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Synthesis, characterization and *in vitro* antimicrobial activity of some novel 4,5-dihydro-1*H*-pyrazoline derivatives

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The present study deals with the synthesis of some novel pyrazoline derivatives as antimicrobial agents. The antimicrobial activities have been performed against a panel of gram-positive and gram-negative bacteria [*Staphylococcus aureus* (ATCC-25923), *Bacillus Subtilis* (ATCC-6633), *Pseudomonas aeruginosa* (ATCC-25619), *Escherichia coli* (MTCC-739) and *Klebsiella pneumoniae* (MTCC-109)] and fungal strains [*Candida albicans* (ATCC-10231), *Aspergillus niger* (MTCC-4344), *Aspergillus fumigatus* (MTCC-343), *Penicillium Chrysogenum* (MTCC-2725) and *Tricophyton rubrum* (MTCC-296)]. Compounds **4d** and **4i** have shown remarkable and excellent activity against three multi-resistant strains *viz*. *P. aeruginosa*, *S. aureus* and *E. coli* with diameter of zone of inhibition 40, 32, 28 and 44, 34, 31 mm and MIC values 1.56 for *P. aeruginosa*, 6.25 and 3.12 µg/mL for *S. aureus* and *E. coli* respectively. Compounds **4e** and **4h** have shown better activity against *S. aureus* and *P. aeruginosa*. **4a** and **4g** have been shown to display moderate activity against *S. aureus* and *P. aeruginosa* respectively. The newly synthesized compounds have been characterized by elemental analysis, Infrared (IR), ^{1}H and ^{13}C NMR and mass spectrometry.

Keywords: Antimicrobial activity, Mannich bases, pyrazoline, sodium borohydride

Heterocyclic nitrogenous compounds and their fused analogues represent an important class of heterocyclic compounds. These compounds hold a special place in organic chemistry. Their role as lead candidates in drug design cannot be overstated and the appearance of heterocyclic motifs in natural products is astronomically frequent. It is known that 4,5-dihydro-1*H*-pyrazole (pyrazoline) and its derivatives exhibit extensive biological and pharmacological activities *viz*., anticonvulsant[1,2], antimicrobial[3], antitubercular[3], antitumor[4] and antidepressant[5,6]. Thus considerable efforts have been devoted to design and synthesize functional pyrazoline derivatives over the past few years. Although there are reports of synthesis of *these* substituted heterocycles, the development of synthetically important functionalized new pyrazolines is still a challenge and has become a much attempted research endeavor. In addition, compounds containing active hydrogen atoms yielded aminobenzylated Mannich bases with secondary amines and aromatic aldehydes. Mannich bases are important compounds owing to their wide range of biological and industrial applications. They are also employed as intermediates[7,8] in chemical synthesis and polymer chemistry. Several important therapeutic compounds have been synthesized *via* the Mannich reaction[9-12]. They are potent biological agents finding application in pharmaceutical chemistry as antibacterial, antimalarial, vasorelaxant, anticancer, analgesic as well as in agricultural chemistry as pesticides against various plant pathogens[13-16]. These observations have prompted us to design and synthesize a new series of Mannich bases containing pyrazoline moiety, in their molecular structure to explore their potency as chemotherapeutic agents. Based upon the above findings and in continuation of our work on heterocycles[17-23] 1-(3-(4-(1-(4-substituted phenyl)-3-morpholino propylamino) phenyl)-5-(3/4-substitutedphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (**4a-c**) and 1-(3-(4-(3-(benzyl-(methyl)amino)-1-(4-substitutedphenyl) propylamino) phenyl)-5-(3/4-substituted phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (**4d-i**) were synthesized and screened against a panel of bacterial strains, *viz*: *Staphylococcus aureus* (ATCC-25923), *Bacillus subtilis* (ATCC-6633), *Pseudomonas aeruginosa* (ATCC-25619), *Escherichia coli* (MTCC-739), *Klebsiella pneumoniae* (MTCC-109) and, unicellular and multicellular pathogenic fungi such as *Candida*

albicans (ATCC-10231), *Aspergillus niger* (MTCC-4344), *Aspergillus fumigatus* (MTCC-343), *Penicillium chrysogenum* (MTCC-2725) and *Tricophyton rubrum* (MTCC-296).

