Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Design and Development of Some Pyrimidine Analogues as an Anthelmintic Agent

Smita J. Pawar*, Rahul Dorugade, Amol P. Kale, Dhanashri Zope

PDEA's Department of Pharmaceutical Chemistry, Seth Govind Raghunath Sable College of Pharmacy, Pune, Maharashtra, India.

ARTICLE INFO

Article history:

Received: 07 September, 2023

Revised: 26 December, 2023

Accepted: 30 December, 2023

Published: 30 January, 2024

Keywords:

Anthelmintics, Pyrimidine, Aldehydes, Microparasitic diseases, Molecular docking, Earthworms, Nematode, Trematode, Cestode.

DOI:

10.25004/IJPSDR.2024.160102

ABSTRACT

Anthelmintic drugs are used to treat parasitic infections and acknowledge the challenge in developing effective anthelmintics due to the significant homology between parasites and their hosts. Despite the existence of various anthelmintic drugs in the market, the emergence of drug resistance necessitates the continuous development of new and more efficient drugs to combat parasitic infections. The development of anthelmintic drugs involves a multi-faceted process that aims to create effective treatments against parasitic infections. Pyrimidines have been investigated for their potential anthelmintic activity. Therefore, the present study involves the synthesis of derivatives based on pyrimidine. The series of 4-amino-2-hydroxy-6-substituted phenyl pyrimidine-5-carbonitrile was synthesized by treating substituted benzaldehyde with malononitrile and urea. The synthesized compounds were subsequently screened for their anthelmintic efficacy. The chemical structures were confirmed by infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The anthelmintic activity was performed on the adult Indian earthworm *Pheretima posthuma*. *In-vitro* anthelmintic studies revealed that, among all the screened compounds, compound 1f demonstrated significant or appreciable anthelmintic properties. Molecular docking was conducted on quinol-fumarate reductase to elucidate potential interactions between the newly synthesized pyrimidine derivatives and the specific cavity of the quinol-fumarate reductase enzyme. This analysis aimed to gain insights into the binding interactions and the possible mechanism of action of the synthesized compounds.

Introduction

Anthelmintics are pharmaceuticals used to treat and prevent microparasitic diseases like parasitic nematode, trematode, and cestode infections in humans as well as animals.[1,2] Our capacity to disrupt the life cycles of these parasites has been hampered by the lack of efficient vaccinations and poor sanitation in some endemic places.[3,4] High costs and small global markets for antiparasitic medications and chemicals are barriers to the development of novel anthelmintics. For animals and people, the expenses of developing new medications are expected to be $400 million and over $800 million, respectively. According to estimates, there is a $12 billion global market for antiparasitic medications and chemicals for plant pathogens, a $11 billion market for livestock and companion animals, and a $0.5 billion industry for human health.[5] The market offers a wide range of anthelmintics or anthelmintic medications to eliminate such parasitic worms from the body by either killing or stunning them without significantly harming the host cell.[6, 7] Due to the persistence of the recurring establishment of resistance, well-known marketing pyrimidine-derived medicines pyrantel and morantel are frequently employed as anthelmintic medications with broad-spectrum activity and high cure rates.[8] But utilizing currently available medications, some infectious disorders that are already present cannot be entirely cured in humans.[9] Parasites can develop resistance to anthelmintic drugs.[10]

However, 4- (1H-benzimidazol-2-yl)-6- (2-chloroquinolin-3-yl) pyrimidine-2-amine derivatives showed good anthelmintic activity against *Pheretima posthuma* using albendazole as a standard.[11] The presence of

***Corresponding Author:** Dr. Smita J. Pawar

Address: PDEA's Department of Pharmaceutical Chemistry, Seth Govind Raghunath Sable College of Pharmacy, Pune, Maharashtra, India.

