ORIGINAL RESEARCH ARTICLES

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL BIS-HETERO CYCLIC DERIVATIVES

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ABSTRACT

The present research outlines a series of bis-hetero cyclic derivatives (a1-6) synthesized from methyl-1-(2, 5-dioxopyrrolidin-1-yl) -6- methyl -2- oxo -4- phenyl -1, 2, 3, 4- tetrahydro pyrimidine -5- carboxylate treated with different aromatic aldehydes under acidic environment. The synthesized titled derivatives were confirmed by determination of physicochemical properties, by different spectral data and they were evaluated for *in vitro* antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger* and *Candida albicans* organisms at 25, 50, 100 µg mL^{-1} concentrations using streptomycin and fluconazole as reference standard drug respectively, through cup plate method. The *in vitro* antimicrobial assay results indicated that the derivatives a1, a2 and a3 showed significant antimicrobial activity, whereas the remaining derivatives showed moderate antimicrobial activities compared to the standard drugs. Further extension of this research to the cellular level is required to describe the mechanism of action, efficacy, and structural activity of these derivatives for antimicrobial activity.

Keywords: Pyrrole, Pyrimidine, antibacterial, antifungal, cup plate method, Bis-Heterocyclic

INTRODUCTION

Bacteria and fungi are the frequent cause of organ dysfunction and infections, which can create life threats to humans[1]. Some of the microbes may become resistant to the existing drug due to their unnecessary usage and their infections may not be controlled with this drug[2]. Moreover, in current days, novel strains of microbial infections are coming to attack humans. Sometimes these microbial infections may or may not be controlled by the existing antimicrobial agents in the market[3]. So, there is an enormous scope for the development of novel antimicrobials to fight against novel and resistant microbial infections.

Heterocyclic chemistry is a special area of chemistry, which comprises the physicochemical, synthetic procedures, and various applications of heterocyclic compounds. Since the majority of the drug chemical structures are composed of heterocyclic compounds, it is known that these heterocyclic compounds are best and can be considered for drug development[4]. Compounds having two heterocycle rings fused with spacers are called bis-heterocyclic compounds. The utilization of bis-heterocycles has drastically increased in the field of medicinal chemistry for the design of biologically active compounds[5]. The bis-heterocyclic compounds containing nitrogen impart properties like biologically active, electronic, highly reactive, and wide range of solubility[6], these properties favor the medicinal chemist to select bis-heterocycles containing nitrogen like imidazole, pyrrole, pyrimidine, indole, triazoles, etc. scaffolds for design and development of novel biologically active compounds.

Pyrrole (Fig.1) is the most prominent scaffold among heterocyclic compounds and gains significant value in drug design and discovery. The pyrrole derivatives

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were proved as the predominant class of biologically active compounds since they can exhibit wide range of pharmacological activities, available naturally, and also can be synthesized[7].

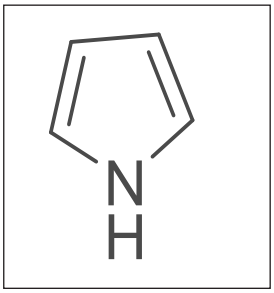


Fig. 1: Molecular structure of pyrrole

To date, a vast array of synthetic methods has been created to synthesize pyrroles[8]. Pyrrole derivatives are versatile and their derivatives were reported for biological activities[9] like anti-inflammatory, antioxidant, anticancer, antimicrobial, anti-diabetic and enzyme inhibition activities. Some of the marketed drugs proven for antimicrobial activity possessing pyrrole ring[10] are ertapenam, cefepime, linezolid, sitafloxacin, etc (Fig. 2).

