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SYNTHESIS, CHARACTERIZATION, *IN SILICO* STUDIES AND BIOLOGICAL EVALUATION OF *N*-(2,5-DIMETHYL-1*H*-PYRROL-1-YL) ISONICOTINAMIDE

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ABSTRACT

N-(2,5-Dimethyl-1*H*-pyrrol-1-yl)isonicotinamide **3** was produced in a single step of synthesis by refluxing isonicotinic acid hydrazide **1** with acetonyl acetone **2** in the presence of acetic acid. The synthesized compound **3** was assessed for antitubercular, antibacterial, and enzyme inhibitory activity investigations after being characterized by IR, ^{1}HNMR and Mass spectral analysis. To ascertain the likely binding manner, *in silico* molecular docking experiments were carried out, and the synthetic compound **3**'s ADMET characteristics were computed.

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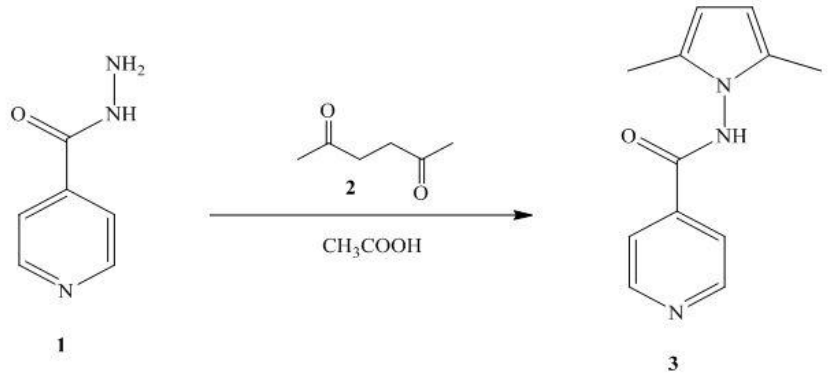
INTRODUCTION

A highly contagious and sometimes fatal infection, tuberculosis (TB) is brought on by the bacterial strain *Mycobacterium tuberculosis*, which can cause serious sickness. TB is a multi-systemic disease, but its most prevalent site of infection is the lung. Over the past few decades, TB has become the biggest cause of death worldwide, with millions of people contracting the disease each year. The global campaign to end TB has been successful in reducing the disease's mortality rate and spread [1].

A well-known biological moiety with important characteristics is pyrrole. Because of its wide range of pharmacological properties, pyrrole and its derivatives have been designed and synthesized in large quantities [2-5]. The first-line medication most frequently used to treat TB is isoniazid. Because of its potency, excellent selectivity, and favorable pharmacokinetic characteristics, isoniazid is a first-line treatment for TB resistance [6]. This program of synthesis of pyrrole analogue has envisaged serving as a new scaffold for evaluation as antitubercular agent by *in silico* molecular docking studies.



SCHEME1: Synthesis of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl) isonicotinamide 3:

EXPERIMENTAL SECTION

Materials and Methods

All chemicals and solvent were purchased from SD fine chemicals India. The uncorrected melting points of synthetic compounds were determined using the SHITAL-Digital programmable melting point apparatus [SSI -22(B)], and Thiele's Tube sometimes. IR spectra were acquired using KBr pellets on the Bruker-T spectrophotometer. The ^{1}H NMR spectrum was recorded on Bruker Avance II ^{1}NMR 500 MHz instrument using dimethylsulfoxide (DMSO-d_6) as the solvent and TMS as the internal standard. Chemical shifts are reported as δ values (ppm). The Schimadzu QP 20105 GC-Mass spectrometer and the WATERS Q-T of premier mass spectrometer were used to record the mass spectra. Thin-layer chromatography (TLC) was performed on precoated TLC sheets of silica gel 60 F254 (Merck, Darmstadt, Germany) and detected using an UV lamp at 253 nm.

