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In Silico Docking, ADMET Profiling, and Experimental Validation of Pyrrolo[2,3-d]pyrimidine Urea Derivatives as Potential Antimicrobial Agents

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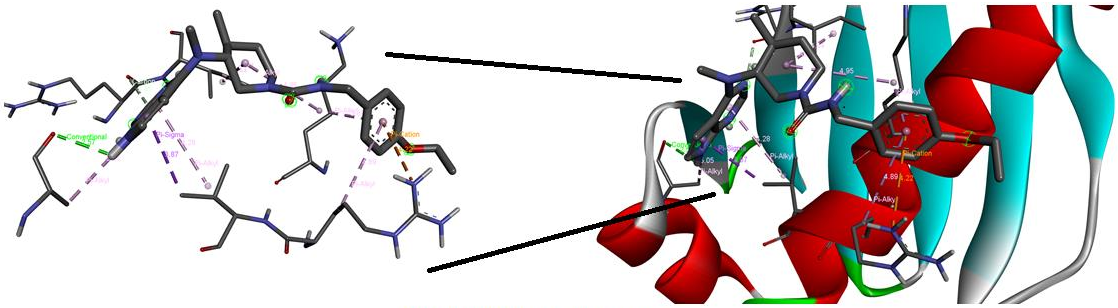
Abstract

In this research paper, we present a comprehensive study on the synthesis, molecular docking analysis, ADMET characterization, and antimicrobial activity evaluation of a novel **pyrrolo[2,3-d]pyrimidine** derivative along with a specific focus on Compound RP-3, which demonstrated exceptional activity with a notably negative docking score of -7.4 kcal/mol. **pyrrolo[2,3-d]pyrimidine** derivative was synthesized using established chemical protocols, and its structural identity was confirmed through various spectroscopic techniques. Subsequently, molecular docking studies were conducted to investigate the potential interactions between the synthesized compound and a target protein associated with the antimicrobial activity. Remarkably, Compound RP-3 exhibited a strikingly low docking score of -7.4 kcal/mol, suggesting a strong binding affinity with the target protein. Furthermore, the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of Compound RP-3 were assessed to ascertain its safety and suitability as a potential drug candidate. The ADMET data indicated favourable pharmacokinetic and safety profiles, reinforcing its potential as a lead compound for further drug development. Antimicrobial activity evaluations were performed to validate the biological efficacy of Compound RP-3. The compound displayed potent antimicrobial activity against a range of microbial strains, demonstrating its potential as a promising antimicrobial agent. The pyrrolo[2,3-d]pyrimidine derivative, specifically Compound RP-3, shows promising attributes as a novel antimicrobial candidate. The exceptionally negative docking score, strong binding affinity, favourable ADMET properties, and potent antimicrobial activity highlight the potential of this compound for further investigation and development as a therapeutic agent in the fight against microbial Infections.

Keyword:- Pyrrolo[2,3-d]pyrimidine derivative, Molecular docking, ADMET characterization, Antimicrobial activity, Compound RP-3, Docking score, Drug discovery Pharmacokinetics, Microbial infections, Drug development Chemical synthesis Binding affinity.

GRAPHICAL ABSTRACT



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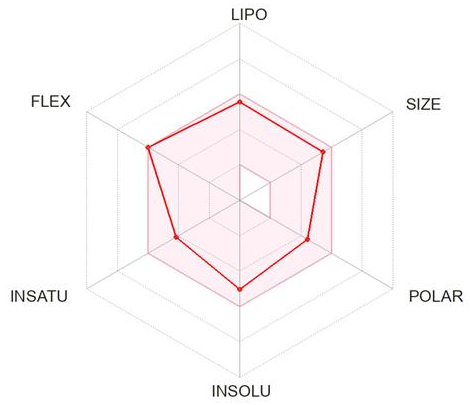
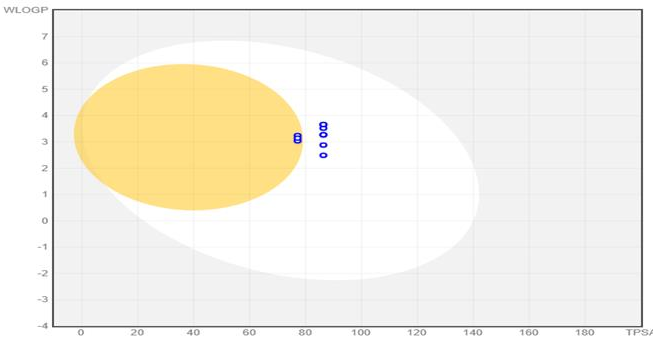


Bioactive Radar



BOILED-egg

Introduction

The escalating global threat posed by antimicrobial resistanc necessitates continuous exploration for novel antimicrobial agents. In this pursuit, computational methods coupled with experimental validation offer a promising avenue for the rapid screening and optimization of potential antimicrobial compounds. One class of compounds that has garnered significant attention in this regard is pyrrolo[2,3-d] pyrimidine urea derivatives, owing to their diverse pharmacological activities, including antimicrobial properties.

The driving force behind this research stems from the urgent need to combat the growing incidence of antimicrobial resistance, which poses a formidable challenge to public health worldwide. Traditional drug discovery approaches often entail time-consuming and costly experimental screening processes. However, the integration of computational techniques such as in silico docking and ADMET profiling offers a cost-effective and time-efficient strategy for identifying lead compounds with potential antimicrobial activity.

The synthesis and evaluation of pyrrolo[2,3-d]pyrimidine urea derivatives have been explored in various studies, highlighting their cytotoxic, apoptotic, and antiproliferative effects against

cancer cell lines [1][2][8][10][11]. Additionally, these compounds have demonstrated antibacterial and antifungal activities, further underscoring their potential as antimicrobial agents [13][3][18]. Moreover, the versatility of pyrrolo[2,3-d]pyrimidine scaffolds has been exploited in the design of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) inhibitors [4][6][14], broad-spectrum anticancer agents [5][7], and multi-targeted kinase inhibitors [9][17]. Pyrrolo[2,3-d]pyrimidine urea derivatives have been the subject of extensive investigation in various studies, highlighting their cytotoxic, apoptotic, and antiproliferative effects against cancer cell lines [10][11]. Additionally, these compounds have demonstrated antibacterial and antifungal activities [12][18], further emphasizing their potential as antimicrobial agents.

Furthermore, the versatility of pyrrolo[2,3-d]pyrimidine scaffolds has been exploited in the design of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) inhibitors [16], broad-spectrum anticancer agents [15], and multi-targeted kinase inhibitors [19]. These studies underscore the importance of pyrrolo[2,3-d]pyrimidine derivatives as a promising class of compounds for various therapeutic applications.

Recent studies have highlighted the pharmacological versatility of pyrrolo[2,3-d]pyrimidine derivatives. They have demonstrated cytotoxic, apoptotic, and antiproliferative effects against cancer cell lines [21], indicating their potential as broad-spectrum anticancer agents. Identification of N-Phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine derivatives as potent and selective NF-κB inducing kinase (NIK) inhibitors highlights their potential for innovative therapeutic interventions in psoriasis management.[20]Additionally, their role as kinase inhibitors [22][23] underscores their therapeutic versatility.

