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Proceeding Paper

Multi-Target In Silico Evaluation of New 2-Pyrazolines as Antimicrobial Agents †

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Abstract: The world today, is being ravaged by the emergence and re-emergence of microbial infections caused by antimicrobial-resistant strains, brought about primarily by the frequent and perhaps unnecessarily using antimicrobial agents. A need therefore arises to develop new antimicrobial drugs that can combat these pathogens resistant to currently available antibiotics. This present study has adopted a multi-enzyme in silico approach in evaluating new 2-pyrazolines as antimicrobial agents, targeting and aiming to inhibit three pivotal enzymes in the bacteria's life cycle. A library of 2-pyrazolines was tailored to achieve the desired activity. The library of compounds and amoxicillin, a standard antimicrobial drug, were docked into the molecular target enzymes. They were also subjected to toxicity and drug-likeness tests, using PROTOX and swissADME respectively. A moderate toxicity profile was indicated, as more than 90% of the ligands were in ProTox class 4. The majority exhibited advantageous ADME characteristics. A significant number of them demonstrated a binding affinity for the target proteins that was stronger than both the native ligand and the binding affinity of amoxicillin. Ligands 30, 20, and 8 are the notable ones across all target enzymes. These results suggest that these novel ligands may be powerful inhibitors, particularly when it comes to interfering with the formation of bacterial cell walls, folic acid, and nucleotide metabolism. Additional in vivo and in vitro research is required to confirm these results and evaluate their therapeutic potential.

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1. Introduction

Life is impacted by microorganisms. These microbes can either be non-pathogenic, assisting ecology, or pathogenic, causing harm to life (Boudou et al., 2023). Pathogens are primarily responsible for infectious diseases by exploiting a host's immune system. However, since Fleming, A. (1929) reported his accidental discovery of Penicillin, their first combatant, other drugs classed antibiotics (i.e., ciprofloxacin, ampicillin, sulfamethazine) have been adopted in clinical use (Mansuso et al., 2023).

Despite the immense progress made in the discovery of drugs to treat microbial infections, antimicrobial resistance (AMR) still poses a significant challenge to this day (El-Naggar et al., 2023). Hence these AMRs or emerging and re-emerging microbial diseases continue to compel scientists to search for newer antibiotics with exceptional therapeutic safety and efficacy (Aslam et al., 2018; Chen and Bajorath, 2023).

Among the numerous classes of compounds being investigated, (Brown, 2018; Ebenezer et al., 2022) recognize pyrazoline derivatives as fascinating chemical entities with diverse biological activities and great promise for drug development. (Ismail et al., 2021) also pointed out that over a decade ago, published papers reported 2-pyrazolines as pharmacophore scaffolds with paramount pharmacological potency. A number of

them have been identified to possess biological activities, such as antimicrobial, antitumor, anticancer, etc.

(Liu et al., 2023) and (Smith, T. J., & Zhang, H. 2024) emphasized that Glucosamin-6-Phosphase synthase, GlcN-6-P is a crucial enzyme in the synthesis of peptidoglycan, a primary component of the bacterial cell wall. Its inhibition can prevent cell wall synthesis and subsequently, bacterial cell death. Likewise, inhibition of the bacteria enzyme Dihydrofolate Reductase, DHFR which is responsible for the synthesis of folic acid can equally be detrimental to the bacteria (Silver, 2022). Similarly, Thymidine Phosphorylase, TP which is responsible for DNA synthesis and repair can be targeted so that antibiotics can disrupt the nucleotide metabolism in bacteria, leading to impaired DNA synthesis and ultimately bacterial cell death (Cui et al., 2023).