Results and Discussion

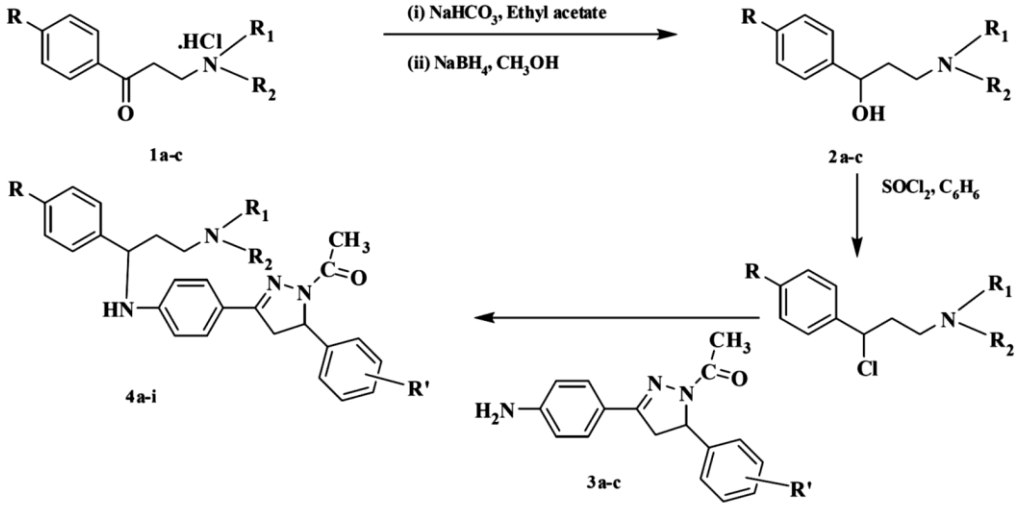
Chemistry

In this work, we have described synthesis of a novel series of some novel 4,5-dihydro-1*H*-pyrazoline derivatives 1-(3-(4-(1-(4-substituted phenyl)-3-morpholino propylamino)phenyl)-5-(3/4-substituted-phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone **4a-c** and 1-(3-(4-(3-(benzyl(methyl) amino)-1-(4-substituted phenyl) propylamino) phenyl)-5-(3/4-substituted phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) ethanone **4d-i**. As outlined in Scheme I, the preparation of new compounds involved three steps: (i) Mannich reaction of different acetophenones and secondary amines, (ii) reduction of the keto compounds by sodium boro-hydride to the corresponding 3-*N*-substituted amino-1-phenyl-propanols **2a-c** and (iii) the reaction of alcohol derivatives with thionyl chloride followed by addition of 1-(3-(4-aminophenyl)-5-(3/4-substituted-phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) ethanone **3a-c** in alcohol to give compounds **4a-c** and **4d-i** (Scheme I).

The structures of newly synthesized compounds were confirmed by IR and, ^{1}H and ^{13}C NMR spectral studies. All the synthesized compounds **4a-c** and **4d-i** showed characteristic absorption bands at 3447-3427, 2964-2886, and 1655-1620 cm^{-1} assigned to NH, CH and C=N functionalities respectively in IR. CO stretching band of $-COCH_3$ group is observed at 1662-1653 cm^{-1}. The ^{1}H NMR spectra of the final compounds exhibited ABX pattern for the presence of three hydrogen atoms attached to pyrazoline system and a singlet in the range of δ 2.04-2.17 indicative of the presence of an N-acetyl group. The other signals were also found in accordance to the structure of **4a-c** and **4d-i**. The ^{13}C NMR spectra of the final compounds revealed a peak of C=O carbon at δ 167.3-169.2 and formation of pyrazole ring in **4a-i** was evidenced by the peak of C=N carbon at δ 149.5-152.2.

Antimicrobial Activity

The compounds belonging to this series were screened for their antibacterial activity against five bacterial strains *viz*. *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 25619), *Escherichia coli* (MTCC 739) and *Klebsiella pneumoniae* (MTCC 109). In addition, the compounds were also evaluated for their antifungal activity against animal fungus *viz*. *Candida albicans* (ATCC 10231), *Aspergillus niger* (MTCC 4344), *Aspergillus fumigatus* (MTCC 343), *Penicillium*



Synthesis of compounds **4a-i**. The substituents R, R_1 and R_2 in compounds 1a-c to 2a-c. a: R= chloro, R_1& R_2= morpholino; b: R= chloro, R_1=Methyl & R_2= N-benzyl; c: R= bromo R_1= Methyl, R_2=N-benzyl. The nature of substituents R' in compound 3 are a: R'=3-nitro; b: R'=4-chloro; c: R'= 4-methoxy. The nature of R, R_1, R_2 and R' in compounds 4 are a: R= chloro R_1, R_2= morpholino and R'=3-nitro; b: R= chloro R_1, R_2= morpholino and R'= 4-chloro; c: R= chloro R_1, R_2= morpholino and R'= 4-methoxy; 4d: R= chloro R_1=methyl, R_2= N-benzyl and R'=3-nitro; 4e: R= chloro R_1=methyl, R_2= N-benzyl and R'= 4-chloro; 4f: R= chloro R_1=methyl, R_2= N-benzyl and R'= 4-methoxy; 4g; R= bromo R_1=methyl, R_2= N-benzyl and R'=3-nitro; 4h: R= bromo R_1=methyl, R_2= N-benzyl and R'= 4-chloro; 4i: R= bromo R_1=methyl, R_2= N-benzyl and R'= 4-methoxy.