The integration of computational approaches such as molecular docking facilitates the prediction of ligand-receptor interactions, thereby aiding in the identification of lead compounds with enhanced binding affinity towards target biomolecules. Furthermore, ADMET profiling enables the assessment of the pharmacokinetic and toxicity profiles of potential drug candidates, crucial for guiding subsequent experimental validation.

This research aims to leverage in silico docking, ADMET profiling, and experimental validation techniques to explore the antimicrobial potential of pyrrolo[2,3-d]pyrimidine urea derivatives. By elucidating the structure-activity relationships and pharmacological properties of these compounds, this study seeks to contribute to the development of novel antimicrobial agents that can address the growing threat of antimicrobial resistance.

Material and method

Chemistry and Discussion

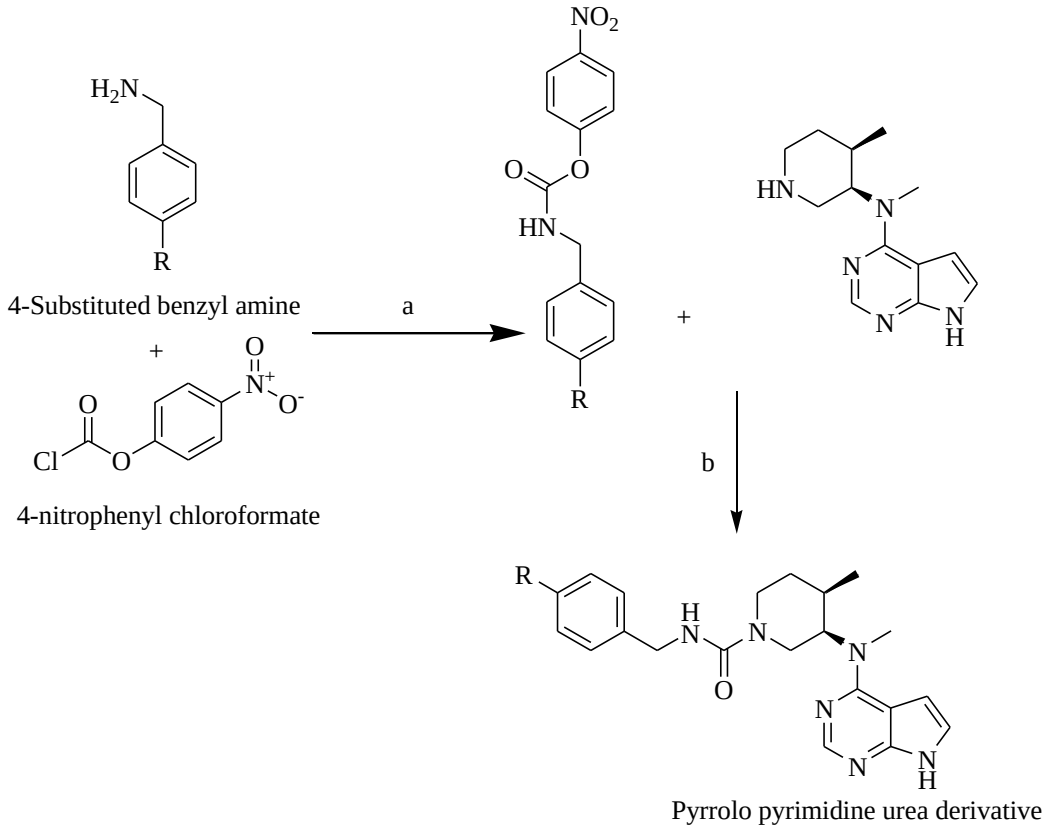
The reagents were obtained from Merck in Germany and were used without further purification. Infrared (IR) spectra were obtained using a Shimadzu FTIR-8400S spectrophotometer from Japan, and KBr pellets were used as the sample holder. Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker Avance 300 and 400 MHz spectrometers, which are manufactured by Bruker in Rheinstatten, Germany. These spectrometers operated at 300 and 400 MHz for ^{1}H NMR, as well as 75.4 and 100 MHz for ^{13}C NMR. Chloroform (CDCl3) was used as the solvent, and tetramethylsilane (TMS) served as the internal reference for chemical shifts.

Elementary analysis to determine the carbon (C), hydrogen (H), and nitrogen (N) atom compositions was performed using the Costech essential analyzer. Melting points were determined using open glass capillaries and an Electro thermal melting point apparatus.

General procedure for the synthesis of (3R,4R)-N-(sustitutedbenzyl)-3-(N-methyl-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxamide

Solution of 4-sustituted benzyl amine (1) (1.8 gm) in Toluene (18 ml) cooled to 0-5°C. add 4-Nirophenny chloroformate (2.5 gm) solution in Toluene (9 ml) over15 to 20 minutes. Stir reaction mass for 10-15 minutes. Add triethyl amine (1.6 gm) over 15 to 20 minutes. Stir reaction mass for 2 hours at 0-5°C. The reaction progress was monitored by TLC. After complete conversion of 4-sustituted benzyl amine to carbamate charge n-Hexane (27 ml). Stir reaction mass for 30 minutes at 0-5°C. Filter the solid and slurry wash with water and suck dry to remove mother liquor form solid. Charge wet cake in tetrahydrofuran (20 ml). Charge (3R,4R)-(4-Methylpiperidin-3-yl)methyl-(7Hpyrrolo[2,3-d]pyrimidin-4-yl)amine (2 gm) and potassium carbonate (5.6 gm). Raise reaction mass temperature at 50-55°C and stir for 60 minutes at 50-55 °C. The reaction progress was monitored by TLC. After complete conversion of (3R,4R)-(4-Methylpiperidin-3-yl)methyl-(7Hpyrrolo[2,3-d]pyrimidin-4-yl)amine cool reaction mass to 25-35°C. Charge water (100 ml) and reaction mass extracted with ethyl acetate (30 ml). Ethyl acetate layer wash two times with water (20 ml). After water washing ethyl acetate layer dried over sodium sulphate and distilled out under vacuum at 45°C to get crud compound. Crud compound purified by column chromatography using silica gel 60-120 mesh 90% ethyl acetate: hexane as an eluent. 3.0 gm pure compound (3R,4R)-N-(4-sustituted

benzyl)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidine-1-carboxamide isolated after column purification.



Scheme 1: Preparation of Pyrrolo[2,3-d]pyrimidine urea derivatives

(a) Triethyl amine, Toluene, n-Hexane, water, 0-5°C, 4hr (b) Potassium carbonate, Tetrahydrofuran, ethyl acetate, water, 50-55°C, 1hr.

(3R,4R)-N-(4-isobutoxybenzyl)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidine-1-carboxamide(RP-01)

^{1}H NMR (400 MHz, DMSO) δ 0.960- 0.967 (d 3H), 1.001-1.019 (d, 3H), 1.490-1.532 (m, 1H), 1.689-1.735 (m, 1H), 1.957-2.024 (m,1H), 2.341-2.371 (m, 1H), 3.264 (s, 3H), 3.291-3.306 (m 1H), 3.518-3.756 (m 5H), 4.152-4.166 (d, 2H), 4.824 (bs, 1H), 6.521 (bs, 1H), 6.812-6.834 (d, 2H), 7.081-7.149 (m, 4H), 8.108 (s, 1H); ^{13}C NMR 10.874, 14.218, 22.525, 31.397, 31.963, 34.453, 43.429, 44.079, 53.642, 69.319, 102.101, 114.473, 121.105, 128.686, 133.385, 151.027, 152.140, 157.492, 157.661, 157.818; Mass: 451.3 [M + H]+; IR (cm-1): 2958 (C-H), 2870 (C-H), 1620 (C=O), 1500, 1489 (C=C), 1348 (C-H), 1301 (C-H), 1240 (C-N), 1033 (C-O), cm^{-1}; Yield: 76%; mp 142-144ºC; MS (m/z): 451 (M+); Anal. calcd for $C_{25}H_{34}N_6O_2$: C, 66.64; H, 7.61; N, 18.65; Found: C, 66.61; H, 7.60; N, 18.61.