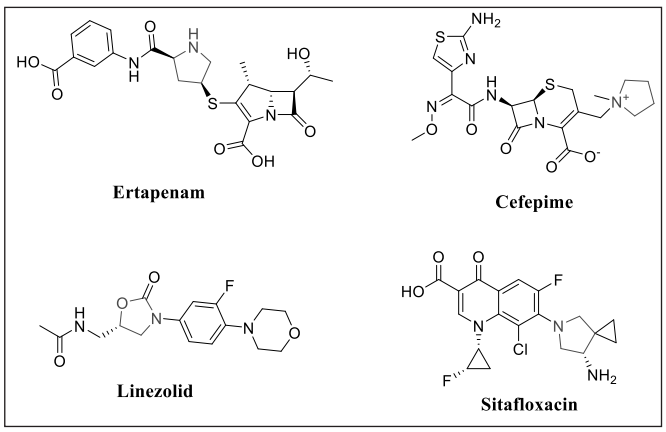


Fig. 2: Marketed antimicrobial drugs having pyrrole ring

Pyrimidine (Fig. 3) is another aromatic heterocyclic organic biologically active substance that has nitrogen at one and third positions in the ring.

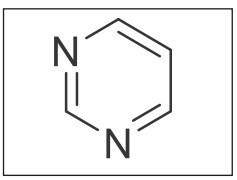


Fig. 3: Molecular structure of pyrimidine

Since the pyrimidine nitrogen bases are present in nucleotides of nucleic acids, a wide range of therapeutic activities can be exhibited by them. To date, the pyrimidine derivatives were reported for a huge number of biological activities[11] like anti-inflammatory, antioxidant, antimicrobial, anticonvulsant, antihypertensive, antihistaminic, analgesic, anticancer and CNS depressant activities. Some of the marked drugs[11] with proven antimicrobial activity having pyrimidine ring are sulfadiazine, sulfamerazine, sulfamethazine, sulfameythoxydiazine, sulfadoxine, sulfadimethoxine, amicetin, plicacetin, bacimethrin and gourgetin (Fig. 4).

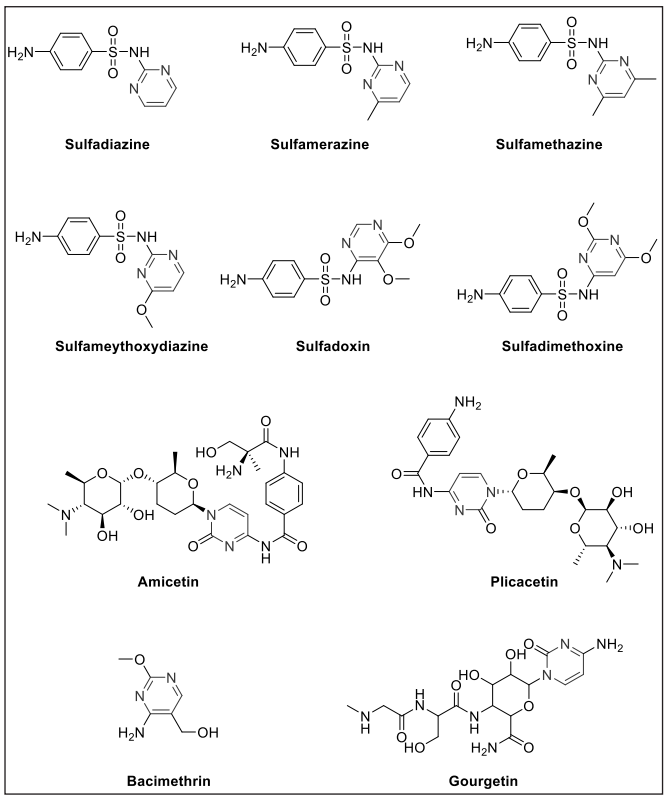


Fig. 4: Marketed antimicrobial drugs having pyrimidine ring

The antimicrobial efficacy of these marketed drugs may be due to the presence of pyrrole or pyrimidine in their chemical structures. Hence, for developing the novel highly potent antimicrobial agents, fusing these pyrrole and pyrimidine heterocyclic scaffolds (Fig. 5) through spacer may be a good choice[12].

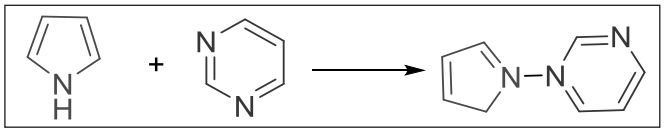


Fig. 5: Bis-heterocycle containing pyrrole and pyrimidine

The fusing of pyrrole and pyrimidine rings into bis-heterocycle derivatives may synergise the individualus antimicrobial activity. The present work aimed to synthesize, characterize and evaluate for *in vitro* antimicrobial activity of some bis-heterocyclic compounds containing pyrrole and pyrimidine moieties.