Email ✉: sjpawar4477@gmail.com

Tel.: +91-9970705513

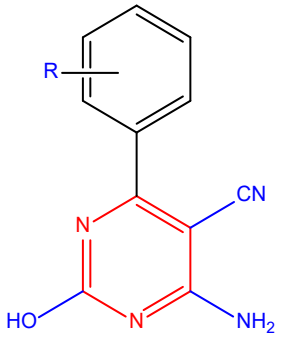
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

various substitutions such as hydroxy, methoxy, and nitro groups on the aromatic ring of 4,6-disubstituted pyrimidine-2-one derivatives provides significant activity.[12] 2-methyl/propyl4 (2 (substitutedbenzylidene) hydrazinyl)5,6,7,8 tetrahydrobenzo[4,5]thieno[2,3] pyrimidines were synthesized and evaluated for anthelminthic activity against adult Indian earthworms (*P. posthuma*). Compounds with propyl group at the 2-position of the thieno[2,3-d] pyrimidine scaffold found to be favorable for the anthelminthic activity of the exhibiting mean paralytic time of 2.5, 2.81 minutes and helminthicidal time of 21, 20.03 minutes, respectively, at the same concentration (100 µg/ml, 259.7 µM).[13] 2- (N, N-dimethyl guanidinyl)-4,6-diaryl pyrimidines exhibited good anthelmintic activity inducing paralysis in 36 to 48 minutes and death in 40 to 51 minutes as compared to the standard drug albendazole.[14] Anthelmintics, a category of antiparasitic medications, often present significant challenges for individuals infected with parasites. Anti-parasitic drugs effectively eliminate parasitic worms without harming the host. David I Ugwu *et al.* (2018) synthesized new pyrimidine analogs bearing carboxamide and sulphonamide moieties and tested them for anthelmintic activity. Among all compounds (44) exhibited satisfactory anthelmintic activity (Paralyzing time: 37, 26 and 19 minutes) compared to that of albendazole (Paralyzing time: 28, 20 and 10 minutes). The increased activity could potentially be attributed to the existence of both the nitrophenyl and pyrimidine scaffold.[15] Based on literature findings suggesting the potential anthelmintic properties of pyrimidine, we undertook the design and synthesis of 4-Amino-2-hydroxy-6-substitutedphenylpyrimidine-5-carbonitrile and conducted an evaluation of its efficacy against parasites. The chemical structures were confirmed by infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR). The molecular docking was performed on quinol-fumarate reductase to gain insight into the possible interactions between newly synthesized pyrimidine derivatives and the selected cavity of quinol-fumarate reductase enzyme.

Materials And Method

The chemicals utilized in the synthesis were all of laboratory-grade quality. The determination of melting points was carried out using an open capillary equipped with the Veego electronic apparatus (model: VMP-D). The IR spectra of the synthesized compounds were obtained using a Shimadzu 8400-S fourier-transform infrared spectroscopy (FTIR) spectrophotometer with potassium bromide. The ^{1}H-NMR spectra were recorded in $CDCl_3$ and DMSO using an NMR BRUKER 500 MHz instrument, and the chemical shift values were expressed in parts per million, referenced downfield from tetramethyl silane (TMS) as the internal standard. Thin layer chromatography (TLC) was conducted on precoated aluminum sheets (Silica gel 60F254, 6x2.5 cm) employing a solvent mixture of chloroform: ethyl acetate (4:1). The spots were visualized under ultraviolet light. To assess the purity of the synthesized compounds, Rf values were calculated for each compound using the following formula:

RF= Distance travelled by the compound/Distance travelled by the solvent front



Scheme 1: Phenyl derivative of pyrimidine-5-carbonitrile

Scheme of Synthesis

The target derivatives of pyrimidine were synthesized by treating equimolar quantity of malononitrile, sodium ethoxide and substituted benzaldehyde (Scheme 1). The mixture was acidified with glacial acetic acid and the product was separated. The detailed procedure is as follows.

Procedure for synthesis of 4-amino-2-hydroxy-6-substituted phenylpyrimidine-5-carbonitrile (1a-f)

A solution of 0.01 mole of sodium ethoxide, the equimolar quantity of malononitrile 0.01 mole of substituted benzaldehyde and 0.015 mole of urea was stirred and heated under reflux until the reaction completes. Following this, the reaction mixture was cooled by pouring it onto crushed ice, and 3 to 4 drops of glacial acetic acid were introduced. The resulting solid was separated by filtration, and dried, and the crude product was further purified by recrystallization using aqueous ethanol.