2. Methods

In creating the 2D structures of the library of compounds, Chem Draw was used. Spartan 14 was used to convert the structures to 3D, minimize their energies, and save them as .mol2 files. The target enzymes Glucosamine-6-Phosphase synthase, Thymidine Phosphorylase, and Dihydrofolate Reductase with respective PDB IDs: 1MOQ, 4EAD, and 7MQP were downloaded from rcsb.org. Deletion of solvent molecules and co-crystallized ligands was done using UCSF Chimera. Subsequently, UCSF Chimera was used for the dock prep of the receptor, ligands; including native ligand and amoxicillin. AutoDock was used to change the receptor and ligands' file format to pdbqt and to set the grid box. Binding energies were determined using Cygwin Terminal. And postdock analysis was done using UCSF Chimera and Discovery Studio 2020.

3. Results and Discussion

3.1. Drug-Likeness and Bioavailability

None of the ligands has more than one violation of Lipinski's rule, with most of them complying fully with it, demonstrating desired drug-likeness properties. The ligands' Bioavailability score is 0.55 indicating they can be orally administered.

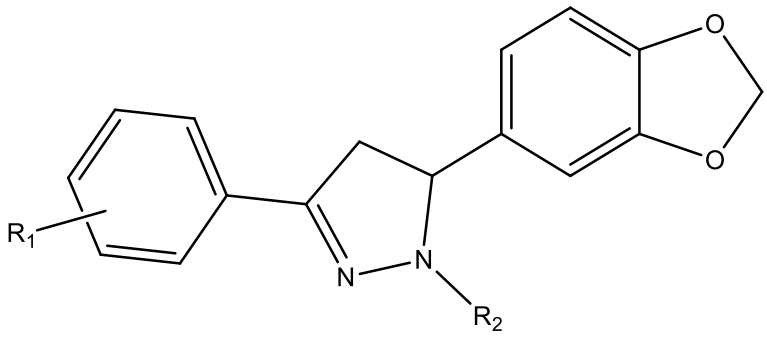

Figure 1. General structure of the library of compounds.

3.2. Synthetic Accessibility

Their synthetic availability scores are well within the range of 3.43 to 3.93 which underscores their plausible synthesis in the laboratory with ease.

3.3. LogP and Water Solubility

Their logP value ranges from 2.35 to 4.52, and most of them are moderately soluble or higher in water, which means they exhibit the crucial balance between hydrophilicity and lipophilicity, necessary for drug absorption within the biological entity.

Table 1. Show extracts from SwissADME output.

Lig	Formula	MW	H-Acceptors	H-Bond Donors	Water Solubility	XLOGP3	Bioavailability Score	Synthetic Accessibility
2	$C_{18}H_{17}N_3O_3$	323.35	4	1	Soluble	2.31	0.55	3.57
3	$C_{19}H_{18}N_2O_3$	322.36	4	0	Soluble	2.88	0.55	3.54
5	$C_{23}H_{20}N_2O_2$	356.42	3	0	Moderately soluble	5.2	0.55	3.7
7	$C_{19}H_{19}N_3O_3$	337.37	4	1	Soluble	2.67	0.55	3.69
8	$C_{20}H_{20}N_2O_3$	336.38	4	0	Soluble	3.24	0.55	3.66
9	$C_{20}H_{20}N_2O_4$	352.38	5	0	Moderately soluble	3.65	0.55	3.8
10	$C_{24}H_{22}N_2O_2$	370.44	3	0	Poorly soluble	5.57	0.55	3.82
12	$C_{17}H_{14}ClN_3O_3$	343.76	4	1	Soluble	2.57	0.55	3.46
13	$C_{18}H_{15}ClN_2O_3$	342.78	4	0	Soluble	3.14	0.55	3.43
15	$C_{22}H_{17}ClN_2O_2$	376.84	3	0	Moderately soluble	5.46	0.55	3.6
17	$C_{17}H_{13}Cl_2N_3O_3$	378.21	4	1	Moderately soluble	3.2	0.55	3.48
18	$C_{18}H_{14}Cl_2N_2O_3$	377.22	4	0	Moderately soluble	3.77	0.55	3.45
19	$C_{18}H_{14}Cl_2N_2O_4$	393.22	5	0	Moderately soluble	4.17	0.55	3.58
20	$C_{22}H_{16}Cl_2N_2O_2$	411.28	3	0	Poorly soluble	6.09	0.55	3.61
25	$C_{22}H_{18}N_2O_3$	358.39	4	1	Moderately soluble	4.48	0.55	3.64
27	$C_{18}H_{16}N_2O_5$	340.33	6	2	Soluble	1.8	0.55	3.53
29	$C_{18}H_{16}N_2O_6$	356.33	7	2	Soluble	2.21	0.55	3.67
30	$C_{22}H_{18}N_2O_4$	374.39	5	2	Moderately soluble	4.13	0.55	3.7
35	$C_{23}H_{20}N_2O_3$	372.42	4	0	Moderately soluble	4.81	0.55	3.75
40	C24H22N2O4	402.44	5	0	Moderately soluble	4.78	0.55	3.93