MATERIALS AND METHODS

Synthetic-grade reagents and solvents were employed for this research without any purification. Contech digital melting point apparatus model CDMP-300 was used to determine the melting point in degree Celsius and were uncorrected or synthesized by open capillary tubes.

The completion of the reaction and end product purity at each step was monitored by thin layer chromatography, a mixture of organic solvents was used for separation.

The Bruker FT-IR model ALPHA-T spectrophotometer was employed for recording the IR spectra through the KBr pellet method. The Bruker AMX400 MHz NMR spectrophotometer was employed for recording ^{1}H and ^{13}C NMR spectra.

Synthesis scheme

Step-1:

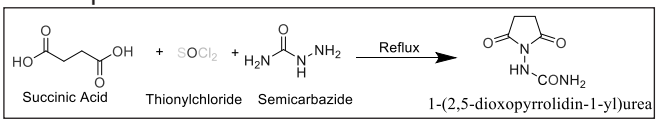


Fig. 6: Synthesis of 1-(2,5-dioxo pyrrolidin -1- yl) urea

Step-2:

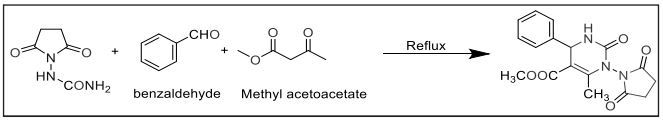


Fig. 7: Synthesis of methyl-1- (2,5- dioxo pyrrolidin-1- yl)- 6- methyl -2-oxo -4- phenyl-1, 2, 3, 4-tetra hydro pyrimidine -5- carboxylate

Step-3:

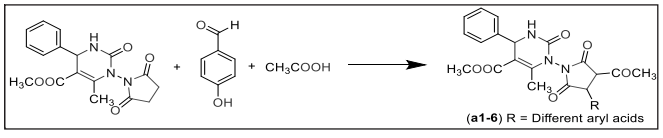


Fig. 8: Synthesis of novel bis-hetero cyclic derivatives

The general method for synthesis of novel bis-hetero cyclic derivatives

Synthesis of 1- (2,5-dioxo pyrrolidin -1- yl) urea (Step-1): A mixture of succinic acid (0.074 M) and thionyl chloride (0.0385 M) was refluxed for 30 min. Semicarbazide hydrochloride (0.01 M) was dissolved in 5 mL of benzene (Fig. 6). The solution of semi-carbazide hydrochloride was added slowly to the above reaction mixture[13]. The reaction mixture was then refluxed till HCl gas was completely evolved. Then the mixture was cooled, wash with brine solution, also treated with drying agent calcium carbonate to remove the entrapped residual water, and the reaction mixture filtered. The product was recrystallized from ethanol.

Synthesis of methyl-1-(2,5-dioxo pyrrolidin -1-yl) -6- methyl -2-oxo-4-phenyl -1, 2, 3, 4 tetra hydro pyrimidine -5- carboxylate (Step-2): The mixture of methyl acetoacetate (0.02 M), benzaldehyde (0.02 M), 1-(2,5- di oxo pyrrrolidin-1-yl) urea (0.02 M), phosphorus pentoxide (0.05 M), ethanol was taken into a 250 mL RBF and refluxed for about 1 h (Fig. 7) to obtained methyl 1-(2, 5-dioxo pyrrolidin-1-yl)-6-methyl -2- oxo-4-phenyl -1, 2, 3, 4 tetra hydro pyrimidine-5-carboxylate[14]. Then, the mixture was cooled, washed with brine solution, also treated with drying agent calcium carbonate to remove the entrapped residual water, and the reaction mixture filtered. The product was recrystallized from ethanol.