Scheme I

chrysogenum (MTCC 2725) and *Tricophyton rubrum* (MTCC 296) respectively. Antimicrobial activities were evaluated by measuring the diameter of zone of inhibition (mm) and minimal inhibition concentration (MIC values, µg/mL). Ciprofloxacin, Ampicillin and Gentamicin were used as standard antibacterial and Fluconazole and Miconazole as standard antifungal drugs.

The antibacterial activity of all the nine compounds **4a-i** displayed interesting results. Each compound showed different activity for a different strain. Two compounds, **4d** and **4i** showed remarkably excellent activity against three multi-resistant strains *viz*. *P. aeruginosa*, *S. aureus* and *E. coli* with diameter of zone of inhibition 40, 32, 28 and 44, 34, 31 mm and MIC values 1.56 for *P. aeruginosa*, 6.25 and 3.12 µg/mL for *S. aureus* and *E. coli* respectively. Compounds **4e** and **4h** showed better activity against *S. aureus* and *P. aeruginosa*. Two compounds, **4a** and **4g** were shown to display moderate activity against *S. aureus* and *P. aeruginosa* respectively. All the other compounds displayed moderate to minimal antibacterial activity. It can therefore be inferred that the active compounds may either be able to penetrate the peptidoglycan bacterial cell wall easily or may have a better fit at the receptor site. Compounds **4d** and **4i** exhibited promising antibacterial activity against *S. aureus* and *P. aeruginosa* and are the lead drug candidates and further work is being carried out at CSIR-Central Drug Research Institute, Lucknow, India, concerning their toxicological evaluation. As far as antifungal activity is concerned, compounds **4d** and **4i** bearing electron withdrawing (Cl, NO_2 and Br, $-OCH_3$) groups in their molecular structure showed excellent results having maximum zone of inhibition (28 and 30 mm) and MIC (3.12 and 1.56 µg/mL) which are quite comparable with that of the standard drug Fluconazole and Miconazole against *C. albicans* and *A. fumigatus*. Other compounds (**4d**, **4g** and **4i**) exhibited promising activity against filamentous fungi *A. fumigatus* and *P. chrysogenum*. Compounds **4b**, **4e**, **4f** and **4h** showed moderate activity against *C. albicans*, *A. fumigatus*, *P. chrysogenum* and *T. rubrum*. All the other compounds displayed moderate to minimal antifungal activity. The diameter of zone of inhibition (mm) and Minimal Inhibition Concentration (MIC values) results are illustrated in Table I and Table II. The graphical representations (MIC value) are shown in Figure 1 and Figure 2.

Experimental Section

All chemicals were purchased from Alfa Aesar, Sigma-Aldrich, Merck, SD Fine, Qualigens, and Spectrochem. Solvents and reagents were used without further purification, unless otherwise specified. IR spectra (KBr) were recorded on Perkin-Elmer FTIR spectrophotometer (λ max in cm^{-1}); ^1H and ^{13}C NMR spectra were recorded on Bruker NMR spectrometer at 400 MHz using $CDCl_3$ as solvent. Chemical shifts (δ) are reported in ppm using TMS as internal standard. EI mass spectra were recorded on a Va 70–70H mass spectrometer at 70 eV. Elemental analysis was performed on a Perkin-Elmer 2400 series

Table I — Antibacterial activity of newly synthesized compounds 4a-i: diameter of zone of inhibition (mm) by disc-diffusion assay (µg/disc) and MIC values (µg/mL) by two fold serial dilution technique