- *4-Amino-2-hydroxy-6-phenylpyrimidine-5-carbonitrile* 1a

Yield: 82%. m.p.: 179–180°C. FTIR: OH (3564.57), NH (3495.13), ArCH (3093.92), CN (2314.66), C=N (1647.27), C=C (1543.10), C-N (1219.21); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.733–7.747 Ar-CH (d, 2H), 7.726–7.730 Ar-CH (t, 1H),7.952–7.961 Ar-CH (d, 2H), 5.108 NH_2 (s, 2H), 8.559 OH (s, 1H).

- *4-Amino-6- (4-chlorophenyl)-2-hydroxypyrimidine-5-carbonitrile* 1b

Yield: 68%. m.p.: 162–164°C. FTIR: OH (3371.68), NH (3302.24), ArCH (3032.20), CN (2229.79), C=N (1581.33), C=C (1496.81), C-N (1095), C-Cl (825.56); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.723–7.737 Ar-CH (d, 2H), 7.947–7.952 Ar-CH (d, 2H), 5.108 NH_2 (s, 2H), 8.559 OH (s, 1H).

- *4-Amino-2-hydroxy-6- (4-nitroyphenyl)pyrimidine-5-carbonitrile* 1c

Yield: 78%. m.p.: 221–223°C. FTIR:OH (3363.97), NH (3209.30) ArCH (3035.91), CN (2206.64), C=N (1635.69), C=C (1519.96), N-O (1257.63), C-N (1126.47); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.615–7.723 Ar-CH (d, 2H), 7.923–7.934 Ar-CH (d, 2H), 5.108 NH_2 (s, 2H), 8.559 OH (s, 1H).

- *4-Amino-2-hydroxy-6- (4-hydroxyphenyl)pyrimidine-5-carbonitrile* 1d

Yield: 61%. m.p.: 173-175°C. FTIR:OH (3402.54), NH (3302.24), ArCH (3032.20), CN (2229.79), C=N (1566.25), C=C (1450.52), C-N (1296.21; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.723-7.737 Ar-CH (d, 2H), 7.947-7.952 Ar-CH (d, 2H), 8.455 OH (s, 1H) 5.108 NH_2 (s, 2H), 8.559 OH (s, 1H).

- *4-Amino-6- (3-bromophenyl)-2-hydroxypyrimidine-5-carbonitrile* 1e

Yield: 81%. m.p.: 178–180°C. FTIR:OH (3402.54), NH (3302.24), ArCH (3032.20), CN (2229.79), C=N (1597.11), C=C (1512.34), C-N (1242.20), C-Br (840.99); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.723–7.737 Ar-CH (d, 2H), 7.947–7.952 Ar-CH (d, 2H), 8.455 OH (s, 1H) 5.108 NH_2 (s, 2H), 8.559 OH (s, 1H).

- *4-Amino-6- (4-fluorophenyl)-2-hydroxypyrimidine-5-carbonitrile* 1f

Yield: 91%. m.p.: 226–228°C. FTIR:OH (3379.40), NH (3333.10) ArCH (3066.21),CN (2206.64), C=N (1604.83), C=C (1527.67), C-N (1350.22), C-F (1111.03); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.739–7.745 Ar-CH (d, 2H), 7.937–7.942 Ar-CH (d, 2H), 5.110 NH_2 (s, 2H), 8.549 OH (s, 1H).

Biological Evaluation

Experimental animal

The anthelmintic activity was evaluated in vitro using adult earthworms (*P. posthuma*). Earthworms were collected from moist soil and subsequently cleansed with normal saline to eliminate any fecal matter or debris adhering to their bodies. Earthworms measuring 6–8 cm in length and 0.3–0.5 cm in width were utilized for the anthelmintic activity test. The identification of the earthworms was conducted at the Department of Zoology, Waghire College, Saswad. *P. posthuma* was chosen as a model for anthelmintic activity due to its similarity in anatomy and physiology to the intestinal roundworm parasites found in humans. Before the commencement of the experiment, the earthworms underwent a washing process using normal saline. [16,17]

The inclusion criteria for the study were as follows:

- Healthy earthworms within the length range of 6–8 cm were included.
- Earthworms of the same species were selected to prevent errors in the study results.