Synthesis of ethyl 4-(2-substituted)-1-3-[(*E*)-1-(substituted) methylidene]-4-[(*Z*)-1-(substituted) methylidene]-2, 5-dioxotetrahydro-1*H*-1 –pyrrolyl -6- methyl -2- oxo -1, 2, 3, 4 –tetrahydro -5-pyrimidinecarboxylate (Step-3): The mixture of methyl1-(2, 5-dioxo pyrrolidin-1-yl) -6- methyl -2- oxo-4-phenyl -1, 2, 3, 4-tetra hydro pyrimidine -5- carboxylate (0.02 M) add different aromatic aldehydes in acetic acid was boiled in the sand bath for about 15 min (Fig. 8). Then the reaction mixture was kept aside overnight to obtain the final product[15]. Then, the mixture was washed with brine solution and also treated with drying agent calcium carbonate to remove the entrapped residual water and the reaction mixture filtered. The product was recrystallized from ethanol.

Antibacterial activity

The *in vitro* antibacterial activity evaluation of the titled derivatives (**a1-6**) was performed against *B. subtilis*, *E. coli, S. aureus*, *and P. aeruginosa* organisms by the cup-plate method. Inhibition zones were determined in mm by cup-plate method[16] using streptomycin as the standard drug. The sterilized (autoclaved at 121 °C for 20 min) agar (potato dextrose) medium was inoculated with cultured test organisms, incubated for 18 h, and transferred into sterile petri dishes. The solidified cultured medium was bored for making cups of 8 mm diameters. Each 50 µL of the test (different concentrations) and the standard

drug was transferred into cups by sterilized pipette. The treated Petri plates were incubated at 37 °C for about 24 h in the incubator, later the inhibition zone diameters were measured in mm. The average diameter of the inhibition zone was recorded for three replications of the test and streptomycin (50 µg mL^{-1}).

Antifungal activity

On potato dextrose agar medium, the anti-fungal action of each compound was assessed against *A. niger* and *C. albicans*. As a benchmark, 50 µg mL^{-1} of fluconazole was used, and DMSO served as the control. Sterilized potato dextrose medium in the molten state was cooled to 45 °C, inoculated with selected test organisms under aseptic conditions, fully mixed, and then poured into sterile petri plates. These petri plates were treated with sample and standard solutions and incubated at 28 °C for about 48 h, later the diameters of inhibition zones were measured in millimetres, using fluconazole (100 µg mL^{-1}) as the reference standard[17].

RESULTS AND DISCUSSION

The results disclosed that the bis-heterocyclic derivatives were synthesized with satisfactory yields, and were examined for *in vitro* antimicrobial properties. Novel bis-heterocycles containing pyrrole and pyrimidine compounds (Fig. 9) were efficiently synthesized and were confirmed through spectral data analysis, The physicochemical properties and their spectral data are presented in Tables I & II.

Table I: Physicochemical properties of novel bis-heterocyclic derivatives (a1-6)

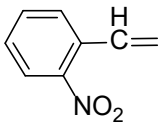
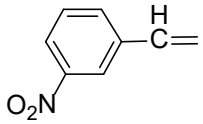
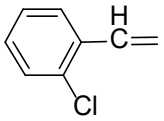
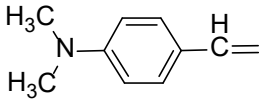
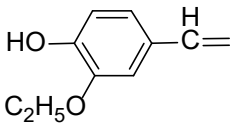
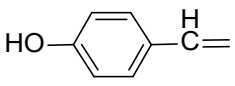
Compound code	R	Molecular formula	Melting point (°C)	Colour of compound	Yield (%)	R_f
a1	 2-Nitro benzaldehyde	$C_{26}H_{22}N_4O_8$	116-118° C	Yellow	95	0.92
a2	 3-Nitro benzaldehyde	$C_{26}H_{22}N_4O_8$	117-119°C	Lemon Yellow	92	0.89
a3	 2-Chloro benzaldehyde	$C_{26}H_{22}ClN_3O_6$	114-116°C	Brown	97	0.86
a4	 p-Di methyl amino benzaldehyde	$C_{28}H_{28}N_4O_6$	118-120°C	Lemon Yellow	87	0.90
a5	 3-Ethoxy 4-hydroxy benzaldehyde	$C_{28}H_{27}N_3O_8$	112-114°C	Light Brown	91	0.87
a6	 4-Hydroxy benzaldehyde	$C_{26}H_{23}N_3O_7$	115-117°C	Yellow	79	0.85