Procedure for the synthesis of *N*-(2, 5-dimethyl -1*H*-pyrrol-1-yl) isonicotinamide 3:

To a suspension of isonicotinic acid hydrazide **1** (0.4 g, 0.003 mol) in ethanol (10 ml) were added acetonyl acetone **2** (0.6 ml, 0.006 mol) and glacial acetic acid (1 ml) and the reaction mixture was heated on a boiling water bath for 4 hr. The reaction mixture was concentrated to half original volume and poured into crushed ice (50 g). The separated solid was filtered, washed with water, dried purified by column chromatography by using Chloroform: Methanol (9:1) as a mobile phase and silica gel as a stationary phase. Compound **3** was obtained as a yellow amorphous solid; Yield 71%. M.p. 130-132^{o}C

FTIR (KBr- cm^{-1}): 3225.71, 1673.28, 1323.17, 1536.43.
^{1}H NMR (DMSO-d_6, 500 MHz, δ ppm): 9.78 (s,1H, amide-NH), 8.70 (d, 2H, *J=8.2*, pyridine-C_2 and C_6-H), 7.63 (m, 2H, pyridine-C_3 and C_5-H), 5.86 (s, 2H, pyrrole-C_3 and C_4-H), 2.09 (s, 6H, pyrrole 2-CH_3 -H).
Mass (m/z): found. 215.41 (M+); Calcd. 215.26

Molecular docking using Surflex-Dock

Surflex-Dock was used for molecular docking to clarify the binding mechanism of compounds in the active sites of the dihydrofolate reductase and ENR enzymes using the patented Sybyl-X 2.0 search engine. For the purpose of future molecule structure optimization, this offers explicit information. The crystal structures of *Mycobacterium tuberculosis* dihydrofolate reductase complexed with NADPH and methotrexate and enoyl acyl carrier protein reductase InhA in association with 4TZK were obtained from the Brookhaven Protein Database (PDB http://www.rcsb.org/pdb). The protein and ligands employed in our docking method were created using the Sybyl-X 2.0 standard production process.

Pharmacological/Biological Assays

PASS

For computational screening of potential properties, such as biological activities like kinase inhibitor along with other relevant activities, i.e., nicotinic alpha2 beta2 receptor antagonist, pterin deaminase inhibitor, cyclic AMP phosphodiesterase inhibitor, etc., we have used PASS (for synthesized compound **3**, available host at http;//www.way2drug.com/PASSOnline/predict.php) and (https://proteins.plus/). A method for assessing an organic drug-like aspirant's typical biological potential was created by these servers program. PASS forecasts a broad spectrum of action based on the structural characteristics of organic molecules. Therefore, the biological activity outline for virtual molecules may be assessed using PASS. This combination of methods produced quantitative structure-activity relationships (SAR) through the close construction of models from bioactive ligands and the breakdown of chemical structures using 2D and/or 3D descriptors. In relation to structures where Pa (probable activity) was higher and Pi (probable inactivity) was taken into consideration for pharmacological activity, the PASS activity was assessed.

ADME Analysis

A drug's potential is determined not only by how quickly it can be put to use, but also by a satisfactory analysis (ADME) that forecasts several significant aspects *in silico* and is useful for analyzing the good attributes of the molecules. The rule is useful while creating new drugs and developing possible medication molecules. For the current study, ADME analysis was performed utilizing the molinspiration predictor and SWISS ADME. Molecular weight, number of rotatable bonds, hydrogen bond acceptor, hydrogen bond donor, molar refractivity, TPSA, water solubility (log S), blood brain barrier, skin permeability (log Kp), synthetic accessibility score (SA), percentage absorption, pharmacokinetics, drug-likeness, and medicinal chemistry friendliness properties of drug molecule are among the attributes that this web server tool assesses for the ADMET. The lipophilicity of compounds is assessed by a score analysis that integrates data from multiple lop P prediction programs, including SILICOS-IT, XLOGP3, MLOGP, and iLOGP. The log of a pharmacological substance's concentration in two solvents in unionized form is a measure of a molecule's lipophilicity; the lower the log P value, the better the lipophilicity. The basic goal is to avoid weakly soluble compounds since log S of a chemical is critical for absorption and distribution qualities, and low water solubility frequently leads to poor absorption.

Antitubercular activity

The newly synthesized compound was evaluated against *M. tuberculosis* strain $H_{37}Rv$ by means of Microplate Alamar Blue assay (MABA) and results are shown in **Table 4** with MIC values.[7]

Antibacterial activity

Antibacterial inhibition study for the synthesized molecule was determined in comparison with ciprofloxacin as reference drug, against *S. aureus* (Gram +ve) and *E. coli* (Gram -ve) by broth micro dilution method.[8] Antibacterial activity data is listed in**Table 4** with their MIC values.