Anthelmintic activity on P. posthuma

The anthelmintic activity was evaluated on adult Indian earthworms (*P. posthuma*) due to their physiological resemblance to the intestinal roundworm parasites found in humans. To prepare the samples, synthesized compounds (100 mg) were triturated with Tween 80 (0.5%) and distilled water, followed by stirring using a mechanical stirrer for 30 minutes. A suspension of the reference drug, albendazole, was also prepared at the same concentration using a similar method.

Three sets of *P. posthuma* of almost identical sizes (2 inches in length) were placed in 4-inch diameter petri plates containing 10 mL suspensions of the test samples and reference drug at concentrations of 50, 100, and 150 mg/mL, maintained at room temperature. Additionally, another set of *P. posthuma* was kept as a control in a 10 mL suspension of distilled water and Tween 80.

The paralyzing and death times of the earthworms were recorded, and the mean values were calculated for triplicate sets. The determination of death time involved placing the earthworms in warm water (50°C) to stimulate movement; if there was no movement, it indicated that the worm had died.

The results obtained from the test samples were compared with those treated with the standard drug, albendazole, to assess and compare the anthelmintic activity.

Molecular Docking

The molecular docking procedure involved utilizing Vlife MDS 4.6 software with the grid batch docking method.

The structures of all the compounds were initially constructed using ChemDraw Ultra 8.0 software. These structures underwent further adjustments, including the addition or removal of hydrogens. Subsequently, the geometries of these compounds were optimized utilizing the Merck molecular force field (MMFF). To explore the conformational space, various conformers of the six-compound set were generated through a systematic conformational search option.

For the receptor, the structure of the quinol-fumarate reductase protein (PDB: 3VR8) was retrieved from the protein data bank (http://www.rcsb.org). This protein structure served as the target for the molecular docking simulations.

Receptor Representation

The receptor representation involved the retrieval of the PDB file from the RCSB protein data bank. The protein initially comprised numerous side chains, which were refined to create a monomer by eliminating chains other than chain A along with ligands. Additionally, water molecules were removed from this monomeric form of the protein structure.

Hydrogens were then added to the protein molecule, and subsequent energy minimization was carried out utilizing the MMFF to optimize the structure and minimize potential energy interactions within the protein.

Generation of Conformers

The generation of conformers involved creating various 3D optimized structures for individual ligands using a systematic conformational search method. In this approach, a predefined set of rotatable bonds within the ligands was identified. For each of these bonds, the software explored all feasible conformations in relation to one another.

The optimization of the generated conformers was carried out utilizing the MMFF force field, which is a commonly used force field for molecular mechanics calculations, to refine and optimize the geometries of these conformers. This process allowed for the exploration of the different possible spatial arrangements or conformations of the ligands.

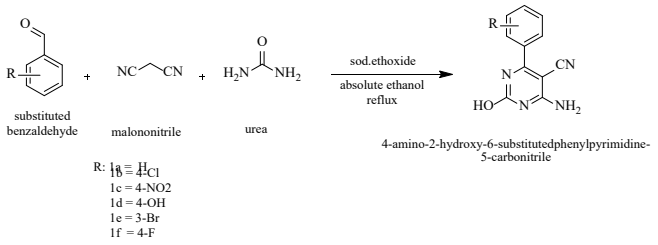
Docking

The docking procedure involved batch grid docking by generating conformers of the test compounds. Cavity number 1 was selected as the target binding site, and the docking simulations were executed utilizing the Biopredicta program. Following the docking simulations, the resulting docked conformations were subjected to scoring using a scoring function, presumably, the dock score. This score likely assessed and ranked the interactions or binding affinities of the compounds within the binding site of the enzyme. Moreover, the binding energy of the complex formed between the compound and the enzyme was also considered as an important parameter for the evaluation of the interactions and potential affinity of the compound with the enzyme.