Table II: Spectral data of synthesized novel bis-heterocyclic derivatives (a1-6)

Compound code	Molecular formula	Spectral data
a1	$C_{26}H_{22}N_4O_8$	**IR (υ in cm^{-1})** 1664.63 (Str, CH=C-), 2827.42 (Str, -Ar-H), 2876.19 (Str, CHO), 3157.47 (Str, -CH_3), 3451.59 (Str, -NH-). **^{1}H NMR ($CHCl_3$, δ in ppm):** 9. 74 (1H, s, HCO); 6.69 (2H, s, Ar-H); 3.09 (3H, s, -NH-); 0.89 (4H, t, CH_3). **^{13}C NMR ($CHCl_3$, δ in ppm) :** 10.5 (CH_3), 24.1 (CH_3), 30.4 (CH_2), 38.4 (C-H in cyclic), 51.7(O-CH_3), 127.5 to 145.2 (C-Ar), 154.7 (N-CO), 168.6 (O-CO), 173.7(N-CO, cyclic), 204.3 (C-CO), MS (m/z, %): 521 (M^{+1}, 7), 245 (47), 275 (31).
a2	$C_{26}H_{22}N_4O_8$	**IR (υ in cm^{-1})** 1665.70 (Str, CH=C-), 2827.71 (Str, -Ar-H), 2876.00 (Str, CHO), 3157.00 (Str, -CH_3), 3157.85 (Str, -NH-). **^{1}H NMR ($CHCl_3$, δ in ppm) :** 9.87 (1H, s, HCO); 6.95 (2H, t, Ar-H); 1.25 (3H,t,-NH-). **^{13}C NMR ($CHCl_3$, δ in ppm) :** 9.5 (CH_3), 22.7 (CH_3), 31.6 (CH_2), 39.7 (CH in cyclic), 54.2(O-CH_3), 125.1 to 143.6 (C-Ar), 154.1 (N-CO), 168.8 (O-CO), 171.6(N-CO, cyclic), 201.5(C-CO), MS (m/z, %): 521 (M^{+1}, 9), 245 (35), 275 (42), 137 (22).
a3	$C_{26}H_{22}ClN_3O_6$	**IR (υ in cm^{-1})** 1666.68 (Str, CH=C-), 2827.72 (Str, CHO), 2875.49 (Str, -Ar-H), 3157.00 (Str, -NH-), 3161.82 (Str, -CH_3); **^{1}H NMR ($CHCl_3$, δ in ppm) :** 10 (1H, s, HCO); 7.25 (2H, t, Ar-H), 1.25 (3H, s, -NH); **^{13}C NMR ($CHCl_3$, δ in ppm) :** 9.2 (CH_3), 26.6 (CH_3), 30.9 (CH_2), 39.5 (CH in cyclic), 52.3(O-CH_3), 125.8 to 147.2 (C-Ar), 153.9 (N-CO), 167.1 (O-CO), 172.8 (N-CO, cyclic), 203.4 (C-CO), MS (m/z, %): 509 (M^+, 12), 211 (M^{+2}, 4), 245 (46), 275 (33), 154 (19), 147 (22), 112 (48).
a4	$C_{28}H_{28}N_4O_6$	**IR (υ in cm^{-1})** 1665.01 (Str, CH=C-), 2824.69 (Str, -Ar-H), 2874.56 (Str, CHO), 3154.36 (Str, -CH_3), 3392.13 (Str, -NH-); **^{1}H NMR ($CHCl_3$, δ in ppm) :** 9.74 (1H, s, HCO); 6.69 (2H,t,Ar-H); 1.25 (3H,t,-NH-); ^{13}C NMR (**$CHCl_3$**, δ/ppm): 10.6(CH_3), 24.7 (CH_3), 32.3 (CH_2), 38.8 (CH in cyclic), 42.5 (-N-CH_3), 54.6(O-CH_3), 123.1 to 142.7 (C-Ar), 156.9 (N-CO), 165.8 (O-CO), 169.9 (N-CO, cyclic), 202.6 (C-CO), MS (m/z, %): 519 (M^{+1}, 15), 245 (37), 275 (72), 154 (26), 120 (19).
a5	$C_{28}H_{27}N_3O_8$	**IR (υ in cm^{-1})** 1662.87 (Str, CH=C-), 2825.85 (Str, -Ar-H), 2827.97 (Str, CHO), 3149.16 (Str, -CH_3), 33932.13 (Str, -NH); **^{1}H NMR ($CHCl_3$, δ in ppm):** 9.81 (1Hs, CHO); 6.24 (2H,q, Ar-H); 1.46 (3H,t,-NH-); ^{13}C NMR (**$CHCl_3$**, δ/ppm): 10.7(CH_3), 15.9(CH_3), 28.6 (CH_3), 34.2 (CH_2), 40.3 (CH in cyclic), 51.7(O-CH_3), 63.8 (CH_2), 121.2 to 148.6 (C-Ar), 152.2 (N-CO), 161.6 (O-CO), 178.2 (N-CO, cyclic), 200.6 (C-CO), MS (m/z, %): 536 (M^{+1}, 10), 245 (39), 291 (42), 155 (24), 138 (36).
a6	$C_{26}H_{23}N_3O_7$	**IR (υ in cm^{-1})** 1663.19 (Str, CH=C-), 2827.20 (Str, -Ar-H), 2874.16 (Str, CHO), 3155.90 (Str, -CH_3), 3392.13 (Str, -NH-); **^{1}H NMR ($CHCl_3$, δ in ppm):** 9.87 (1Hs, CHO); 6.93 (2H,t, Ar-H); 1.58 (3H,s,-NH-); ^{13}C NMR (**$CHCl_3$**, δ/ppm): 9.2(CH_3), 26.4 (CH_3), 35.1 (CH_2), 39.4 (CH in cyclic), 52.4(O-CH_3), 124.5 to 146.4 (C-Ar), 152.1 (N-C=O), 156.3 (OH-C), 160.8 (O-CO), 179.4 (N-CO, cyclic), 203.2 (C-CO), MS (m/z, %): 492 (M^{+1}, 16), 246 (52), 247 (39), 154 (29), 94 (32).

