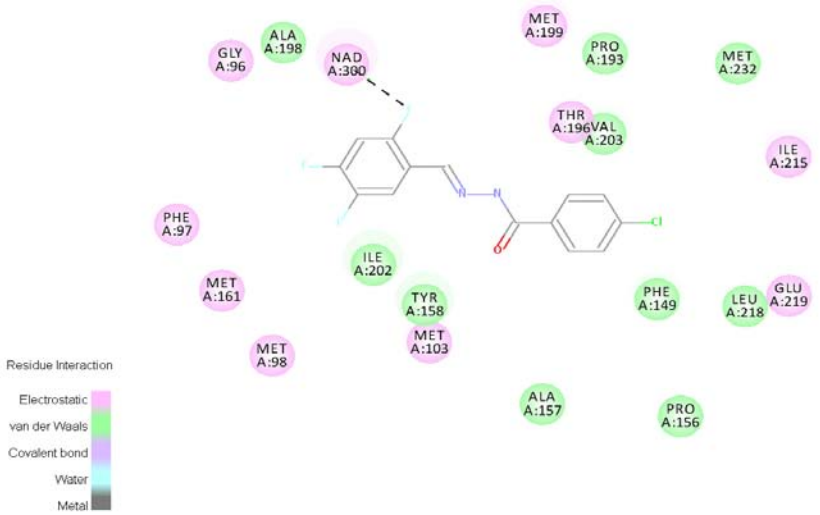


Fig. 2: Binding configuration of P1 with 2NSD

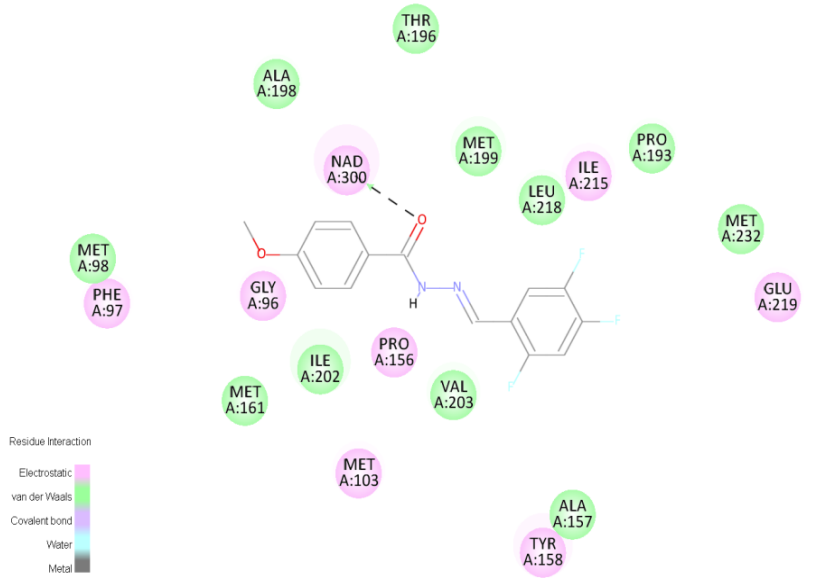


Fig. 3: Binding configuration of J1 with 2NSD

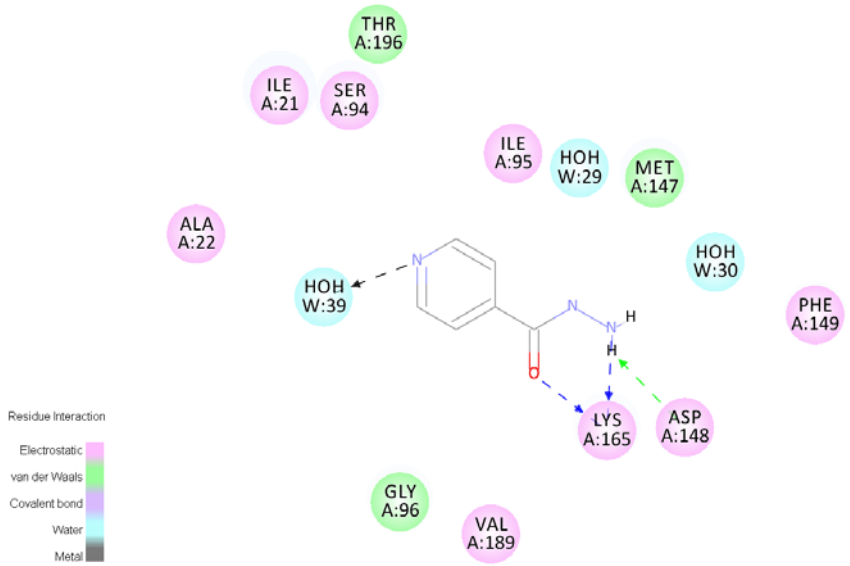


Fig. 4: Binding configuration of isoniazid with 2NSD

Table 2: Statistical docking results of the aroylhydrazones [ADS version 2.5 software]

Compound id	Potential energy	Van der walls energy	Electrostatic energy	Initial RMS gradient	Final RMS gradient	CDocker energy	CDocker interaction energy
B1	13.15033	3.12177	1.32932	43.22383	0.09303	-22.9303	-37.3152
P1	11.98248	3.60418	-2.98297	41.26503	0.09358	-27.9953	-40.7347
J1	14.03885	2.92098	1.03532	43.24664	0.08363	-29.6707	-44.3367
Isoniazid (standard)	-6.67385	1.78410	-11.80107	42.51513	0.07183	-22.1896	-27.3771

Table 3: Molecular interactions of aroylhydrazones with active site residues of 2NSD

Compound id	Interaction details		
	Type	Number	Residues involved
B1	Electrostatic	2	(1) Between–C=O-of benzo hydrazide and of amino acid residue LYS A: 165 (2) Between fluorine of aldehyde and of amino acid residue ILE A: 21
J1	Electrostatic	1	Between fluorine of aldehyde and NAD 300 of amino acid residue
P1	Electrostatic	1	Between–C=O-of benzo hydrazide and NAD 300 of amino acid residue
Isoniazid	Electrostatic	2	(1) Between–C=O-of benzo hydrazide and and of amino acid residue LYS A: 165 (2) Between hydrogen of benzohdrazide and LYS A: 165 of amino acid residue
	Van der Walls	1	Between hydrogen of benzohydrazide and of amino acid residue ASP: 148

From the results of docking, it was found that CDocker score of compound B1 was more negative. This implied that B1 needed only less energy to bind with the active site of the pathogen. At the same time, it made two numbers of electrostatic interactions with amino acid residue LYS A: 165 which was comparable with that of standard drug isoniazid. Therefore compound B1may be suitable to overcome the drug resistance of enoyl acyl carrier protein reductase (InhA) from *Mycobacterium tuberculosis*.

CONCLUSION

Novel aroylhydrazones were synthesised using the simple and convenient method. The antimicrobial activities of the synthesized compounds screened against gram positive *S. aureus* and gram-negative *E. coli* bacterial and *A. niger* fungi strains reveals that all the compounds may acts as a potential antimicrobial activity. To expand the knowledge about anti-tuberculosis activity against *Mycobacterium tuberculosis* InhA, molecular docking study was performed. Compound B1 shows good binding ability with two electrostatic interactions, which is comparable with that of isoiazid, standard drug. Therefore, B1 was found to possess the good inhibitory capability and it may be suitable to overcome the drug resistance of *Mycobacterium tuberculosis* InhA protein.

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CONFLICTS OF INTERESTS

All authors have none to declare

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