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Research Article

SYNTHESIS OF NOVEL ANTIMICROBIAL DERIVATIVES OF 3-SUBSTITUTED PYRROLIDINE2, 5-DIONES USING PHARMACOPHORE HYBRID APPROACH: PART-I

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ABSTRACT

Objective: A series of substituted benzo[*d*]thiazol-2yl- 3- substituted pyrrolidine 2,5-dione compounds, P(1-18) were synthesized and evaluated for their antimicrobial activity against gram positive, gram negative bacteria and fungi.

Methods: Titled compounds were synthesized by fusion reaction. The minimum inhibitory concentration was determined using two fold dilution method. The batch grid docking was performed to analyze the probable interactions of synthesized compounds with DNA gyrase.

Results: The compound, P14 was found to be active against *E. coli* and *S. aureus* at 3.125 and 6.25µg/ml MIC respectively and compound P15 was found to be active against *S. epidermis* and *E. coli* at 6.25 and 3.125 µg/ml MIC respectively. The compound P6, P12, and P18 were found to possess MIC at 6.25 and 12.5µg/ml.

Conclusion: Though, the relationship between the activities shown by these compounds in, *in vivo* and *in vitro* study is still to be established, these results suggest the suitability of the designed molecular framework as a potential anti-microbial lead.

Keywords: Benzothiazole, Antimicrobial activity, Pyrrolidine-2, 5-dione.

INTRODUCTION

The irrational use of antimicrobial drugs and their combinations for last two decades has been major concern for increasing drug resistance to several microorganisms, especially for Gram positive bacteria [1]. Emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens has been one of the challenges in the management of infectious diseases in recent times [2]. Therefore, there has been always need for development of newer antibacterial and antifungal agents with mechanism of action possibly different from that of existing antimicrobial agents [3]. Benzothiazole derivatives are of considerable interest due to their important biological properties.

The substituted benzothiazoles have been associated with antitumour, antimicrobial [4] and antifungal [5] activities. Literature survey revealed that synthetic cyclic imides, such as succinimide, phthalimide, maleimide possess structural features, which confer potential antibacterial, antifungal, analgesic and antitumor activity. Pyrrolidine-2, 5-dione derivatives have been screened for its antibacterial activity [6]. Pyrrolidine-2,5-diones synthesized by microwave assisted synthesis method have shown to posses anti-proliferative and antimicrobial potency [7]. In view of these findings, the rational approach to lead discovery has prompted a better insight in developing small molecule, substituted benzo[*d*]thiazol-2yl-3-substituted pyrrolidine-2,5-dione and screening for antimicrobial potential. DNA gyrase is a major bacterial protein that is involved in replication & transcription and catalyzes the negative supercoiling of bacterial circular DNA. The DNA gyrase is a known target for antibacterial agents since its blocking induces bacterial death. Hence, the studies are further extended to check probable interactions with this mostly preferred bacterial target.

MATERIALS AND METHODS

Chemistry

The titled compounds were synthesized as outlined in scheme-1. The starting compounds 6-substituted 2- amino benzothiazoles B (1-6) were prepared using previously reported method [8]. Then, substituted benzo[d]thiazol-2yl-3- substituted pyrrolidine2,5-dione was obtained on controlled heating of 2-amino 6- substituted benzothiazole with 3-substituted succinic anhydride by simple fusion.

Experimental

All the chemicals used in the synthesis were of laboratory grade. Melting point was determined in open capillary on Veego (Model: VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FTIR spectrophotometer using potassium bromide.

The 1H NMR spectra were recorded in CDCl3 using Varian-Mercury 300MHz spectrometer and chemical shifts are given in units as parts per million, downfield from tetra methyl silane (TMS) as an internal standard.

To monitor the reactions, as well as to establish the identity and purity of reactants and products, thin layer chromatography was performed on precoated aluminum sheets using chloroform: methanol solvent system. The spots were visualized under ultra- violet light.