Protein Complex Optimization

The process of protein complex optimization involves allowing the ligand to adopt a minimum energy pose within the active site cavity of the protein. This optimization is achieved by merging the docked molecule (ligand) into the protein structure. Subsequently, the ligand-enzyme complex undergoes another round of minimization, refining the arrangement and interactions between the ligand and the enzyme. During this complex optimization, particular attention is paid to observing and noting the interactions of the compounds with the amino acid residues present in the cavity of the protein. This analysis aims to understand the specific interactions between the ligand and the active site residues, shedding light on the binding mode and potential mechanisms of action within the protein's active site.

Binding energy = Optimized docked complex energy – (energy of optimized apo receptor without ligand + energy of optimized ligand



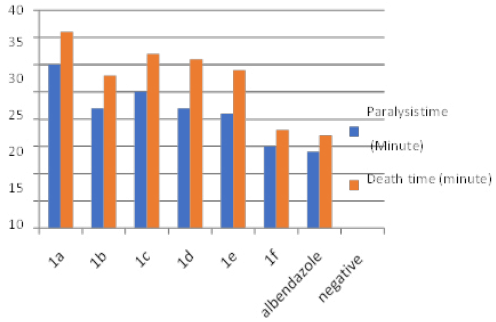
Scheme 2: Synthesis of 4-amino-2-hydroxy-6-substitutedphenylpyrimidine-5-carbonitrile (1a-f)

Results

The new derivatives, 4-Amino-2-hydroxy-6-substituted phenylpyrimidine-5-carbonitrile (1a-f), were successfully synthesized by reacting pyrimidine, malononitrile, and urea with selected benzaldehydes in absolute ethanol according to Scheme 2. The IR spectrum of synthesized compounds showed the NH stretch between 3301 to 3495 cm^{-1}. The broad stretch of hydroxyl OH was observed between 3564 to 3402 cm^{-1}

Anthelmintic activity was observed in a dose-dependent manner. Compound 1f exhibited the highest anthelmintic activity at concentrations of 50, 100, and 150 mg/mL, surpassing the standard drug albendazole (10 mg/mL). Compounds 1b, 1d, and 1e also demonstrated modest yet significant activity at a concentration of 150 mg/mL against the *P. posthuma* worm. Statistical analysis using 'Dunnett's test' with one-way ANOVA showed significant differences ($p < 0.001$) compared to both the control and standard group. Table 1, Figs 1, and 2 summarize the anthelmintic activity results.

Molecular docking was performed for the synthesized compounds using mitochondrial quinol-fumarate reductase enzyme, referencing pyrental. The interactions were compared, and satisfactory results were obtained, providing insight into the binding interactions of the synthesized compounds with the mitochondrial quinol-fumarate reductase enzyme of *P. posthuma*. Table 2 summarizes the dock score and binding energies of all compounds and the standard. Figs 3 and 4 illustrate the interactions of compound 1f and the standard, respectively.



p-value =0.00027, (p value ≤ 0.05 is significant.)

Fig. 1: Graph showing paralysis time and death time for synthesized compounds showing anthelmintic activity

Table 1: Anthelmintic activity of the synthesized compounds

S. No.	*Compounds*	*Dose (mg/mL)*	*Paralysis time (minute)*	*Death time (minute)*
			Means ± S.E.M	*Mean ± S.E.M*
1	1a	50	32 ± 0.5	38 ± 0.41
		100	31 ± 0.65	37 ± 0.32
		150	30 ± 0.45	36 ± 0.96
2	1b	50	25 ± 0.529	32 ± 0.36
		100	23 ± 0.26	31 ± 0.54
		150	22 ± 0.74	28 ± 0.32
3	1c	50	30 ± 0.763	34 ± 0.61
		100	28 ± 0.341	32 ± 0.38
		150	25 ± 0.23	32 ± 0.54
4	1d	50	27 ± 0.76	38 ± 0.8
		100	25 ± 0.95	35 ± 0.56
		150	22 ± 0.16	31 ± 0.43
5	1e	50	25 ± 0.36	35 ± 0.72
		100	23 ± 0.04	32 ± 0.56
		150	21 ± 0.69	29 ± 0.403
6	1f	50	20 ± 1.2	25 ± 0.78
		100	18 ± 0.56	22 ± 0.62
		150	15 ± 0.75	18 ± 0.12
8	Albendazole	50	17 ± 1.1	21 ± 2.1
		100	16 ± 1.2	20 ± 0.78
		150	14 ± 0.8	17 ± 0.62