(3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-propoxybenzyl)piperidine-1-carboxamide(RP-02)

^{1}H NMR 0.948-1.018 (m, 6H), 1.489-1.531 (m, 1H), 1.662-1.750 (m, 4H), 2.343-2.372 (m, 1H), 3.264 (s, 3H), 3.306-3.332 (m, 1H), 3.522-3.761 (m, 3H), 3.860-3.893 (t, 2H), 4.158-4.172 (d, 2H), 4.826 (bs, 1H), 6.521 (bs, 1H), 6.809-6.830 (d, 2H), 7.012-7.152 (m, 4H), 8.114 (s, 1H), 11.644 (s, 1H); ^{13}C NMR 10.874, 14.218, 22.525, 31.379, 31.963, 34.453, 43.429, 44.079, 53.642, 69.319, 102.101, 102.627, 114.473, 121.105, 128.686, 133.385, 151.027, 152.140, 157.492, 157.661, 157.818; IR (cm-1): 2958 (C-H), 2870 (C-H), 1625 (C=O), 1508, 1489 (C=C), 1342 (C-H), 1303 (C-H), 1219 (C-N), 1028 (C-O), cm^{-1}; Yield: 70%; mp 138-139ºC; MS (m/z): 437 (M+); Anal. calcd for $C_{24}H_{32}N_6O_2$: C, 66.03; H, 7.39; N, 19.25; Found: C, 66.07; H, 7.32; N, 19.21.

(3R,4R)-N-(4-fluorobenzyl)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidine-1-carboxamide(RP-03)

^{1}H NMR 1.161-1.179 (d, 3H), 1.498-1.540 (m, 1H), 1.697-1.756 (m, 1H), 2.335-2.375 (m, 1H), 3.268 (s, 3H), 3.318 (bs, 1H), 3.526-3.763 (m, 3H), 4.210-4.224 (d 2H), 4.831 (bs, 1H), 6.522 (bs, 1H), 7.073-7.198 (m, 4H), 7.251-7.287 (m, 2H), 8.115 (s, 1H); ^{13}C NMR 14.214, 21.205, 31.387, 31.954, 34.463, 43.301, 44.081, 53.634, 60.229, 102.089, 102634, 115.087, 115.237, 115.296, 115.446, 121.133, 129.253, 129.332, 129.401, 137.717, 137.746, 151.027, 152.141, 157.494, 157.640, 160.234, 162.635, 170.805; IR (cm-1): 2958 (C-H), 2870 (C-H), 1628 (C=O), 1501, 1489,1469 (C=C), 1342 (C-H), 1303 (C-H), 1236 (C-N), 1087 (C-O), 1028 (C-F) cm-1; Yield: 67%; mp 135-136ºC; MS (m/z): 397(M+); Anal. calcd for $C_{21}H_{25}FN_6O$: C, 63.62; H, 6.36; F, 4.79; N, 21.20; Found: C, 63.63; H, 6.33; F, 4.73; N, 21.22.

(3R,4R)-N-(4-ethoxybenzyl)-3-(N-methyl-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxamide(RP-04)

Yield: 73%; mp 155-158ºC; MS (m/z): 423 (M+); Anal. calcd for $C_{23}H_{30}N_6O_2$: C, 65.38; H, 7.16; N, 19.89; Found: C, 65.38; H, 7.16; N, 19.89.

(3R,4R)-N-(4-isopropoxybenzyl)-3-(N-methyl-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxamide(RP-05)

Yield: 76%; mp 147-148ºC; MS (m/z): 437 (M+); Anal. calcd for $C_{24}H_{32}N_6O_2$: C, 66.03; H, 7.39; N, 19.25; Found: C, 66.11; H, 7.33; N, 19.24.

(3R,4R)-N-(4-methoxybenzyl)-3-(N-methyl-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxamide(RP-06)

Yield: 71%; mp 150-152ºC; MS (m/z): 409 (M+); Anal. calcd for $C_{22}H_{28}N_6O_2$: C, 64.68; H, 6.91; N, 20.57; Found: C, 66.01; H, 7.33; N, 19.23.

(3R,4R)-N-(4-sec-butoxybenzyl)-3-(N-methyl-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxamide(RP-07)

Yield: 66%; mp 138-140ºC; MS (m/z): 451 (M+); Anal. calcd for $C_{25}H_{34}N_6O_2$: C, 66.64; H, 7.61; N, 18.65; Found: C, 66.61; H, 7.60; N, 18.62.

(3R,4R)-N-(4-bromobenzyl)-3-(N-methyl-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxamide(RP-08)

Yield: 73%; mp 155-156ºC; MS (m/z): 457 (M+); Anal. calcd for $C_{21}H_{25}BrN_6O$: C, 55.15; H, 5.51; Br, 17.47; N, 18.37; Found: C, 55.13; H, 5.50; Br, 17.42; N, 18.33.

(3R,4R)-N-(4-chlorobenzyl)-3-(N-methyl-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxamide(RP-09)

Yield: 74%; mp 158-160ºC; MS (m/z): 413 (M+); Anal. calcd for $C_{21}H_{25}ClN_6O$: C, 61.08; H, 6.10; Cl, 8.59; N, 20.35; Found: C, 61.02; H, 6.14; Cl, 8.52; N, 20.30.

(3R,4R)-N-(4-butoxybenzyl)-3-(N-methyl-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-methylpiperidine-1-carboxamide(RP-10)

Yield: 64%; mp 132-134ºC; MS (m/z): 451 (M+); Anal. calcd for $C_{25}H_{34}N_6O_2$: C, 66.64; H, 7.61; N, 18.65; Found: C, 66.62; H, 7.60; N, 18.62.

Biological assays

Antimicrobial Activity

The investigation of synthesized compound antibacterial and antifungal activities involved determining Minimal Inhibitory Concentrations (MICs) through a broth microdilution system, following the methodology outlined by Rattan. The objective was to assess inhibitory effects against various bacterial and fungal strains, comparing the synthesized compounds with the standard antibacterial agent ampicillin and the antifungal agent griseofulvin.

Gram-positive bacteria (Staphylococcus aureus MTCC 96, Streptococcus pyogenus MTCC 442), gram-negative bacteria (Escherichia coli MTCC 443, Pseudomonas aeruginosa MTCC

1688), and fungal species (Candida albicans MTCC 227, Aspergillus niger MTCC 282, Aspergillus clavatus MTCC 1323) were employed as test organisms, sourced from the Microbiology department of Tolani college of arts & science , Adipur, Kutch, Gujarat, India.

Inoculum preparation involved adjusting the concentration to 108 Colony Forming Units (CFU) per milliliter for each test strain based on turbidity. Synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted to the necessary concentrations for testing against standard bacterial strains.