Compd	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*E. coli*	*K. pneumoniae*
4a	22 (25.0)	30 [b](3.12 [b])	09 [a](100 [a])	20 (25.0)	14 (100 [a])
4b	23 (>12.5)	27 (12.5)	10 (100 [a])	22 (25.0)	12 (100 [a])
4c	22 (25.0)	28 (6.25)	11 (100 [a])	24 (>12.5)	18 (>25)
4d	32 [b](6.25[b])	40 [b](1.56 [b])	17 (>50.0)	28 [b](6.25 [b])	16 (>50.0)
4e	34 [b](3.12 [b])	36 b(3.12 [b])	10 (100[a])	26 (6.25)	08 [a](100 [a])
4f	26 (12.5)	27 (12.5)	09 [a](100 [a])	21 (>12.5)	10 (100 [a])
4g	30 [b](6.25)	28 (>6.25)	08 [a](100 [a])	20 (25.0)	20 (25.0)
4h	33[b](>3.12[b])	38 [b](3.12 [b])	18 (50.0)	24 (>3.12[b])	22 [b](12.5 [b])
4i	34 [b](3.12 [b])	44 [b](1.56 [b])	25 [b](6.25[b])	31 [b](3.12 [b])	08 [a](100 [a])
Control	06	06	06	06	06
Gentamicin	22	23	22	20	–
Ampicillin	29	20	18	19	–
Ciprofloxacin	20-28	22-30	–	26	–

[a] No activity.

[b] Entries in bold font indicate better activity than reference drugs Gentamicin, Ampicillin and Ciprofloxacin[24].

Table II — Antifungal activity of newly synthesized compounds 4a-i: diameter of zone of inhibition (mm) by disc-diffusion assay (µg/disc) and MIC values (µg/mL) by two fold serial dilution technique

Compd	*C. albicans*	*A. fumigatus*	*A. niger*	*P. chrysogenum*	*T. rubrum*
4a	11 (100 [a])	20 [c](12. 5 [c])	18 (12.5)	12 (100 [a])	13 (100 [a])
4b	18 (>12.5)	22 [c](6.25 [c])	21 [c](6.25 [c])	09 [a](100 [a])	10 (100 [a])
4c	12 (50.0)	11 (100 [a])	09 [a](100 [a])	14 (50.0)	11 (100 [a])
4d	28 [c](3.12 [c])	27 [c](>3.12[c])	10 (100 [a])	24 [c](6.25 [c])	21 (12.5)
4e	22 (6.25)	24 [c](6.25 [c])	11 (100 [a])	18 (12.5)	24 [c](6.25 [c])
4f	10 (100 [a])	28 [c](>3.12[c])	09 [a](100 [a])	11 (100 [a])	19 (>12.5)
4g	08 [a](100 [a])	30 [c](>1.56 [c])	08 (100 [a])	13 (100 [a])	09 (100 [a])
4h	17 (>12.5)	28 [c](3.12 [c])	12 (100 [a])	19 (12.5)	26 [c](6.25 [c])
4i	30 [c](3.12 [c])	36 [c](1.56 [c])	09 (100 [a])	23 [c](6.25 [c])	12(100 [a])
Control	06	06	06	06	06
Fluconazole	18 (6.25)	–	–	–	–
Miconazole	22	–	–	–	–

[a] No activity

[c] Entries in bold font indicate better activity than reference drugs Fluconazole and Miconazole[25]

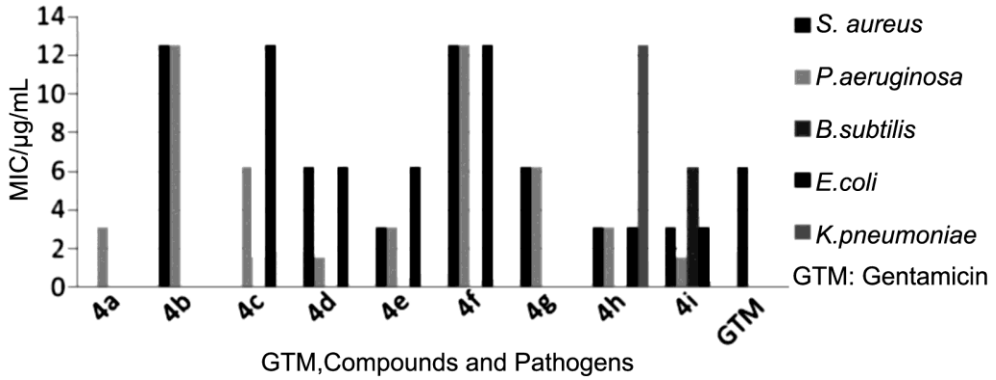


Figure 1 — A graphical representation of MIC values (µg/mL) of compounds **4a-i** against various bacterial strains