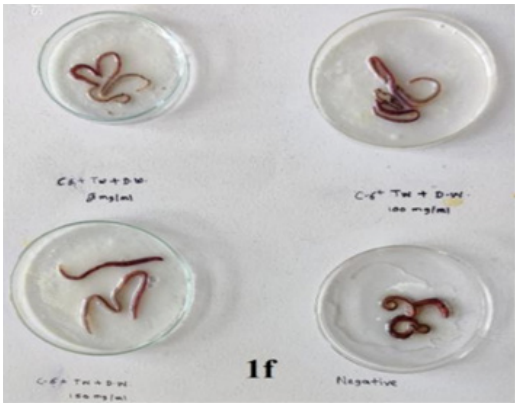


Fig. 2: Anthelmintic activity of compound 1f

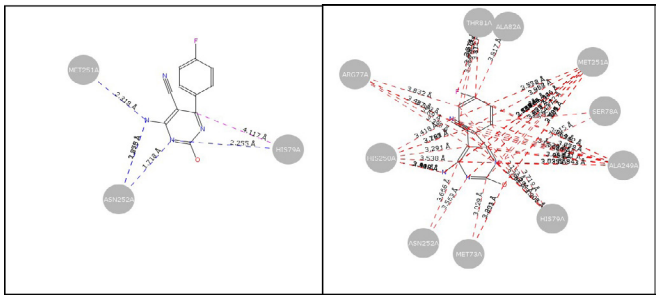


Fig. 3: Interactions of compound1fwith mitochondrial quinol-fumarate reductase enzyme

Table 2: Dock score and different binding interactions of target compounds with *Homo sapien dhfr*

Compound	*No of conformer*	*Conformer No.*	*Dock score*	*Binding energy (kcal/mol)*	*Interactions*
1a	122	C96	-3.5915	-98.83	HB-SER78A, HIS79A HP-HIS79A VDW-MET73A, ARG77A, SER78A, HIS79A, THR80A, ALA249A, HIS250A, MET251A, ASN252A
1b	122	C2	-3.2552	-21.87	HB-ALA82A HP- HIS79A VDW-MET73A, ARG77A, SER78A, HIS79A, THR81A, THR247A, ALA249A, HIS250A, MET251A
1c	122	C10	-3.4920	-68.70	HB- HIS79A, ASN252A HP-HIS79A, MET73A, ARG77A, SER78A, HIS79A, ALA249A, HIS250A, MET251A, ASN252A
1d	122	C122	-3.8572	-24.68	HB-HIS79A, ASN252A, ASN252A HP-HIS79A VDW-MET73A, ARG77A, HIS79A, ALA249A, HIS250A, MET251A, ASN252A
1e	122	C92	-3.7800	-56.59	HB-ALA82A HP- HIS79A VDW-MET73A, ARG77A, SER78A, HIS79A, THR81A, ALA82A, ALA249A, HIS250A, MET251A,
1f	122	C28	-3.8958	-96.83	HB- ASN252A, MET251A, HIS79A HP-HIS79A VDW-MET73A, ARG77A, SER78A, HIS79A, THR80A, ALA249A, HIS250A, MET251A, ASN252A
Pyrantel	130	C57	-3.4009	-24.62	HB-HIS79A HP-LYS72A, MET73A, SER78A, ALA249A VDW-LYS72A, ARG77A, SER78A, HIS79A, THR248A, ALA249A, HIS250A, MET251A

HB-Hydrogen bond interaction, HP-Hydrophobic interaction, VDW-Vander Waal's interaction.

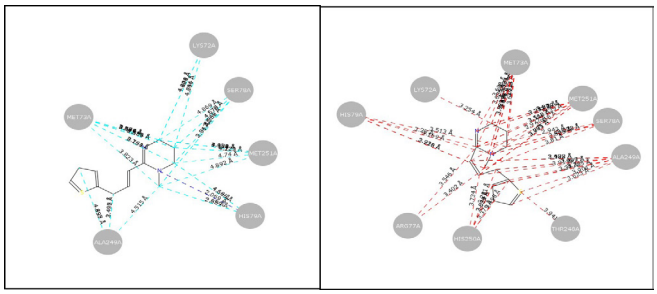


Fig. 4: The interaction of pyrantel with mitochondrial quinol-fumarate reductase enzyme.