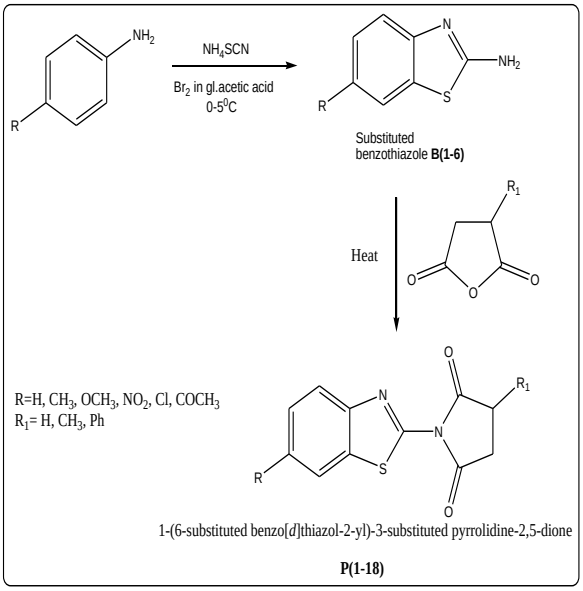
General procedure for synthesis of 2-amino benzothiazole B (1-6)

Aniline (0.078mol) was taken in a 250 ml round bottom flask; ammonium thiocyanate (0.156mol) and 100 ml acetic acid were added to it. Bromine solution (0.02mol) in acetic acid was added to the reaction mixture till an orange-yellow color appeared. The temperature of reaction mixture was maintained below 5^0C till the addition of bromine solution.

The slurry was kept overnight (20 hrs) with constant stirring. Then precipitate was dissolved in water (200 ml), filtered to remove any undissolved matter and was basified with concentrated ammonia solution. The precipitate obtained **B (1-6)** was filtered, washed with water, dried and recrystallized using ethanol and water mixture (70:30) [8, 9, 10].

General procedure for synthesis of 6-substituted benzo[*d*]thiazol-2yl-3-substituted pyrrolidine-2, 5-dione P (1-18)

0.15 mol of succinic anhydride and 0.1 mol of 6- substituted 2-amino benzothiazole **B (1-18)** were taken in a beaker heated at 110^0 C. The contents of reaction were mixed occasionally during the first 10 minutes and reaction continued till there was complete fusion. The mixture was kept undisturbed for 5 minutes, till the liquid mass solidifies. The solid mass was then suspended in water to remove unreacted succinic anhydride. The solid obtained was filtered, dried and recrystallized from 50% ethanol [11].



Scheme 1: Synthetic route for target compound

Biology

The synthesized compounds were evaluated for their antimicrobial potential against gram positive, gram negative bacteria and fungi by in vitro determination of minimal inhibitory concentrations (MICs). Antibacterial activity of synthesized compounds was evaluated against three gram positive (*Staphylococcus aureus* ATCC 9144, *Bacillus subtillis* ATCC 6633, *S. epidermis* ATCC 12228) and three gram negative bacteria (*E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *S. typhi)* using ciprofloxacin as standard antibacterial agent. Antifungal activity was evaluated against *Candida albicans* ATCC 10231 *and Aspergillus niger* ATCC 10594 using fluconazole as standard.

Minimal Inhibitory Concentration (MICs)

The double dilution method was used for the determination of antibacterial and antifungal activity. Nutrient broth was prepared & added in microtiter plates except first well in which inoculum was not added and taken it as negative control. Stock solutions of test compounds were prepared in DMSO at concentration of 200 µg/ml followed by two fold dilution at concentrations of (100, 50, 25….3.125µg/ml).

The inoculums were added to the other wells with test antimicrobial compounds ranging from 100, 50, 25….3.125µg/ml. The micro titer plates were then incubated at 37°C for 48 hrs and minimal inhibitory concentration were measured for the growth in the form of turbidity.

Docking Studies

The docking process was carried out to analyze the possible interaction between newly synthesized compounds and the selected cavity of DNA gyrase enzyme. The high-resolution (2.30 A°) X-ray structure of DNA gyrase complexed with pyrazolthiazole (PDBid code 3G75) was imported into Vlife 3.5 MDS, and the ligand was extracted to leave a cavity. The protein was a dimer which was converted in to monomer by deleting one-chain. Water molecules were removed from the monomer.

The hydrogens were added in the protein molecule and energy was minimized using Merck Molecular Force Field (MMFF). The structure of pyrazolthiazole extracted from the 3G75 protein was energy minimized using MMFF. The conformers of pyrazolthiazole, were generated conformation and docked back to the corresponding binding pocket to determine the ability of Biopredicta tool to reproduce the orientation and position of the inhibitor observed in the crystal structure. The docked conformations were further scored using dock score. The interaction between synthesized compounds and DNA gyrase pocket was recorded.