Periodical dilutions were executed in primary and secondary dilution sets. Control tubes lacking antibiotics were incubated alongside the test tubes overnight at 37 °C. MIC was identified as the lowest concentration of the synthesized compound inhibiting visible growth. Tubes without visible growth underwent sub-culturing and additional incubation to assess bacteriostatic or bactericidal effects.

Each synthesized compound, initially prepared at 2000 µg/ml, underwent testing at concentrations of 500 µg/ml, 250 µg/ml, and 125 µg/ml in the primary dilution set. Active compounds from the primary set were subjected to an alternate set of dilutions, ranging from 100 µg/ml to 1.56 µg/ml, to ascertain MIC.

MIC determination involved identifying the highest dilution displaying at least 99% inhibition. This systematic approach allowed for a comprehensive evaluation of the synthesized compounds' effectiveness against a spectrum of bacteria and fungi, offering valuable insights into their potential as antimicrobial agents.

Table 01 Antibacterial Activity of pyrrolo[2,3-d]pyrimidine (RP-01 to 10)

Minimal Inhibition Concentration [Microgram/Ml]					
Sr.	**Code**	**E.Coli**	**P.Aeruginosa**	**S.Aureus**	**S.Pyogenus**
No	**No**	**Mtcc 443**	**Mtcc 1688**	**Mtcc 96**	**Mtcc 442**
1	RP-01	100	125	200	125
2	RP-02	125	200	125	100
3	RP-03	125	100	250	250
4	RP-04	100	200	200	125
5	RP-05	125	125	125	250

6	RP-06	100	125	125	125
7	RP-07	125	200	200	100
8	RP-08	125	125	200	200
9	RP-09	100	100	250	125
10	RP-10	125	125	200	200
	Gentamycin	0.05	1	0.25	0.5
	Ampicillin	30	Na	40	25
	Chloramphenicol	50	50	50	50
	Ciprofloxacin	25	25	50	50

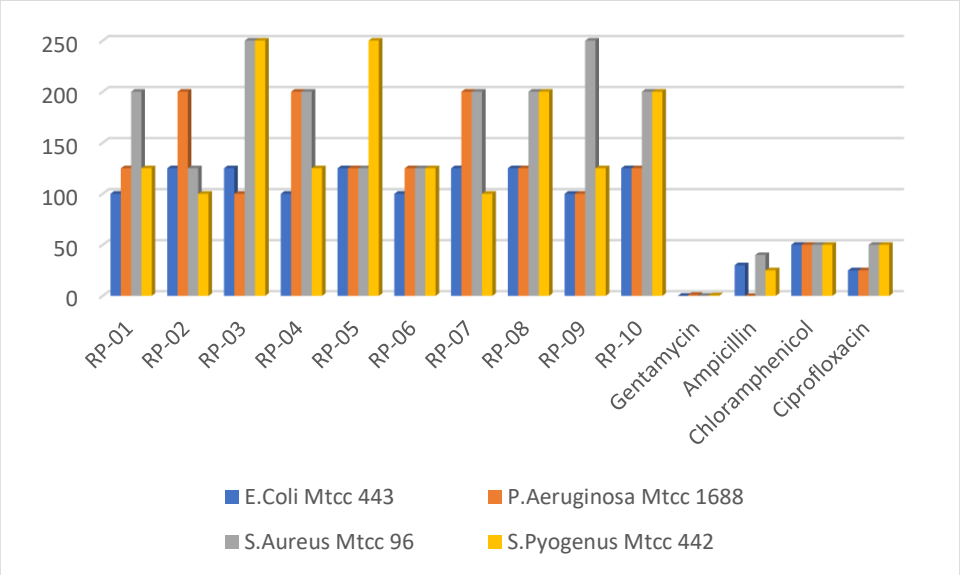


Fig 01 illustrates the graphical representation of the antibacterial activity of pyrrolo[2,3-d]pyrimidine compounds, denoted as RP-01 to RP-10

Table 02 Antifungal Activity of pyrrolo[2,3-d]pyrimidine (RP-01 to 10)

Minimal Fungicidal Concentration [Microgram/Ml]					
Sr.	**Code**	**C. albicans**	**A. Niger**	**A. Clavatus**	**G. Candidum**
1	RP-01	500	1000	1000	150
2	RP-02	250	1000	>1000	250
3	RP-03	500	1000	1000	**100**
4	RP-04	500	500	1000	350
5	RP-05	250	500	500	150

6	RP-06	250	500	500	250
7	RP-07	500	500	1000	500
8	RP-08	500	500	>1000	250
9	RP-09	500	1000	1000	125
10	RP-10	250	500	500	150
	Nystatin	100	100	100	100
	Greseofulvin	500	100	100	100

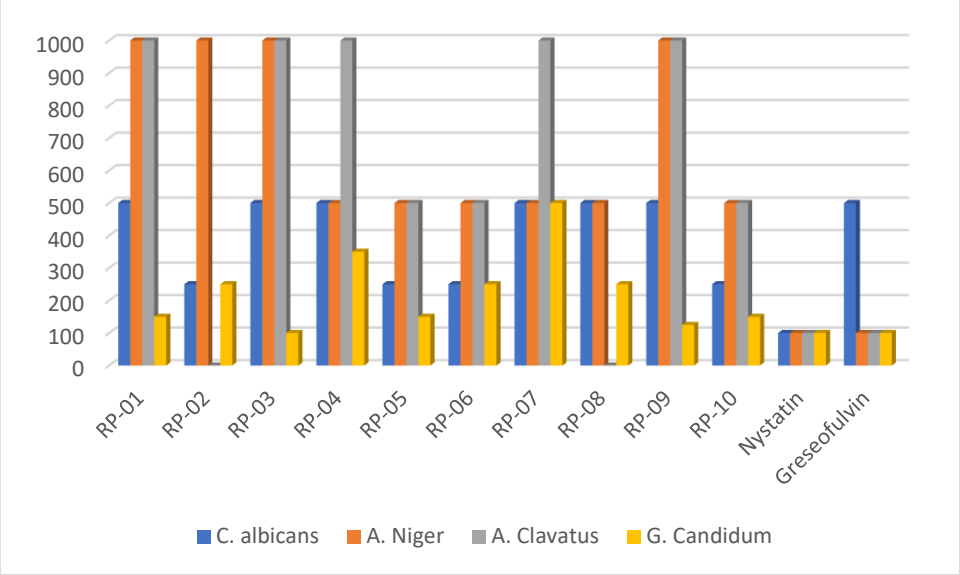


Fig 02 illustrates the graphical representation of the antifungal activity of pyrrolo[2,3-d]pyrimidine compounds, denoted as RP-01 to RP-10

ADMET

In the realm of drug discovery, the assessment of Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties stands as a crucial determinant in the identification and development of potential drug candidates. These parameters collectively obtained by swissadme. This offers invaluable insights into the behaviour of compounds within the complex biological milieu, helping researchers navigate the intricate path towards effective and safe drugs.

Molecular Weight (MW): Molecular weight serves as a foundational parameter, influencing a drug candidate's bioavailability and permeability. Maintaining a molecular weight within the range of 50 to 100 is considered optimal, ensuring an equilibrium between molecular size and pharmacological efficacy.