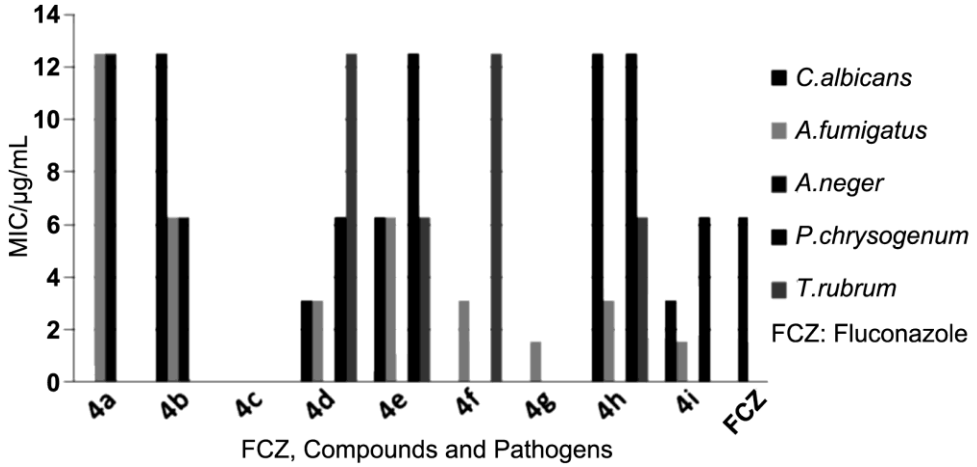


Figure 2 — A graphical representation of MIC values (µg/mL) of compounds **4a-i** against various fungal strains

II elemental CHNS analyzer. Melting points were determined in an open capillary tube and are uncorrected. The TLCs were visualized in an iodine chamber. 3-N-substituted aminopropiophenones **1a-c** and 3-N-substituted amino-1-phenylpropanols **2a–c** were prepared according to known procedures[26]. 1-(4-Aminophenyl)-3-(3/4-substitutedphenyl)prop-2-en-1-one were prepared by Claisen condensation reaction which is very well known in literature[27,28]. Compounds **3a-c** were synthesized according to literature procedures[29].

General procedure for the preparation of 1-(3-(4-(1-(4-substituted phenyl)-3-morpholino propylamino) phenyl)-5-(3/4-substitutedphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone 4a-c and 1-(3-(4-(3-(benzyl (methyl)amino)-1-(4-substituted phenyl) propylamino) phenyl)-5-(3/4-substituted phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone 4d-i

Thionyl chloride (0.04 mol) in benzene (10 mL) was added to 3-*N*-substituted amino-1-phenylpropanol **2a-c** (0.01 mol) and the reaction mixture was heated on a water bath for about 7 h. Excess of thionyl chloride was removed by azeotropic distillation with dry benzene. The chloride derivatives of **2a-c** were mixed without purification with 1-(3-(4-aminophenyl)-5-(3/4-substitutedphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone **3a-c** (0.01 mol) in absolute ethanol and refluxed for 10-15 h, on a water bath. The reaction mixture was concentrated under reduced pressure and the crude product purified by recrystallization from ethanol. The TLCs were performed using hexane: ethyl acetate (in various ratios v/v) as mobile phase.

1-(3-(4-(1-(4-Chloro phenyl)-3-morpholino propyl-amino) phenyl)-5-(3-nitro phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 4a: m.p.122-24°C. Yield: 67%. R_f value 0.43 (8.5:1.5, Hexane: Ethyl acetate); IR (KBr): 3429 (NH), 2932 (CH), 1654 (C=O), 1620 (C=N), 1519, 1347 (NO_2 asymm and symm.), 1190 (C-O), 731 cm^{-1} (C-Cl); ^{1}H NMR (400MHz, $CDCl_3$): δ 1.67-1.73 (m, 2H, 2-CH_2), 2.07 (s, 3H, $COCH_3$), 2.41 (m, 4H, -CH_2-N-CH_2-), 2.59 (m, 2H, 3-CH_2), 3.21 (dd, 1H, CHa of CH_2), 3.43 (dd, 1H, CHb of CH_2), 3.51-3.57 (m, 4H, -CH_2-O-CH_2-), 3.72-3.81 (m, 1H, 1-CH), 4.31-4.36 (dd, 1H, CH-Ar), 4.98 (s, 1H, NH), 6.82-6.88 (m, 2H, 2′6′ NH-ArH), 6.93-8.12 (m, 10H, ArH); ^{13}C NMR (100MHz, $CDCl_3$): δ 29.2 (CH_3), 37.2 (2-CH_2), 38.9 (CH_2, pyrazoline ring), 48.9 (3-CH_2), 57.1 (1-CH), 59.2 (CH, pyrazoline ring), 60.6 (C-N-C), 65.6 (C-O-C), 113.1, 123.2-131.4, 144.9, 147.3, 148.9 (phenyl ring C), 151.2 (C=N), 167.9 (C=O); MS: *m/z* 561 (M^+), 563 (M+1), 546, 518, 515, 293, 280, 266, 253, 238, 220, 191, 152. Anal. Calcd for $C_{30}H_{32}ClN_5O_4$: C, 64.11; H, 5.69; N, 12.46. Found: C, 65.69; H, 4.92; N, 11.64%.