Table III: Antibacterial activity (zone of Inhibition in mm)

Derivative ID	Concentration of sample	Inhibition zone (mm)*			
		Gram +ve		Gram (-ve)	
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
a1	25 µg mL^{-1}	11.4	13.1	15.9	17.3
	50 µg mL^{-1}	16.3	14.7	28.5	24.0
	100 µg mL^{-1}	22.6	17.4	30.2	32.4
a2	25 µg mL^{-1}	10.8	10.9	13.9	14.7
	50 µg mL^{-1}	13.9	12.3	25.0	20.8
	100 µg mL^{-1}	21.4	16.5	28.3	26.9
a3	25 µg mL^{-1}	11.2	12.9	15.1	16.8
	50 µg mL^{-1}	15.2	13.3	27.2	24.5
	100 µg mL^{-1}	21.2	17.5	30.1	32.0
a4	25 µg mL^{-1}	8.1	9.5	9.3	8.5
	50 µg mL^{-1}	10.8	11.3	12.4	13.8
	100 µg mL^{-1}	17.5	14.2	17.8	19.1
a5	25 µg mL^{-1}	8.1	9.4	8.9	8.2
	50 µg mL^{-1}	9.6	11.4	12.6	11.1
	100 µg mL^{-1}	10.9	16.6	18.4	19.7
a6	25 µg mL^{-1}	8.4	9.9	10.5	8.6
	50 µg mL^{-1}	12.7	10.8	23.9	24.0
	100 µg mL^{-1}	20.9	16.0	28.9	27.5
Standard (Streptomycin)	25 µg mL^{-1}	10.1	10.2	9.9	10.0