RESULTS AND DISCUSSION

The compounds **B (1-6)** were prepared as per reported method from *para*-substituted anilines by stirring with ammonium thiocynate and bromine in acetic acid. The reaction mainly involves formation of intermediate phenylthiourea and cyclisation of which affords 6-substituted 2-amino benzothiazole. The target compounds **P (1-18)** were synthesized by the route indicated in scheme-1. The compounds were prepared by heating 2-amino benzothiazole with substituted succinic anhydride.

^{1}H NMR, IR and mass spectral studies were carried out to confirm the structures of synthesized compounds and spectral data found to be consistent with the target structures. The solid state spectra (KBr,cm^{-1}) of P **(1-18)** revealed characteristic aromatic C-H stretch between 3027-3132 cm^{-1}. The C=N group present in the benzothiazole ring revealed peaks at 1600-1500 cm^{-1} while the carbonyl (C=O) peak of pyrrolidine-2,5-dione is seen at 1780-1700 cm^{-1}. The IR spectra of target compounds showed the absence of asymmetric strong band for amino group between 3490-3300 cm^{-1} of substituted 2-amino benzothiazole. The absence of broad band of succinic anhydride at 3050-2900 cm^{-1} also confirms the structure.

The ^{1}H NMR spectrum displayed singlet at around 5.38ppm for the free NH_2 proton of substituted 2-amino benzothiazole. The aromatic protons were seen as multiplet signals at 7.43-8.02ppm. Disappearance of NH_2 signal proved the formation of target pyrrolidine-2,5-dione. This was further confirmed with appearance of sharp singlet for CH_2-CH_2 protons of pyrrolidine-2,5-dione at around 3.01ppm. The substituent at 3-position in pyrrolidine-2,5-dione introduces asymmetric center, so proton at 3 position (CH proton) showed multiplets at 3.79ppm. The doublets of doublet signals were observed at around 2.59, 2.66ppm for CH_2 of substituted pyrrolidine-2,5-dione ring. Mass spectra were found in agreement with the chemical structures of newly synthesized compound. The physical and spectral data of P (1-18) compounds is given in **Table 1 and 2**, respectively.

Antimicrobial activity

The minimum inhibitory concentration of **P(1-18)** compounds were determined using double dilution method. The compound **P15** with chlorine on benzothiazole ring and phenyl group on pyrrolidine-2,5-dione ring was appeared with potential inhibitory activity against *S. epidermis* and *E.coli* bacteria at MIC of 6.25 and 3.125 µg/ml. The compound **P6** with methyl group on benzothiazole ring and phenyl group on pyrrolidine-2,5-dione showed antibacterial activity at 6.25 µg/ml. In addition the above mentioned compounds also found to contribute highest inhibition of S*. aureus* at concentration of 25µg/ml. The compounds **P12, P14** and **P18** were found to be active against *S. aureus* at 12.5 µg/ml. The synthesized compound showed good potency against *C. albicans and A. niger*. The compound **P8** found to possess comparable antifungal activity against *C. albicans* at 6.25µg/ml. The compound **P3** was found to be active against *S.epidermis* at 12.5 µg/ml. It was observed from the data that phenyl and methyl substituted pyrrolidine-2,5-dione derivatives were found to be active as compared to un-substituted derivatives **(Table3).**

Docking

To predict the probable interactions of newly synthesized compounds with active site of DNA gyrase enzyme, docking studies were carried out using MDS V-life 3.5 software. The binding score, hydrophobic interactions, Van der Waal interactions and hydrogen bonds formed with surrounding amino acids were used to predict the binding modes, binding affinities and orientation of docked compounds in the active sites of DNA gyrase enzyme. The active site (cavity no 1) was defined to include residues within 10.0 A° radius to any of the interaction. The pyrazolthiazole extracted from PDB was docked and studied for its interaction with the active site. The molecular docking results revealed that dock score of -4.778kcal/mol for pyrazolthiazole and it forms single hydrogen bond with Ser-55. The compound **P-14** and **P15** which are the most active compound as antibacterial possesses dock score of -5.37, -5.27 kcal/mol.