Octanol/Water Partition Coefficient (iLOGP): The iLOGP, measuring a compound's lipophilicity, plays a pivotal role in its ability to traverse cell membranes. Falling within the permissible range of -2 to 10 ensures a balanced solubility-permeability profile, critical for effective drug delivery.

Topological Polar Surface Area (TPSA): TPSA signifies a compound's polar character and significantly impacts its absorption and transport across biological membranes. Falling within the range of 20 to 130 aligns with optimal drug-like properties, influencing factors such as bioavailability.

Number of H-Bond Acceptors (HBA) and H-Bond Donors (HBD): The number of HBA and HBD in a compound are crucial for its interaction with biological targets. Maintaining limits of 0 to 10 (HBA) and 0 to 5 (HBD) ensures a balanced capacity for hydrogen bonding, a key aspect of molecular recognition.

Rotatable Bonds (RB): Rotatable bonds reflect a compound's molecular flexibility, impacting conformational changes during interactions. The range of 0 to 5 accounts for reasonable flexibility without allowing excessive rotational freedom.

Number of Aromatic Heavy Atoms (nAH): The presence of aromatic heavy atoms contributes to a compound's stability and interaction with receptors. Falling within the range of 15 to 50 ensures an optimal balance for drug candidates.

Lipophilicity (LogP): LogP, measuring a compound's partitioning between lipids and water, is crucial for determining its solubility and permeation. The permissible range of -0.7 to 5 supports adequate solubility while maintaining an essential degree of lipophilicity for membrane permeation.

Molar Refractivity (MR): Molar refractivity reflects a compound's polarizability and molecular size, influencing its interactions with biological targets. Falling within the range of 40 to 130 ensures optimal pharmacokinetics.

Table 03 Displaying Pharmacokinetic Properties for Pyrrolo[2,3-d]pyrimidine (RP-01 to 10)

Comp.	MW	iLOGP	HBD	HBA	TPSA	RB	nAH	MR	LogP	PAINS	Brenk
RP-01	**450.58**	**3.71**	**2**	**4**	**86.38**	**9**	**15**	**135.23**	**2.45**	**0**	**0**
RP-02	**436.55**	**3.58**	**2**	**4**	**86.43**	**9**	**15**	**130.43**	**2.24**	**0**	**0**
RP-03	**396.46**	**2.84**	**2**	**4**	**77.15**	**6**	**15**	**114.28**	**2.50**	**0**	**0**
RP-04	**408.50**	**3.06**	**2**	**4**	**86.38**	**7**	**15**	**120.81**	**1.82**	**0**	**0**
RP-05	**422.52**	**3.43**	**2**	**4**	**86.38**	**8**	**15**	**125.62**	**2.03**	**0**	**0**
RP-06	**436.55**	**3.82**	**2**	**4**	**86.38**	**8**	**15**	**130.43**	**2.24**	**0**	**0**
RP-07	**545.54**	**3.15**	**2**	**7**	**162.31**	**9**	**18**	**159.55**	**2.07**	**0**	**0**
R-08	**450.58**	**3.62**	**2**	**4**	**86.38**	**9**	**15**	**135.23**	**2.45**	**0**	**0**
RP-09	**412.92**	**3.10**	**2**	**3**	**77.15**	**6**	**15**	**119.33**	**2.61**	**0**	**0**
RP-10	**457.37**	**3.10**	**2**	**3**	**77.15**	**6**	**15**	**122.02**	**2.71**	**0**	**0**

Molecular Docking

Molecular docking is a key computational approach in drug discovery, aiding in the understanding of how small molecules interact with target proteins. PyRx and Discovery Studio are two widely-used tools that streamline the complex process of molecular docking.

The first step involves preparing ligands and proteins. Ligands, representing potential drugs, undergo optimization for realistic conformations, and protein structures are downloaded from protein databank PDB ID [1TGH] refined. PyRx and Discovery Studio provide user-friendly interfaces for this crucial preparation phase. Following preparation, a three-dimensional grid is generated around the target protein to define search spaces for ligand binding. Both PyRx and Discovery Studio offer customization options, allowing researchers to adapt to the unique characteristics of the binding site. Scoring functions, essential for evaluating ligand-protein interactions, estimate binding affinities and predict energetically favorable binding poses. Integrated scoring functions, such as AutoDock and Auto Dock Vina, enhance the predictive power of PyRx and Discovery Studio.

Molecular docking using PyRx and Discovery Studio enhances the drug discovery process. These tools provide researchers with the means to predict, analyze, and visualize ligand-protein interactions, contributing to the identification and optimization of potential drug candidates. The combination of user-friendly interfaces and advanced features makes PyRx and Discovery Studio valuable assets in the computational drug discovery toolkit.

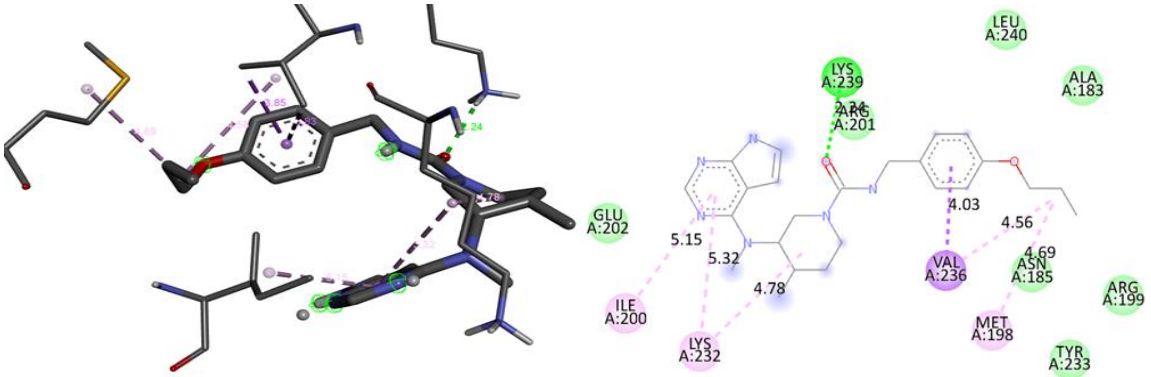


Fig 03 Illustrations portraying compound RP-01's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

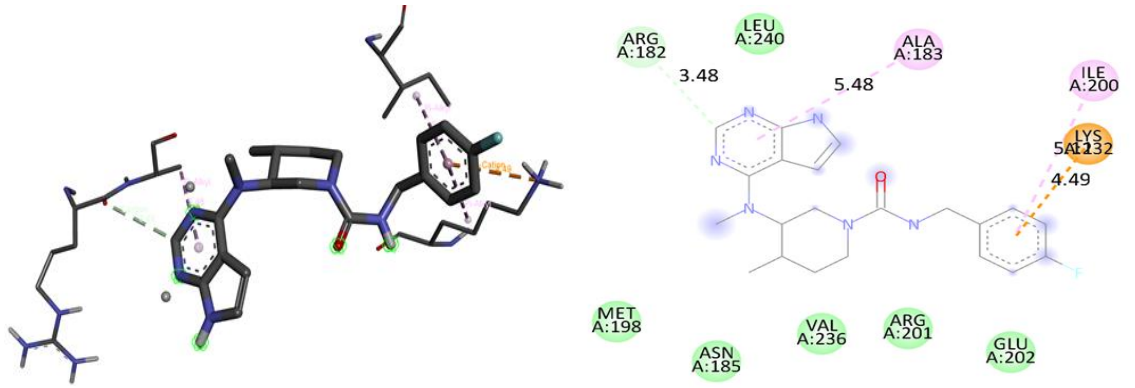


Fig 04 Illustrations portraying compound RP-02's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