1-(5-(4-Chlorophenyl)-3-(4-(1-(4-chlorophenyl)-3-morpholinopropylamino)phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 4b: m.p.134-35°C. Yield: 73%. R_f value 0.39 (8.5:1.5, Hexane: Ethyl acetate); IR (KBr): 3422 (NH), 2922 (CH), 1651 (C=O), 1618 (C=N), 1187 (C-O), 753 cm^{-1} (C-Cl); ^{1}H NMR (400MHz, $CDCl_3$): δ 1.77-1.84 (m, 2H, 2-CH_2), 2.09 (s, 3H, $COCH_3$), 2.56 (m, 4H, -CH_2-N-CH_2-), 2.79 (m, 2H, 3-CH_2), 3.16 (dd, 1H, CHa of CH_2), 3.47 (dd, 1H, CHb of CH_2), 3.55-3.61 (m, 4H, -CH_2-O-CH_2-), 3.83-3.99 (m, 1H, 1-CH), 4.36-4.40 (dd, 1H, CH-Ar), 4.93 (s, 1H, NH), 6.91-7.02 (m, 2H, 2′6′ NH-ArH), 6.99-8.20 (m, 10H, ArH); ^{13}C NMR (100MHz, $CDCl_3$): δ 28.7 (CH_3), 39.1 (2-CH_2), 38.0 (CH_2, pyrazoline ring), 47.9 (3-CH_2), 56.8 (1-CH), 57.9 (CH, pyrazoline ring), 61.3 (C-N-C), 66.4 (C-O-C), 112.1,124.2-133.4, 145.9, 146.3, 149.9 (phenyl ring C), 150.8 (C=N), 168.3 (C=O); MS: *m/z* 551 (M^+), 553 (M+1). Anal. Calcd for $C_{30}H_{32}Cl_2N_4O_2$: C, 65.33; H, 5.80; N, 10.16. Found: C, 65.03; H, 5.86; N, 10.06%.

1-(3-(4-(1-(4-Chloro phenyl)-3-morpholino propylamino) phenyl)-5-(4-methoxy phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 4c: m.p.110-15°C. Yield: 63%. R_f value 0.38 (8.5:1.5, Hexane: Ethyl acetate); IR (KBr): 3441 (NH), 2940 (CH), 1657 (C=O), 1623 (C=N), 1255 (C-O-C), 739 cm^{-1} (C-Cl); ^{1}H NMR (400MHz, $CDCl_3$): δ 1.703-1.889 (m, 2H, 2-CH_2), 2.04 (s, 3H, $COCH_3$), 2.53-2.62 (m, 4H, -CH_2 -N-CH_2-), 2.99-3.012 (m, 2H, 3-CH_2), 3.17 (dd, 1H, CHa of CH_2), 3.66-3.69 (m, 4H, -CH_2-O-CH_2-), 3.78 (s, 3H, OCH_3), 3.81-3.90 (m, 1H, 1-CH), 4.14 (dd, 1H, CHb of CH_2), 4.51-4.55 (dd, 1H, CH-Ar), 4.93 (s, 1H, NH), 6.77-6.79 (m, 2H, 2′6′ NH-ArH, J = 8 Hz), 6.85-688 (d, 2H, 2′, 6′, MeO-ArH, J = 12 Hz), 7.28-7.96 (m, 12H, ArH); ^{13}C NMR (100MHz, $CDCl_3$): δ 27.6 (CH_3), 35.9 (2-CH_2), 40.1 (CH_2, pyrazoline ring), 46.6 (3-CH_2), 54.8(OCH_3), 56.3 (1-CH), 58.2 (CH, pyrazoline ring), 62.5 (C-N-C), 67.1 (C-O-C), 112.3, 124.6-132.2, 148.7, 157.9 (phenyl ring C), 152.2 (C=N), 169.1 (C=O); MS: *m/z* 546 (M^+), 548 (M+1), 444, 417, 308, 238, 220, 191, 132, 131. Anal. Calcd for $C_{31}H_{35}ClN_4O_3$: C, 68.06; H, 6.40; N, 10.24. Found: C, 67.16; H, 6.99; N, 10.48%.