Table 1: The physical data of P (1-18) compounds.

Compound	R	R1	Yield %	M.P.- ^{0} C	Rf
P1	H	H	60	217-220	0.65
P2	H	CH_3	45	186-188	0.71
P3	H	Ph	50	191-195	0.60
P4	6-CH_3	H	55	123-125	0.52
P5	6- CH_3	CH_3	45	181-183	0.66
P6	6- CH_3	Ph	50	125-128	0.78
P7	6-O CH_3	H	55	220-223	0.76
P8	6-O CH_3	CH_3	45	203-205	0.65
P9	6-O CH_3	Ph	50	219-221	0.68
P10	NO_2	H	60	189-192	0.71
P11	NO_2	CH_3	45	196-198	0.70
P12	NO_2	Ph	55	92-98	0.53
P13	Cl	H	60	170-172	0.60
P14	Cl	CH_3	45	123-126	0.55
P15	Cl	Ph	50	124-126	0.62
P16	-$COCH_3$	H	57	210-213	0.56
P17	-$COCH_3$	CH_3	58	198-201	0.53
P18	-$COCH_3$	Ph	62	123-125	0.52

Table 2: The IR and ^{1}H NMR spectral data of P (1-18) compounds

Compound	IR (KBr) cm-1	^{1}H NMR($CDCl_3$) δ ppm	MS(m/z)
P1	3020(C-H), 1748(C=O),	7.43-7.51(m, 2H, Ar-H),7.87(d,1H,Ar-H, J= 7.6), 8.10(d, 1H, Ar-H, J= 8.1), 3.001(s, 4H, CH_2-CH_2)	231(M-1), 151, 102
P2	1720(C=O), 3130(C-H)	7.41-7.55(m, 2H, Ar-H), 7.88(d, 1H, Ar-H, J= 8.1), 8.11(d, 1H, Ar-H, J=8.1), 1.49(d, 3H, CH_3, J=7.1), 2.59(dd, 2H, CH_2 J=2.8, 7.4), 3.21(m, 1H, CH)	247(M+1), 151, 102
P3	1722(C=O), 3034(C-H)	7.41-7.55(m, 5H, Ar-H), 7.88(d, 2H, Ar-H, J=7.9), 8.11(d, 2H, Ar-H, J=8.2), 3.10,3.44(dd,2H, CH_2 J=5.3, 10), 4.29(m, 1H, CH)	
P4	1795(C=O), 3063(C-H)	7.03(d, 1H, Ar-H, J= 7.0), 7.29(s, 1H, Ar-H), 7.67(d, 1H, Ar-H, J=8.8), 2.79(s, 3H, CH_3), 3.71(s, 4H, CH_2-CH_2)	
P5	1748(C=O), 3134(C-H)	7.31(d, 1H, Ar-H, J= 8.1), 7.679s, 1H, Ar-H), 7.97(d, 1H, Ar-H, J= 8.6), 1.49(d, 3H, CH_3 J= 4.3), 2.50(s, 3H, CH_3), 2.59, 2.66(dd, 2H, CH_2 J=2.4, 12.9), 3.15(m, 1H, CH)	
P6	1758(C=O), 3032(C-H)	7.33-7.60(m, 6H, Ar) 7.66(s, 1H, Ar-H) 7.97(d, 1H, Ar-H, J=8.1), 2.49(s, 3H, CH_3), 3.14, 3.41(dd, 2H, CH_2 J=2.4, 7.6), 4.28(m, 1H, CH)	324(M+2), 102
P7	1799(C=O), 3063(C-H),	7.12(d, 1H, Ar-H, J= 2.3), 7.32(s, 1H, Ar-H) 7.97 (d, 1H, Ar-H, J= 8.8), 2.99 (s, 4H, CH_2-CH_2), 3.88(s, 3H, OCH_3)	
P8	1734(C=O), 3030(C-H)	7.102(d,1H,Ar-H, J=6.2), 7.32(s,1H,Ar), 7.98(d,1H, Ar-H,J=8.6), 1.49(m,3H, CH_3), 2.57,3.12(dd,2H, CH_2, J= 3.8,4.8), 3.23(m, 1H,CH), 3.89(s,3H, OCH_3)	278(M+2), 181, 102
P9	1725(C=O) 3132(C-H)	7.130(d, 1H, Ar-H, J= 2.4), 7.269-7.42(m, 6H, Ar-H), 7.96(d, 1H, Ar-H, J= 9.0), 3.89(s, 3H, OCH_3), 4.264(m, 1H, CH), 3.14, 3.42(dd, 2H, CH_2, J= 5.2, 10)	