3.4. Binding Affinity and Ligand-Receptor Interaction

The binding affinity data from Table 2 indicates that several ligands have a stronger binding affinity to the target enzymes compared to the native ligand and amoxicillin. Ligands 8, 20, and 30 notably showed the highest binding affinities across the three targets. These binding energies suggest that these ligands have a strong potential to inhibit the target enzymes more effectively than the current standard drug, amoxicillin. Data indicates that certain substituents, particularly CH_3 and Cl groups, significantly enhance binding, which is consistent with findings in existing literature.

Table 2. Showing ligands' substituents, report, protox class, and the binding energies across the three targets.

LIGANDS	SUBSTITUENTS		REPORT	PROTOX CLASS	BINDING ENERGY		
	R_1	R_2			1MOQ	4EAD	7MQP
NATIVE LIGAND					−7.5	−6.8	−9.6
AMOXYCILLIN					−7.9	−8.6	−6.8
2	4-CH_3	ONH_2	REPORTED	4	−8.9	−8.7	−9.2
3	4-CH_3	$(CO)CH_3$	REPORTED	4	−8.9	−8.7	−9.2

5	4-CH_3	C_6H_5	NEW	4	−7.9	−9.5	−9.9
7	3,5-CH_3	ONH_2	NEW	4	−9.1	−8.9	−9.3
8	3,5-CH_3	$(CO)CH_3$	NEW	4	−9.2	−9.0	−9.8
9	3,5-CH_3	$(CO)OCH_3$	NEW	4	−8.7	−8.9	−9.1
10	3,5-CH_3	C_6H_5	NEW	4	−7.9	−9.6	−9.2
12	4-Cl	ONH_2	NEW	4	−8.8	−8.2	−9.2
13	4-Cl	$(CO)CH_3$	NEW	4	−8.8	−8.6	−9.1
15	4-Cl	C_6H_5	NEW	4	−7.9	−9.3	−9.8
17	3,5-Cl	ONH_2	NEW	4	−8.9	−8.5	−9.2
18	3,5-Cl	$(CO)CH_3$	NEW	4	−9.0	−8.7	−9.5
19	3,5-Cl	$(CO)OCH_3$	NEW	4	−8.7	−8.7	−9.5
20	3,5-Cl	C_6H_5	NEW	4	−7.8	−9.4	−10.1
25	4-OH	C_6H_5	NEW	4	−7.6	−9.1	−9.2
27	3,5-OH	ONH_2	NEW	4	−8.6	−8.7	−9.5
29	3,5-OH	$(CO)OCH_3$	NEW	4	−8.2	−8.7	−9.3
30	3,5-OH	C_6H_5	NEW	4	−8.2	−9.2	−10.2
35	4-$(CO)OCH_3$	C_6H_5	NEW	4	−7.5	−9.1	−8.6
40	3,5-$(CO)OCH_3$	C_6H_5	NEW	4	−7.8	−8.9	−8.5

4. Conclusions

The study demonstrates that 2-pyrazoline derivatives show promising potential as antimicrobial agents. These compounds exhibited strong binding affinities to target enzymes, surpassing the standard drug, amoxicillin. The ligands also displayed favorable drug-likeness and bioavailability properties, indicating their potential for oral administration. However, further in vivo and in vitro studies are necessary to confirm these findings and evaluate their therapeutic potential.

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