InhA and DHFR enzyme inhibition activity:

The newly synthesized compound was evaluated for InhA activity in comparison with triclosan as reference and DHFR activity in reference with trimethoprim and the results were given in **Table 4.**

ADMET studies

ProTox-II was used to predict toxicities (results are presented in **Table 6**) and Molecular ADME properties were calculated using *in silico* Swiss ADME online programme and the results are presented in **Table 5.**

RESULTS AND DISCUSSION

Chemistry

The target compound **3** was made according to **Scheme 1**. In order to synthesize the intended compound **3,** isonicotinic acid hydrazide **1** was refluxed with acetonyl acetone **2** in presence of glacial acetic acid. The structure of the compound **3** was validated by spectral and analytical data.

Pharmacology

Biological activity spectrum PASS analysis

Predicted biological activity profile of the synthesized compound was determined by the online server of PASS. The synthesized compound *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)isonicotinamide **3** showed the highest Pa for Taurine dehydrogenase inhibitor activities 0,925 (**Table 1**).

Table 1: The activity spectrum of *N*-(2, 5-dimethyl-1*H*-pyrrol-1-yl) isonicotinamide, pa represents probability to active and pi represents probability to be inactive.

S. No	Activity name	Pa	pi
1	Taurine dehydrogenase inhibitor	0,925	0,003
2	Amine dehydrogenase inhibitor	0,901	0,003
3	Glutamine-phenylpyruvate transaminase inhibitor	0,839	0,004
4	Threonine aldolase inhibitor	0,795	0,005
5	Phosphatidylserine decarboxylase inhibitor	0,787	0,004
6	CYP2A8 substrate	0,772	0,004
7	Corticosteroid side-chain-isomerase inhibitor	0,733	0,007
8	Isopenicillin-N epimerase inhibitor	0,728	0,004
9	Antimycobacterial	0,719	0,005
10	PfA-M1 aminopeptidase inhibitor	0,711	0,003
11	Serine-pyruvate transaminase inhibitor	0,703	0,003

Molecular Docking

The docking analysis on PDB ID: 1DF7 revealed that molecule had exceptional docking scores (**Table 2**) against the mixture of NADPH and methotrexate that MTB dihydrofolate reductase creates. In accordance with **Fig. 1A**, compound **3** establishes one hydrogen bonding contacts in the active site of the enzyme (PDB ID: 1DF7) that involve the oxygen atom of GLN28 and the hydrogen atom of the NHCO group (O---H-GLN28, 2.43Å). Considering the crystal structure, 3D molecular information of 4TZK, a thorough investigation was done to analyze binding affinities of ligands and target protein using Surflex-Dock program (**Table 3**). Processing of the protein included the deletion of the ligand and the solvent moieties as well as the addition of hydrogen atoms. Compound **3** was docked to the active spot-on enzyme as described in **Fig.1B**. As shown in **Fig. 1B,** compound **3** showed two H-bonding interactions at the dynamic site of the enzyme, H-bonding connections were raised from the nitrogen atom of pyridine with hydrogen atom of amino acid residue ALA198 (N---H-ALA198, 2.56Å), hydrogen atom of SER20 (O---H-SER20, 1.83Å) with oxygen atom of CONH group.The docked picture of the binding interaction of 1DF7_ligand with enzyme active sites in **Fig. 2A** shows fourteen bonding interactions. The docked image of the 4TZK ligand's binding contact with the enzyme active sites is shown in **Fig. 2B**.

Table 2: Surflex Docking score of compound 3 on the PDB ID: 1DF7 in kcal/mol:

Compound	Total Score[a]	Crash Score[b]	Polar Score[c]	D score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
2NSD_ligand	9.25	-0.93	1.54	-150.083	-63.091	-250.958	-46.922
3	3.61	-1.44	0.71	-69.960	14.979	-158.834	-17.948

Table 3: Surflex Docking score of compound 3 on the PDB ID: 4TZK in kcal/mol:

Compound	Total Score[a]	Crash Score[b]	Polar Score[c]	D score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
4TZK_ligand	7.19	-2.06	1.36	-159.93	-54.08	-285.50	-39.55
3	3.59	-0.42	0.11	-66.115	-35.809	-148.693	-18.745