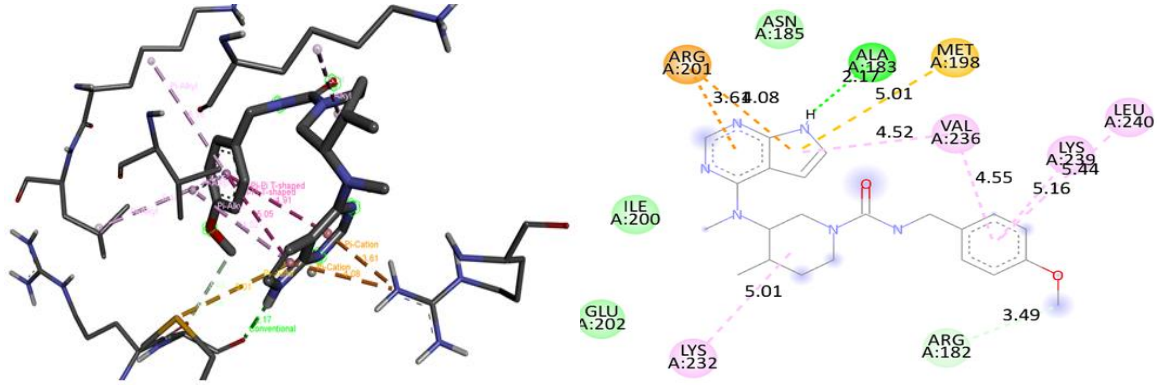


Fig 05 Illustrations portraying compound RP-03's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

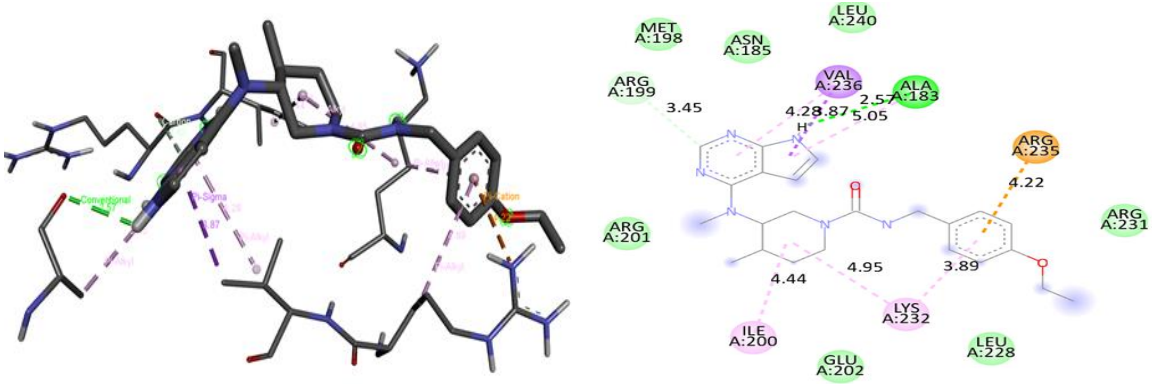


Fig 06 Illustrations portraying compound RP-04's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

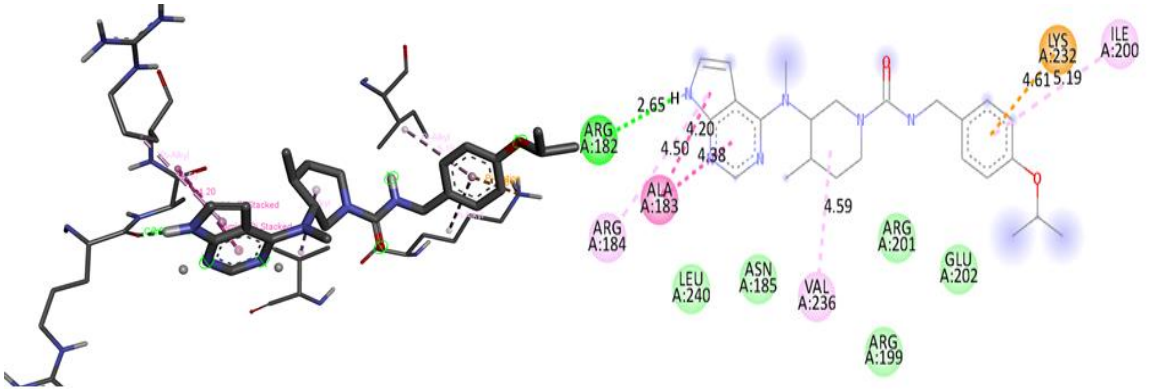


Fig 07 Illustrations portraying compound RP-05's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

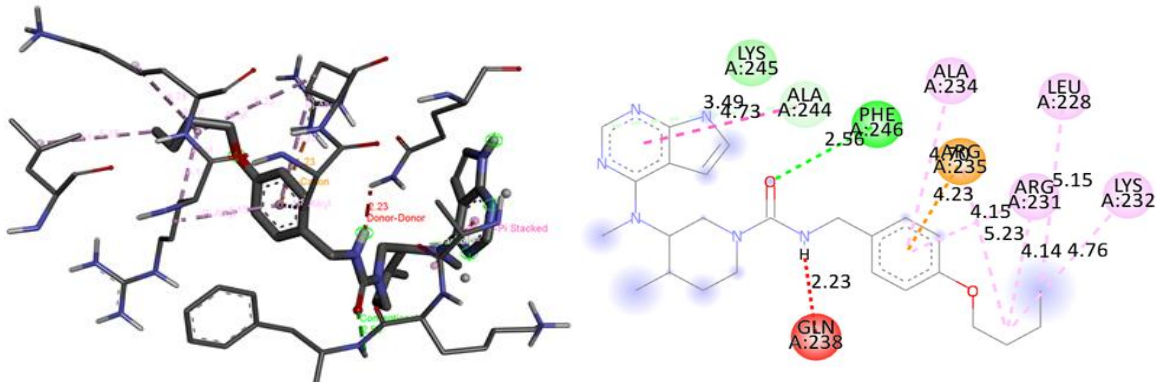


Fig 08 Illustrations portraying compound RP-06's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

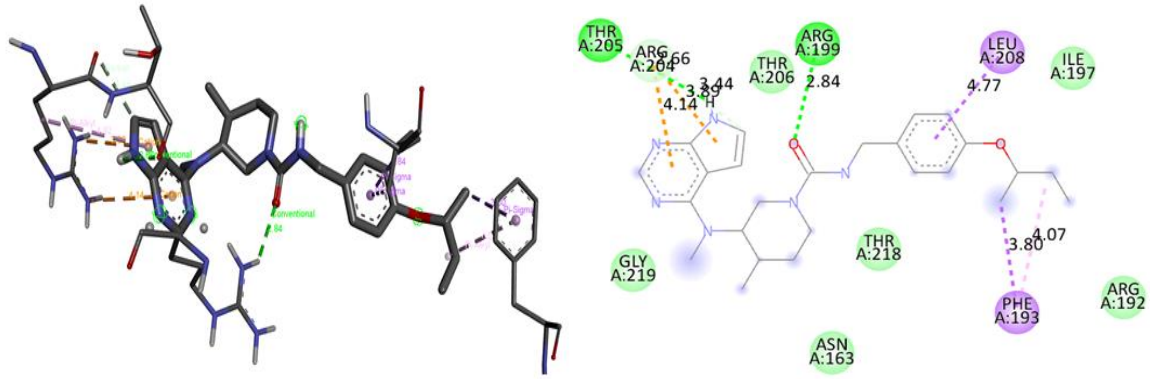


Fig 09 Illustrations portraying compound RP-07's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