Discussion

All synthesized compounds underwent thorough characterization using IR and ^{1}H-NMR spectroscopy. The biological data, expressed as mean ± SEM, was statistically analyzed using One-way ANOVA. The results indicated that the test compounds exhibited significant activity comparable to the standard drug, Albendazole. Among the series of synthesized compounds, compound 1f, characterized by fluoro substitution on the phenyl ring of the pyrimidine structure, demonstrated promising results. Its activity was notably similar to that of the standard drug, albendazole. This suggests the potential of compound 1f as an effective anthelmintic agent. Comparative molecular docking studies were conducted to assess the interactions between the synthesized compounds (1a-f) and the reference drug pyrantel with the mitochondrial quinol-fumarate reductase enzyme. Compound 1f, distinguished by fluoro substitution on the phenyl ring of the pyrimidine structure, displayed the highest dock score compared to the standard drug, pyrantel. These interactions were characterized by hydrophobic interactions, indicating the potential contribution of aromatic rings to the compounds' anthelmintic activity. Additionally, strong hydrogen bonding further highlighted the high affinity of compound 1f with the mitochondrial quinol-fumarate reductase enzyme.

Overall, the study suggests that compound 1f, specifically with fluoro substitution on the phenyl ring of the pyrimidine

structure, holds promise as a potent anthelmintic agent. Its interactions with the target enzyme further support its potential as a candidate for further investigation and development in the field of anthelmintic therapy.

Conclusion

The synthesized series of 4-amino-2-hydroxy-6-substituted phenyl pyrimidine-5-carbonitrile derivatives underwent in-vitro evaluation for their pharmacological activity. Among all the synthesized compounds, compound 1f demonstrated significant anthelmintic properties, showing promising activity in this study. Comparative molecular docking studies were conducted to analyze the interactions between the synthesized compounds and the standard drug, pyrantel, using the mitochondrial quinol-fumarate reductase enzyme. Notably, compound 1f, distinguished by fluoro substitution on the phenyl ring of the pyrimidine structure, exhibited the highest dock score compared to the standard drug pyrantel. These docking results aligned with the observed biological activity, indicating a correlation between the docking predictions and the compounds' actual anthelmintic properties.

In conclusion, the study highlights compound 1f, with its fluoro substitution on the phenyl ring of the pyrimidine structure, as a promising candidate with appreciable anthelmintic activity. The correlation observed between the results of molecular docking studies and biological evaluations strengthens the potential of compound 1f as a significant lead compound warranting further exploration and development in the realm of anthelmintic research and drug discovery.

Acknowledgement

The authors gratefully acknowledge the support received from Pune district Education Association's Seth Govind Raghunath Sable College of Pharmacy, Saswad (Maharashtra) 412301.