**Average of triplicate*

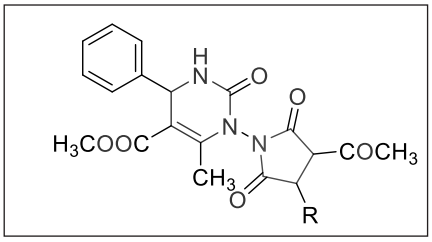


Fig. 9: Basic structure of titled bis-heterocyclic derivatives

Biological evaluation of synthesized compounds

Antimicrobial Activity: Few of the novel bis-heterocyclic derivatives comprising pyrrole and pyrimidine (**a1-6**) were examined for *in vitro* antibacterial properties through the cup plate method against Gram +ve bacteria i.e, *S. aureus and B. subtilis* and two Gram -ve bacteria i.e*., E. coli* and *P. aeruginosa* and for antifungal property against *A. niger* and *C. albicans*. Streptomycin and fluconazole were used as reference standards in the assay. DMSO, which is ineffective in the growth of microbes, was used as solvent and blank. The antimicrobial property of titled derivatives was examined at 25, 50, and 100 µg mL^{-1} concentrations.

The antimicrobial results indicated that the derivatives **a1 a2**, and **a3** showed significant antimicrobial activity against selected test organisms for this test, moreover, the derivatives **a1** and **a3** had electronegative group (nitro and chloro, respectively) substitutions at the ortho position of substituent groups attached at 3 positions on pyrrole ring exhibits extreme antimicrobial activity whereas the derivative **a2** with electronegative group (nitro) substitutions at meta position of substituent groups attached at the third position on pyrrole ring exhibits somewhat less antimicrobial activity when compared to derivatives **a1**, and **a3**, but shown better antimicrobial activity compared to standard drugs. On another side the derivatives **a4, a5**, and **a6** having electropositive group (like dimethyl amino at para position, ethoxy at meta position, and hydroxyl at para position, respectively) substitutions to the substituent groups attached at 3 positions on pyrrole ring exhibit less antimicrobial activity even compared to standard drugs. The results of antibacterial and antifungal studies are presented in the Table III & IV, respectively.

Table IV: Antifungal activity (zone of Inhibition in mm)

Derivative ID	Concentration of sample	Inhibition zone (mm)*	
		A. niger	*C. albicans*
a1	25 µg mL^{-1}	17.5	18.3
	50 µg mL^{-1}	25.3	22.6
	100 µg mL^{-1}	33.6	28.2
a2	25 µg mL^{-1}	14.4	10.2
	50 µg mL^{-1}	20.8	19.4
	100 µg mL^{-1}	26.4	25.1
a3	25 µg mL^{-1}	16.9	17.6
	50 µg mL^{-1}	24.4	21.9
	100 µg mL^{-1}	29.3	32.8
a4	25 µg mL^{-1}	7.5	8.5
	50 µg mL^{-1}	13.3	14.3
	100 µg mL^{-1}	17.5	20.6
a5	25 µg mL^{-1}	12.0	13.5
	50 µg mL^{-1}	17.6	14.2
	100 µg mL^{-1}	18.5	19.6
a6	25 µg mL^{-1}	10.9	14.8
	50 µg mL^{-1}	14.3	16.0
	100 µg mL^{-1}	17.8	18.2
Standard (Fluconazole)	25 µg mL^{-1}	15.2	14.9

**Average of triplicate*

CONCLUSION

Because of the importance and advantages promoted by pyrroles and pyridine scaffolds in medicinal chemistry, the present work elaborates on the synthesis of novel bis-heterocyclic derivatives and *in vitro* evaluation of potential antimicrobial properties through the cup-plate method. The synthesis of new bis-heterocyclic derivatives (**a1-6**) has been done using reported synthetic procedures, and characterized by physicochemical properties and ^{1}H-NMR, and IR spectrophotometric studies. The novel titled derivatives were tested for antimicrobial activity. The *in vitro* antimicrobial results disclosed that the derivatives **a1** and **a3** showed significant antimicrobial activity when compared to standard drugs. Further extensive research into the cellular mechanisms is required to elucidate the efficacy and mode of action of potential derivatives.

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