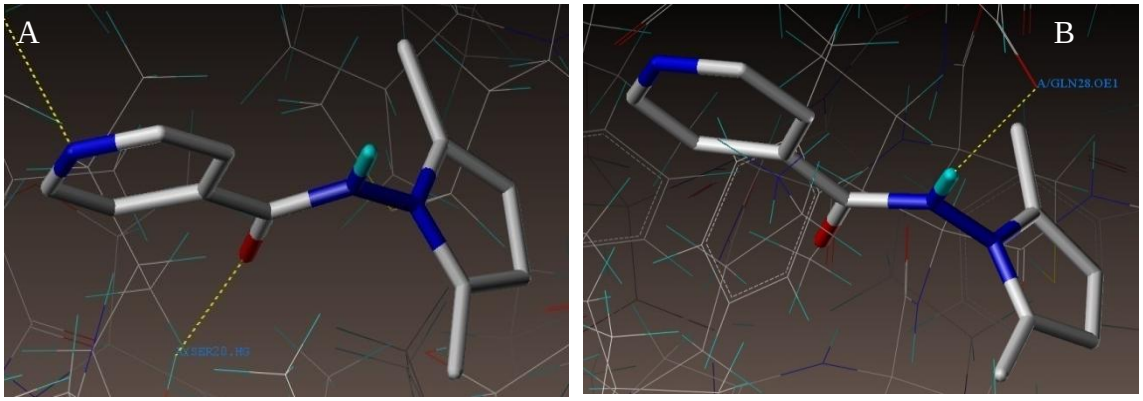


Figure 1(A-B): Docked image of molecule 3 in the enzyme's active site in PDB: 1DF7 and PDB: 4TZK

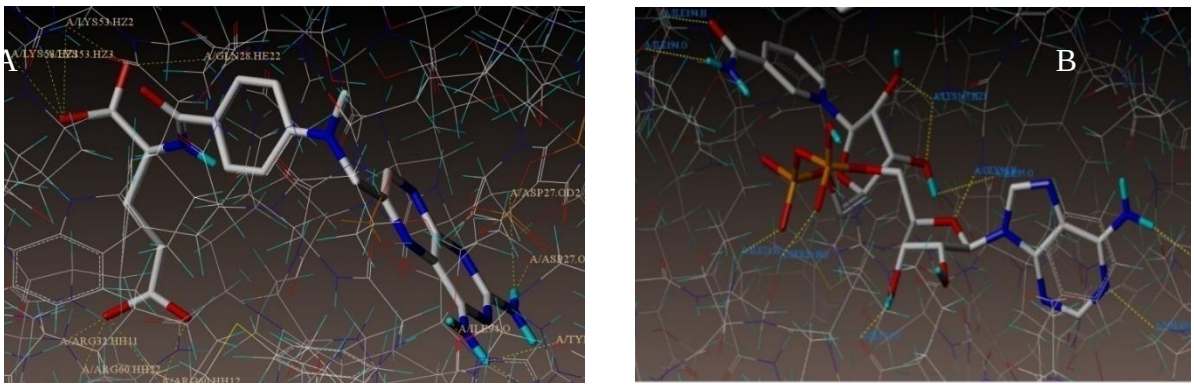


Figure 2 (A-B): Docked views of methotrexate at the enzyme's active site in PDB: 1DF7 and 4TZK_Ligand at the enzyme's active site in PDB: 4TZK.

ADME Analysis

Computational techniques were utilized to evaluate the projected chemo-informatic features. The results clearly indicated that the compounds having drug-like properties did not violate any of the previously established drug-likeness standards. The fact that in the results of the SWISS ADME and Mol inspiration predictor values of log P, molar refractivity, and total polar surface area of these compounds were in perfect accord with the most significant drug-likeness guidelines. Utilizing computational techniques, the anticipated chemo-informatic properties were assessed. The results showed that *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)isonicotinamide **3** had a molecular weight of 215.26 (g/mol), which was within the range value (<500g/mol) of higher molecular weight compounds. Because of this compound's strong hydrophilicity-lipophilicity stability, good gastrointestinal absorption was anticipated, as well as its high lipophilicity. The molecular polar surface area (PSA) is a very useful parameter for drug transport properties; the molecule is well-defined as the surface sum over all polar atoms, primarily oxygen, nitrogen, and attached hydrogen atoms. As a result, passively absorbed molecules with topological polar surface area (TPSA) >140 are assumed to have low oral bioavailability. It has been demonstrated that a very slight correlation exists between this metric and permeability, Caco-2 monolayers, human intestinal absorption, and blood-brain barrier penetration. The PSA parameter is frequently utilized to optimize a drug's capacity to enter cells. Previous studies' findings indicated the PSA standard value (< 89 A^2).

Lipinski's rule RO5 of resulted in 0 violation which showed that *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)isonicotinamide **3**, possesses good molecular weight (g/mol), two Hydrogen Bond Acceptor and one Hydrogen Bond Donor, log p value of0.591there by significantly justifying the drug-likeness behavior of compound **3**. The ADME and toxicity results were depicted in **table 6.**

Biological activity:

Table 4: Preliminary *in vitro* antitubercular, antibacterial activity results of enoyl ACP reductase inhibition values (Results are expressed as % InhA inhibition) of compound 3.