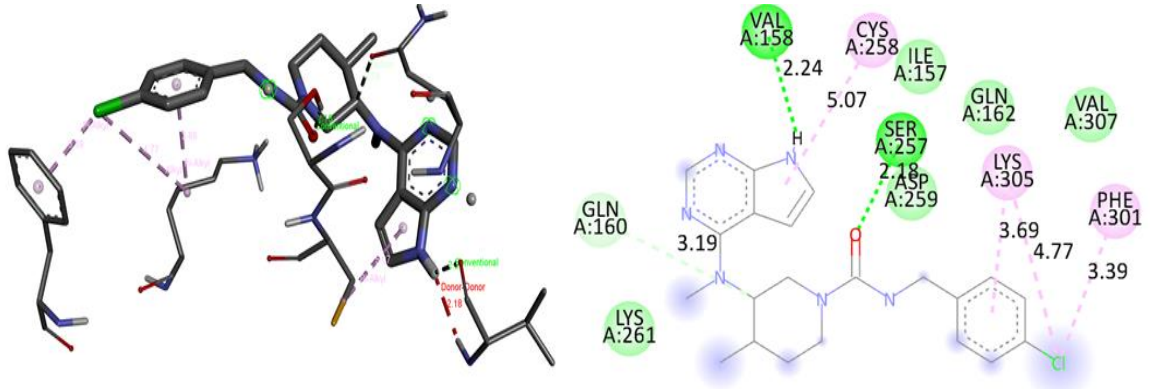


Fig 10 Illustrations portraying compound RP-08's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

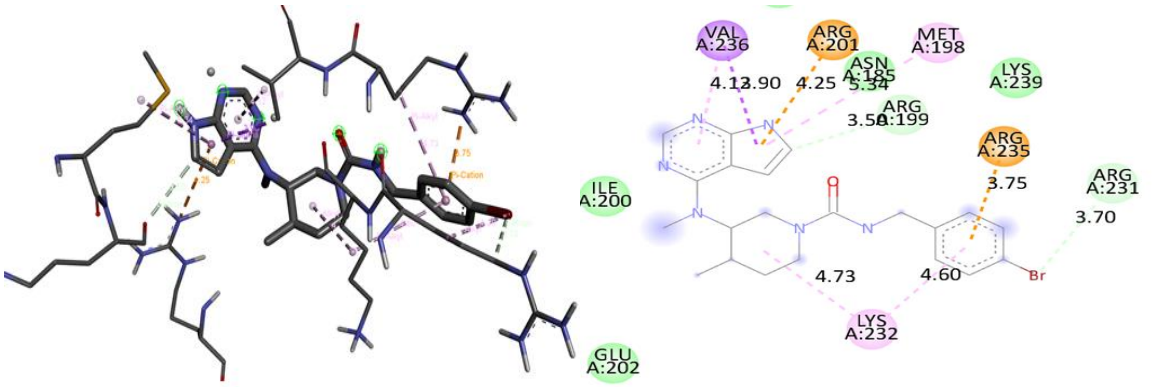


Fig 11 Illustrations portraying compound RP-09's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

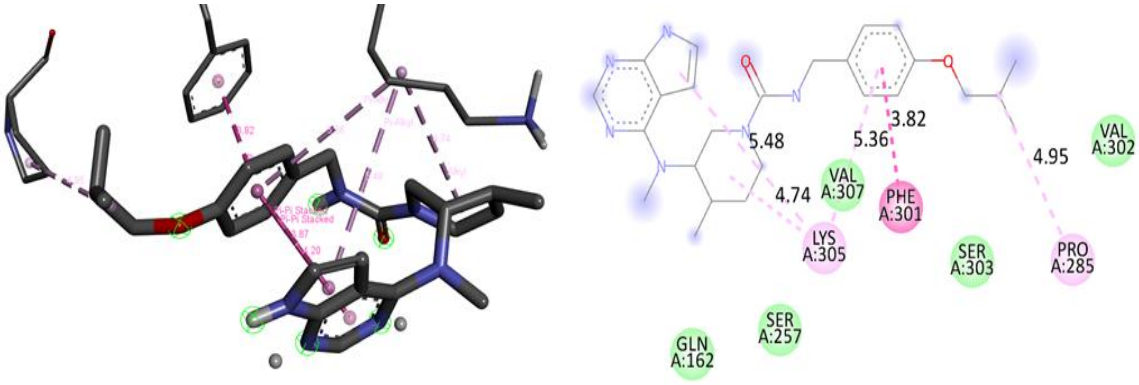


Fig 12 Illustrations portraying compound RP-10's 3D and 2D binding in active pocket of receptor. (PDB: 1TGH)

Table 04 Representation of the results from the molecular docking study of synthesized compounds with receptor 1TGH

Compound	R[function group]	H-bond	π–π Interaction	Hydrophobic Interaction	Docking energy in kcal \mole
RP-01	4-isobutoxy		LYS(A)305, PHE(A)301, PRO(A)285		-6.9
RP-02	4-propoxy	LYS(A)239,	ILE(A)200, LYS(A)232, VAL(A)236, MET(A)198.		-6.9
RP-03	F		ALA(A) 183, ILE(A) 200, LYS(A) 232.	ARG(A)182	-7.4
RP-04	OCH_3	ALA(A)183	ARG(A) 201, MET(A)198, VAL(A)236, LYS(A)239, LEU(A)240, LYS(A0232.	ARG(A) 182	-6.5
RP-05	OCH_2CH_3	ALA (A)183	ALA(A)183, VAL(A)236, ARG(A)235, ILE(A)200, LYS(A)232.	ARG(A)199	-6.5
RP-06	Iso propoxy	ARG(A)182	ALA(A)184, ARG(A)184, LYS(A)232, ILE(A)200, VAL(A)236.		-6.9
RP-07	Butoxy	PHE(A)246	ALA(A)244, ALA(A)234, ARG(A)235, ARG(A)231, LEU(A)228, ARG(A)231, LYA(A)232.	ALA(A)244	-6.7
RP-08	sec-butoxy	THR(A)205, ARG(A)199.	ARG(A)204, LEU(A)208, PHE(A)193	ARG(A)204	-6.3
RP-09	Cl	VAL(A)158, SER(A)257.	CYS(A)258, LYS(A)305, PHE(A)301.	GLN(A)160	-6.5
RP-10	Br		VAL(A)236, MET(A)198, ARG(A)201, ARG(A)235.	ARG(A)199, ARG(A)231.	-7.3