P10	1635(C=O) 3140(C-H)	8.89(s, 1H, Ar), 8.41(d, 1H, Ar, J= 3.0), 8.24(d, 1H, Ar, J= 9.0) 3.05 (s, 4H, CH_2-CH_2)	
P11	1721(C=O) 3257(C-H)	8.88(s,1H, Ar), 8.23(d, 1H, Ar, J= 3.4), 7.53(d, 1H, Ar, J=9.1), 1.28(t, 3H, CH_3, J= 11), 2.53, 2.68(dd, 2H, CH_2, J= 4.2, 13.4), 3.19(m, 1H, CH)	
P12	1733(C=O) 3148(C.-H)	8.89(s, 1H, Ar), 8.41(d, 1H, Ar, J= 3.0), 8.24(d, 1H, Ar, J= 9.0), 7.36-7.49(m,, 5H, Ar), 4.11(m, 1H, CH), 2.68, 3.18(dd, 2H, CH_2,J= 5.3, 6.0)	
P13	1749(C=O), 3145(C-H)	7.47(d,1H,Ar, J=10.5), 7.879s,1H, Ar), 8.02(d,1H,Ar, J=8.6) 3.02(s, 4H, CH_2-CH_2)	
P14	1719(C=O), 3078(C-H)	7.45(d, 1H, Ar, J= 2.4), 7.87(s, 1H, Ar), 8.01(d, 1H, Ar, J= 8.5), 1.50 (d, 3H, CH_3 J= 7.1), 2.60, 2.66 (dd, 2H, CH_2 J=12.8, 1.9), 3.17(m, 1H, CH)	
P15	1796(C=O), 3189(C-H)	7.26-7.50(m, 6H, Ar), 7.89(s, 1H, Ar), 8.02 (d, 1H, Ar, J=8.6), 3.11, 3.45 (dd, 2H, CH_2 J=5.2, 9.5), 4.28(m, 1H, CH)	342(M), 102
P16	1760(C=O), 3023(C-H)	8.38(d,1H,Ar) 8.54(s,1H,Ar), 8.24(d, 1H, Ar, J= 9.0) 2.59(m,3H, CH_3), 3.001(s, 4H, CH_2-CH_2)	
P17	1749(C=O), 3183(C-H)	7.16(d,1H,Ar,J=8.1), 8.087(d,1H,Ar,J=8.1), 8.06(s,1H,Ar),2.61(m,3H,CH_3), 2.57,3.12(dd,2H,CH_2 J=3.5, 3.8), 1.37(s,3H,CH_3)	
P18	1762(C=O), 3049(C-H)	7.21- 7.45(m, 5H,Ar),, 8.38(d,1H,Ar) 8.54(s,1H,Ar), 2.59(m,3H, CH_3), 4.11(m,1H,CH), 2.80, 3.19(dd, 2H, CH_2, J=4.8, 5.2)	

Table 3: Minimum inhibitory concentration (µg/ml)

Comp code	Gram Positive			Gram Negative			Fungus	
	B. subtilis	*S. aureus*	*S.epidermis*	*E. coli*	*P. aeruginosa*	*S. typhi*	*C. albicans*	*A. niger*
P1	25	50	12.5	25	50	25	50	12.5
P2	50	12.5	25	50	100	50	25	6.25
P3	6.25	12.5	12.5	50	25	50	12.5	50
P4	50	25	25	100	25	50	50	12.5
P5	12.5	50	25	12.5	6.25	25	12.5	25
P6	25	6.25	50	25	50	12.5	6.25	12.5
P7	25	50	50	100	100	50	50	12.5
P8	50	25	12.5	50	50	100	6.25	25
P9	100	50	25	100	25	50	25	12.5
P10	50	25	50	50	50	50	50	25
P11	25	12.5	25	25	100	12.5	50	3.125
P12	12.5	50	50	12.5	25	6.25	50	50
P13	50	6.25	25	50	6.25	25	50	50
P14	12.5	6.25	12.5	3.125	12.5	25	12.5	6.25
P15	6.25	12.5	3.125	25	12.5	25	3.125	6.25
P16	50	25	25	25	50	50	50	25
P17	25	50	6.25	12.5	50	50	50	25
P18	6.25	25	25	100	50	12.5	50	6.25
Ciprofloxacin	12.5	6.25	6.25	3.125	3.125	12.5	---	---
Fluconazole	--	--	--	---	----	---	12.5	6.25