Compound	*M. tuberculosis* $H_{37}Rv$ MIC values ($\mu g\ ml^{-1}$)	*S. aureus* (Gram+ve)	*E. coli* (Gram –ve)	IC_{50} (µM) MtDHFR	% Inhibition of InhA at 50 µM
		MIC (µg/mL)	MIC (µg/mL)		
3	0.8	25	1.6	24	86
Pyrazinamide	3.125	-	-	-	-
Streptomycin	6.25	-	-	-	-
Ciprofloxacin	-	2	2	-	-
TCL	-	-	-	-	>99
TMP	-	-		92	-

MIC-Miniumum Inhibitory Concentration, TCL-Triclosan, TMP-Trimethoprim

Table 5: Swiss ADME web tool's ADME properties of synthesized compound 3:

Compound	Log P	Molar refractivity	TPSA	HBA	HBD	RB	GI Absorption	BBB Permeant	Log Kpcm/s	Solubility	CYP inhibitor					Lipinski violation	Synthetic accessibility
											1A2	2C19	2C9	2D6	3A4		
3	1.89	62.11	46.92	2	1	3	High	Yes	-6.43	Soluble	Yes	No	No	No	No	0	2.26

Table 6: Toxicity studies of synthesized compound 3:

Compound	LD50 mg/kg	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Arylhydrocarbon Receptor	Androgen Receptor (AR)	Androgen Receptor Ligand Binding Domain	Aromatase	Estrogen Receptor Ligand Binding Domain	Peroxisome Proliferator Activated Receptor Gamma	Nuclear factor	Heat shock factor response element	Mitochondrial Membrane Potential	Phosphoprotein	ATPase family AAA domain containing protein 5
3	133	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

CONCLUSIONS

Compound **3** showed encouraging *in silico* results, as confirmed by its high protein ligand interaction energy and substantial scoring functions, both of which simultaneously predicted the test compound's activity. The molecule is a promising lead for the development of potent and selective antitubercular agents, as suggested by its *in silico* ADME reporting, toxicity, drug likeness, drug score results, and antitubercular activities.

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REFERENCES

[1] Dheeraj B, Kumud U, Rishendra, K, Ravindra, PS. A recent review on tuberculosis and its treatment. *Int. J. Res. Pharm*. Chem., 2019; 9: 200-210.

[2] Varun B, Divya G, Vikrant A, Saurabh PS. Pyrrole: a resourceful small molecule in key medicinal hetero-aromatics.*RSC adv*. 2015;*20*: 15233-15266.

[3] Alqahtani YS, Avunoori S, Kanimehalli VM, Shaikh IA, Prem Kumar SR, Kulkarni, VH, Mannasaheb BA, Joshi, SD. Synthesis, docking studies and biological evaluation of some new pyrrolyl benzohydrazide derivatives. *Ind. J. Hetero. Chem*. 2024; 34: 35-42.

[4] Mahnashi MH, Kittur B, Prem Kumar SR, Alqahtani A, Ali AA, Alshahrani AA, Shaikh IA, Joshi SD. In silico and in vitro evaluation of a new pyrrolyl benzohydrazide derivatives as potential antimicrobial agents. *Ind. J. Hetero. Chem*. 2022; 32: 523-530.

[5] Avunoori S, Mahnashi MH, Momenah AM, Shaikh IA, Kulkarni VH, Joshi SD. Synthesis, docking studies and biological evaluation of some new 4-(1H-pyrrol-1-yl)benzohydrazide schiff bases. *Ind. J. Hetero. Chem*. 2023; 33: 233-239.

[6] Guilherme FDSF, Herida RNS, Jean LDS. Isoniazid: A review of characteristics, properties and analytical methods. *Crit Rev Anal Chem.* 2017; *47 (4)*: 298-308.

[7] Lourenco MCS, deSouza MVN, Pinheiro AC, Ferreira DM, Goncalves BR, Nogneira TCM. Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues, *ARKIVOCxv*. 2007;181-191.

[8] Goto SK. Jo KT, Kawakita TS, Mitsuhashi ST, Nishino TN, Ohsawa N, Tanami H. Method of minimum inhibitory concentration (MIC) determination. *Chemotherapy*. **1**981; 29: 76-79.