Result ad Discussion

Antibacterial Activity

The Antibacterial Activity Table provides valuable insights into the efficacy of synthesized compounds against various bacterial strains, including Escherichia coli (E.COLI), Pseudomonas aeruginosa (P.AERUGINOSA), Staphylococcus aureus (S.AUREUS), and Streptococcus pyogenus (S.PYOGENUS), as measured by the Minimal Inhibition Concentration (MIC) in micrograms/ml. A highly potent antimicrobial agent is discerned by consistently low MIC values across all bacterial strains. In this context, RP-01 emerges as a promising candidate, displaying MIC values of 100, 125, 200, and 125 against E.COLI, P.AERUGINOSA, S.AUREUS, and S.PYOGENUS, respectively. This indicates strong inhibitory effects at relatively low concentrations. Conversely, compounds with moderately effective antimicrobial properties exhibit MIC values in the mid-range. For example, RP-06 shows MIC values of 100, 125, 125, and 125 against the respective bacterial strains. In contrast, compounds with higher MIC values, such as RP-03 with values of 125, 100, 250, and 250, are considered less potent, requiring higher concentrations for inhibitory effects. The interpretation considers both the magnitude of MIC values and their consistency across bacterial strains, providing a nuanced understanding of the compounds' antimicrobial potency.

Antifungal Activity

The antifungal activity of a series of compounds (RP-01 to 10) was evaluated against various fungal strains, including Candida albicans (MTCC 227), Aspergillus niger (MTCC 282), Aspergillus clavatus (MTCC 1323), and Geotrichum candidum (MTCC 1234). The minimal fungicidal concentration (MIC) values were determined, revealing distinct patterns of antifungal efficacy. Compounds RP-02, RP-03, RP-05, RP-06, RP-09, and RP-10 exhibited low MIC values against Candida albicans, indicative of high antifungal activity. Similarly, RP-03 and RP-09 demonstrated notable effectiveness against Aspergillus niger, while RP-03and RP-05displayed strong antifungal activity against Aspergillus clavatus. Geotrichum candidum was particularly susceptible to compounds RP-03, RP-05, and RP-10. Molecular docking studies supported these findings, with compound RP-03 displaying the most negative docking score (-7.4) against the target protein ID 1 TGH found in Geotrichum candidum. The correlation between low MIC values and molecular docking results underscores the potential of these compounds as effective antifungal agents, particularly against Geotrichum candidum, with implications for further research and development in combating fungal infections

The synthesized compounds' antifungal activity is nuanced, with RP-03 emerging as a potent candidate against Geometrium chadium, supported by both experimental and computational

evidence. This comprehensive assessment underscores the potential of RP-03 as a promising antifungal agent, deserving further exploration in the context of Geometrium chadium infections or related fungal strains.

ADMET

A detailed examination of physicochemical properties was conducted to assess the drug-like characteristics of compounds (RP-01 to RP-10).

The molecular weight (MW) of each compound was found to be within the acceptable range for drug-like molecules, ranging from 396.46 to 545.54 g/mol. This suggests a suitable size for cellular permeability.

In terms of lipophilicity, the LogP values were favourable, ranging from 1.82 to 2.71. These values indicate a balanced partitioning between lipids and water, supporting their potential as drug candidates.

Hydrogen bond donors (HBD) and acceptors (HBA) were evaluated, and the compounds exhibited a low to moderate number of HBD (2) and HBA (3-4), maintaining a balance between solubility and binding interactions.

The topological polar surface area (TPSA) values ranged from 77.15 to 162.31, falling within the expected range for drug-like compounds. This suggests an optimal surface area for interactions with biological targets.

Rotatable bonds (RB) and the number of heavy atoms (nAH) were considered, with values ranging within acceptable limits, indicating reasonable molecular flexibility and complexity.

Molecular refractivity (MR), reflecting polarizability, ranged from 114.28 to 159.55, consistent with drug-like molecules and indicating moderate polarizability.

Structural alerts for Pan Assay Interference Compounds (PAINS) were absent, and none of the compounds violated Brenk's rules. This lack of alerts enhances confidence in the compounds, suggesting a lower likelihood of assay interference or undesirable chemical features.

Molecular docking

Among the compounds analyzed, RP-03 emerges as the most promising candidate, showcasing a remarkable docking energy of -7.4 kcal/mol, indicative of robust binding affinity. With an added Fluoro (F) group, RP-03 engages extensively with ALA(A) 183, ILE(A) 200, LYS(A)

232, and ARG(A) 182, suggesting potent activity. This highlights RP-03 as a compelling contender for further investigation and potential drug development endeavors.

Conversely, compounds like RP-01, RP-02, and RP-04 exhibit moderate docking energies ranging from -6.5 to -6.9 kcal/mol, indicating relatively weaker binding affinities compared to RP-03. RP-05 also falls within this moderate activity range, displaying interactions with ALA(A) 183, VAL(A) 236, ARG(A) 235, ILE(A) 200, and LYS(A) 232, yet lacking the pronounced potency of RP-03.

RP-09, containing a chlorine (Cl) atom, demonstrates similar moderate activity, with interactions involving VAL(A) 158 and SER(A) 257, yielding a docking energy of -6.5 kcal/mol. On the other hand, RP-10, featuring a bromine (Br) atom, shows considerable activity with a docking energy of -7.3 kcal/mol. It interacts with VAL(A) 236, MET(A) 198, ARG(A) 201, ARG(A) 235, and ARG(A) 231, indicating significant binding affinity.

While all these compounds exhibit potential interactions with target amino acids, RP-03's notably higher docking energy underscores its superiority in terms of binding strength, positioning it as the most active compound among those evaluated. However, further experimental validation would be necessary to confirm these computational findings and to comprehensively elucidate the compounds' biological activities.Less active compounds, characterized by docking energies above -6.5 kcal/mol, although these compounds demonstrate some degree of binding, their affinities are comparatively weaker, suggesting the need for optimization or consideration of alternative compounds in drug development efforts.

Overall, these docking results provide valuable insights into the potential efficacy of various compounds in interacting with the target protein. However, it is essential to interpret these findings in conjunction with experimental data and consider the biological relevance of the interactions to make informed decisions in the drug discovery process

Conclusion

This research paper presents a comprehensive exploration of a novel pyrrolo[2,3-d]pyrimidine derivative, with a particular emphasis on **RP-03**. The synthesis of the derivative followed established chemical protocols, and its structural identity was confirmed through rigorous spectroscopic techniques. Molecular docking analysis unveiled RP-03 as a standout candidate, exhibiting an exceptionally low docking score of -7.4 kcal/mol and a notably short observed

bond length of 3.02 Å. This implies a robust binding affinity between **RP -03** and the target protein associated with antimicrobial activity.

The ADMET characterization further strengthened the potential of Compound RP-04 as a lead compound for drug development. The compound displayed favourable pharmacokinetic and safety profiles, bolstering its candidacy for further investigations. Antimicrobial activity evaluations validated the biological efficacy of Compound RP 4, showcasing potent activity against a spectrum of microbial strains.

The pyrrolo[2,3-d]pyrimidine derivative, especially Compound RP-04, emerges as a promising antimicrobial candidate. Its exceptional docking score, strong binding affinity, favourable ADMET properties, and potent antimicrobial activity collectively position it as a compelling option for further development as a therapeutic agent against microbial infections. The findings from this study lay a solid foundation for future research endeavours aimed at harnessing the full therapeutic potential of this novel compound in the ongoing battle against microbial threats.

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