1-(3-(4-(3-(Benzyl(methyl)amino)-1-(4-chloro-phenyl)propylamino)phenyl)-5-(3-nitro phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 4d: m.p.151-53°C. Yield: 59%. R_f value 0.29 (8.5:1.5, Hexane: Ethyl acetate); IR (KBr): 3447 (NH), 2964 (CH), 1653 (C=O), 1631 (C=N), 762 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.59-1.64 (m, 2H, 2-CH_2), 2.11 (s, 3H, $COCH_3$), 2.25 (m, 3H, N-CH_3), 2.56-2.87 (m, 2H, 3-CH_2), 3.35-3.60 (q, 2H, CH_2-benzyl, J_a = 12.63, J_b = 12.64), 3.71 (dd, 1H, CHa of CH_2), 3.84-3.88 (m, 1H, 1-CH), 3.93 (dd, 1H, CHb of CH_2), 4.43-4.50 (dd, 1H, CH-Ar), 5.13 (s, 1H, NH), 6.90-6.93 (d, 2H, 2′6′ NH-ArH, J = 12 Hz), 7.02-8.11 (m, 15H, ArH); ^{13}C NMR (100MHz, $CDCl_3$): δ 25.9 (CH_3), 34.8

(2-CH_2), 39.3 (CH_2, pyrazoline ring), 43.2 (N-CH_3), 48.2 (3-CH_2), 57.1 (1-CH), 57.6 (CH, pyrazoline ring), 63.4 (NCH_2), 111.8, 122.6-131.9, 145.0, 146.7, 148.8 (phenyl ring carbon), 149.5 (C=N), 167.8 (C=O); MS: *m/z* 595 (M^+), 597 (M+1), 580, 489, 418, 323, 277, 272, 206, 191, 152, 132, 120. Anal. Calcd for $C_{34}H_{34}ClN_5O_3$: C, 68.51; H, 5.70; N, 11.75. Found: C, 69.17; H, 5.57; N, 10.75%.

1-(3-(4-(3-(Benzyl(methyl)amino)-1-(4-chlorophenyl)propylamino)phenyl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 4e: m.p.140-41°C. Yield: 74%. R_f value 0.36 (8.5:1.5, Hexane: Ethyl acetate); IR (KBr): 3443 (NH), 2942 (CH), 1658 (C=O), 1635 (C=N), 1456 (C=C, str), 748 cm^{-1} (C-Cl); ^{1}H NMR (400MHz, $CDCl_3$): δ 18.1-1.85 (m, 2H, 2-CH_2), 2.01 (s, 3H, $COCH_3$), 2.33 (m, 3H, N-CH_3), 2.46-2.57 (m, 2H, 3-CH_2), 2.92-3.33 (m, 2H, CH_2-benzyl), 3.44 (dd, 1H, CHa of CH_2), 3.77-3.81 (m, 1H, 1-CH), 4.02-4.12 (dd, 1H, CHb of CH_2), 4.52-4.58 (dd, 1H, CH-Ar), 5.16 (s, 1H, NH), 6.87-6.91 (d, 2H, 2′6′ NH-ArH, *J* = 16 Hz), 7.19-7.88 (m, 15H, ArH); ^{13}C NMR (100MHz, $CDCl_3$): δ 26.3 (CH_3), 33.9 (2-CH_2), 38.7 (CH_2, pyrazoline ring), 42.7 (N-CH_3), 47.1 (3-CH_2), 56.3 (1-CH), 59.2 (CH, pyrazoline ring), 62.6 (N-CH_2), 114.8, 126.6-133.4, 138.7, 149.2 (phenyl ring C), 150.1 (C=N), 168.3 (C=O); MS: *m/z* 584 (M^+), 586 (M+1), 569, 515, 478, 458, 421, 324, 312, 272, 220, 193, 152, 120. Anal. Calcd for $C_{34}H_{34}Cl_2N_4O$: C, 69.74; H, 5.81; N, 9.97. Found: C, 69.42; H, 5.86; N, 10.02%.