The single hydrogen bond forms between carbonyl oxygen of pyrrolidine-2, 5-dione ring and amino group of Arg-144. The benzothiazole moiety is positioned in the hydrophobic pocket surrounded by Asn-54, Ser-55, Ile-86, Glu-58, Asp-81, Thr-173 amino acid residues. The newly synthesized series of compounds docked and observed for its dock score, binding energy and interactions are shown in **Table 4**. It was found that the compound **P4, P5, P7, P11, P12** showed better docking score compared to original ligand. The substituted pyrrolidine-2, 5-dione compounds have shown potential antibacterial activity than un-substituted compounds. The antibacterial activity was found in correlation with dock score. The best docked pose of original and synthesized compound is shown in **Figure 1 and Figure 2** respectively.

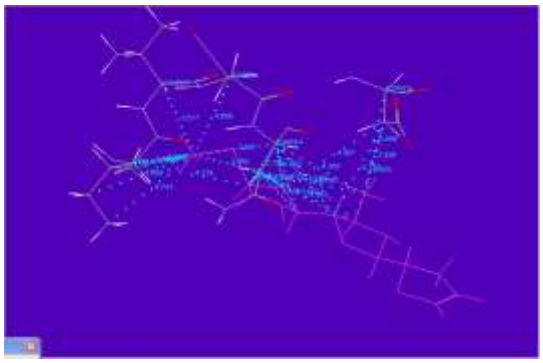


Fig. 1: The pyrazolthiazole ligand from 3G75 is oriented in DNA gyrase binding site. (Hydrophobic interactions)

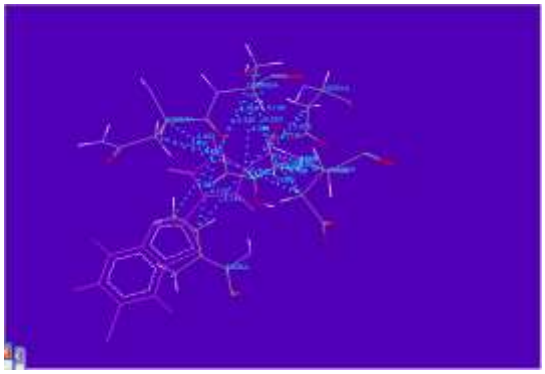


Fig. 2: The P14 compound docked in DNA gyrase active site.

Table 4: Dock scores of newly synthesized compounds P (1-18)

Compound	Dock score (kcal/mol)
Reference ligand	-4.778
P1	-4.94
P2	-5.00
P3	-4.64
P4	-5.019
P5	-5.24
P6	-4.655
P7	-5.08
P8	-4.80
P9	-4.71
P10	-4.77
P11	-5.03
P12	-5.0292
P13	-5.087
P14	-5.37
P15	-5.27
P16	-4.92
P17	-5.002
P18	-4.16

CONCLUSION

Various derivatives of novel pyrrolidine-2, 5-diones were synthesized using simple economic method and screened for their antimicrobial potential. These compounds were evaluated for antibacterial activity against gram positive and gram negative bacterial strains and antifungal activity against *C. albicans* and *A. niger*. The compound, **P14** and **P15** showed MIC at 6.25 and 3.125 µg/ml against *S. epidermis* and *E. coli*. The compound **P6, P12,** and **P18** have shown MIC at 6.25 and 12.5µg/ml.

The results of the antimicrobial activity revealed that compounds particularly with chloro substituents have potential antimicrobial activity. The finding of the study would be useful for the further development of potential antimicrobial compounds. Though, the relationship between the activities shown by these compounds in, *in vivo* and *in vitro* study is still to be established, the results suggest the suitability of the designed molecular framework as a potential anti-microbial lead.

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