References

1. Nixon SA, Welz C, Woods DJ, Costa-Junior L, Zamanian M, Martin RJ. Where are all the Anthelmintics? Challenges and Opportunities on the Path to New Anthelmintics. Int J Parasitol Drugs Drug Resist. 2020; 14: 8–16. Available from: doi: 10.1016/j.ijpddr.2020.07.001
2. Abongwa M, Martin RJ, Robertson AP. A Brief Review on the Mode of Action of Antinematodal Drugs. Acta Vet (Beogr). 2017; 67(2):137–152. Available from: doi: 10.1515/acve-2017-0013
3. Hu Y, Ellis BL, Yiu YY, Miller MM, Urban JF, Shi LZ. An Extensive Comparison of the Effect of Anthelmintic Classes on Diverse Nematodes. Plos One. 2013; 8(7):70702. Available from: DOI: 10.1371/journal.pone.0070702
4. Martin RJ. Modes of action of anthelmintic drugs. Vet J. 1997; 154(1):11–34. doi.org/10.1016/S1090-0233(05)80005-X
5. Shelke PS, Jagtap PN, Tanpure PR. *In-vitro* Anthelmintic Activity of *Boswellia serrata* and *Aloe barbadensis* Extracts on *Pheretima posthuma*: Indian earthworm. Int J Res Med Sci. 2020; 8(5):1843. DOI: https://doi.org/10.18203/2320-6012.ijrms20201939
6. Mottier L, Alvarez L, Ceballos L, Lanusse C. Drug Transport Mechanisms in Helminth Parasites: Passive Diffusion of Benzimidazole Anthelmintics. Exp Parasitol 2006; 113(1): 49-57. DOI: 10.1016/j.exppara.2005.12.004
7. Shruthi N, Poojary B, Kumar V, Bhat M, Joshi H, Revanasiddappa BC. Synthesis, molecular properties and evaluation of anthelmintic activity of new thiazolopyrimidine derivatives. J Chem Pharm Res. 2015; 7(6):181-191.
8. Dixit A, Tanaka A, Greer JM, Donnelly S. Novel Therapeutics for Multiple Sclerosis Designed by Parasitic Worms. Int J Mol Sci. 2017; 18(10):2141. doi: 10.3390/ijms18102141
9. Yadav P, Singh R. A Review on Anthelmintic Drugs and their Future Scope. Int J Pharm Pharm Sci. 2011; 3(3):17-21.
10. Madawali IM, Das K, Gaviraj EN, Navanath V, Shivakumar KB. Synthesis and Evaluation of 4-(1H-Benzimidazol-2-Yl)-6-(2 Chloroquinolin-3- Yl) Pyrimidin-2-Amines as Potent Anthelmintic Agents. J Chem Pharm Res. 2019; 10(11):76–83.
11. Sudha Rani K, Lakshmi Durga J, Srilatha M, Sravani M, Sunand V. Synthesis, Characterization, Anthelmintic and *In-silico* Evaluation of 4,6-Disubstituted Pyrimidine-2- One Derivatives. Pharma Chem. 2018; 10(8):57–61.
12. Chitikina SS, Buddiga P, Deb PK, Mailavaram RP, Venugopala KN, Nair AB. Synthesis and Anthelmintic Activity of some Novel (E)-2-methyl/propyl-4-(2- (substitutedbenzylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidines. Med Chem Res. 2020; 29(9):1600–10. doi.org/10.1007/s00044-020-02586-5
13. Kumarachari R, Peta S, Surur A, Mekonnen Y. Synthesis, Characterization and *In-silico* Biological Activity of Some 2-(N,N-dimethylguanidinyl)-4,6-diarylpyrimidines. J Pharm Bioallied Sci. 2016; 8(3):181-187. DOI: 10.4103/0975-7406.171678
14. Mohanram I, Meshram J. Design, Synthesis, and Evaluation of Isoniazid Derivatives Acting as Potent Anti-Inflammatory and Anthelmintic Agents via Betti Reaction. Med Chem Res. 2014; 23:939–947. DOI:10.1007/s00044-013-0693-2
15. Bolade G, Adeyemi AA. Anthelmintic Activities of Three Medicinal Plants from Nigeria. Fitoterapia. 2008; 79(3): 223-225. DOI: 10.1016/j.fitote.2007.11.023
16. Shaikh A, Meshram J. Novel 1,3,4-Oxadiazole Derivatives of Dihydropyrimidinones:Synthesis, Anti-Inflammatory, Anthelmintic, and Antibacterial Activity Evaluation. J Hetero Chem. 2015; 53(4): 1176-1182. doi.org/10.1002/jhet.2377
17. Deodhar MN, Khopade PL, Varat MG. Sulfonamide Based β -Carbonic Anhydrase Inhibitors: 2D QSAR Study. Med Chem. 2013; 1–8. Available from: doi.org.10.1155/2013/107840

HOW TO CITE THIS ARTICLE: Pawar SJ, Dorugade R, Kale AP, Zope D. Design and Development of Some Pyrimidine Analogues as an Anthelmintic Agent. Int. J. Pharm. Sci. Drug Res. 2024;16(1):9-15. **DOI:** 10.25004/IJPSDR.2024.160102