1-(3-(4-(3-(Benzyl(methyl)amino)-1-(4-chlorophenyl)propylamino)phenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 4f: m.p. 128-31°C. Yield: 61%. R_f value 0.32 (8.5:1.5, Hexane: Ethyl acetate); IR (KBr): 3439 (NH), 2948 (CH), 1662 (C=O), 1627 (C=N), 1460 (C=C, str), 1253 (C-O-C), 743 cm^{-1} (C-Cl); ^{1}H NMR (400MHz, $CDCl_3$): δ 1.47-1.509 (m, 2H, 2-CH_2), 2.08 (s, 3H, $COCH_3$), 2.29 (m, 3H, N-CH_3), 2.39-2.43 (d, 2H, 3-CH_2, *J* = 16 Hz), 3.31-3.42 (m, 2H, CH_2-benzyl), 3.59 (dd, 1H, CHa of CH_2), 3.73 (s, 3H, OCH_3), 3.81-3.84 (m, 1H, 1-CH), 3.98 (dd, 1H, CHb of CH_2), 4.67-4.71 (dd, 1H, CH-Ar), 5.09 (s, 1H, NH), 6.79-6.81 (d, 2H, 2′6′ NH-ArH, *J* = 8 Hz), 6.91-6.94 (d, 2H, 2′,6′, MeO-ArH, *J* = 12Hz), 7.28-7.85 (m, 13H, ArH); ^{13}C NMR (100MHz, $CDCl_3$): δ 24.8 (CH_3), 36.1 (2-CH_2), 37.2 (CH_2, pyrazoline ring), 41.9 (N-CH_3), 49.2 (3-CH_2), 55.9 (1-CH), 57.6 (CH, pyrazoline ring), 54.6 (OCH_3), 61.9 (N-CH_2), 112.3,124.5, 131.8, 148.7, 156.5 (phenyl ring C), 151.3 (C=N), 169.2 (C=O); MS: *m/z* 580 (M^+), 582 (M+1), 565, 511, 474, 460, 308, 293, 272, 209, 194, 152, 132, 120. Anal. Calcd for $C_{35}H_{37}ClN_4O_2$: C, 72.35; H, 6.37; N, 9.64. Found: C, 71.79; H, 6.32; N, 9.47%.

1-(3-(4-(3-(Benzyl(methyl)amino)-1-(4-bromophenyl)propylamino)phenyl)-5-(3-nitro phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 4g: m.p.118-20°C. Yield: 58%. R_f value 0.28 (8.5:1.5, Hexane: Ethyl acetate); IR (KBr): 3432 (NH), 2892 (CH), 1659 (C=O), 1636 (C=N), 570 cm^{-1} (C-Br); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72-1.79 (m, 2H, 2-CH_2), 2.04 (s, 3H, $COCH_3$), 2.22 (m, 3H, N-CH_3), 2.41-2.46 (d, 2H, 3-CH_2, *J* = 20 Hz), 3.46 (m, 2H, CH_2-benzyl), 3.61 (dd, 1H, CHa of CH_2), 3.85-3.89 (m, 1H, 1-CH), 3.92 (dd, 1H, CHb of CH_2), 4.27-4.32 (dd, 1H, CH-Ar), 5.11 (s, 1H, NH), 6.82-6.85 (d, 2H, 2′6′ NH-ArH, *J* = 12 Hz), 7.18-8.08 (m, 15H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.2 (CH_3), 35.5 (2-CH_2), 38.9 (CH_2, pyrazoline ring), 40.8 (N-CH_3), 50.2 (3-CH_2), 56.9 (1-CH), 58.0 (CH, pyrazoline ring), 60.8 (N-CH_2), 114.4, 120.5-134.2, 142.8, 147.2, 157.3 (phenyl ring C), 150.7 (C=N), 167.3 (C=O); MS: *m/z* 639 (M^+), 641 (M+1), 594, 579, 548, 525, 440, 371, 331, 323, 316, 220, 195, 194, 132, 120. Anal. Calcd for $C_{34}H_{34}BrN_5O_3$: C, 63.75; H, 5.31; N, 10.93. Found: C, 64.25; H, 5.11; N, 10.65%.

1-(3-(4-(3-(Benzyl(methyl)amino)-1-(4-bromophenyl)propylamino)phenyl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl)etanone, 4h: m.p.128-31°C. Yield: 67%. R_f value 0.41 (8.5:1.5, Hexane: Ethyl acetate); IR (KBr): 3427 (NH), 2887 (CH), 1656 (C=O), 1638 (C=N), 573 cm^{-1} (C-Br); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73-1.80 (m, 2H, 2-CH_2), 2.09 (s, 3H, $COCH_3$), 2.29 (m, 3H, N-CH_3), 2.51-2.56 (d, 2H, 3-CH_2, *J* = 20 Hz), 3.49 (m, 2H, CH_2-benzyl), 3.58 (dd, 1H, CHa of CH_2), 3.81-3.85 (m, 1H, 1-CH), 3.97 (dd, 1H, CHb of CH_2), 4.17-4.28 (dd, 1H, CH-Ar), 5.17 (s, 1H, NH), 6.87-6.90 (d, 2H, 2′6′ NH-ArH, *J* = 12 Hz), 7.11-8.18 (m, 15H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.01 (CH_3), 36.1 (2-CH_2), 39.02 (CH_2, pyrazoline ring), 41.6 (N-CH_3), 51.4 (3-CH_2), 57.3 (1-CH), 57.9 (CH, pyrazoline ring), 61.2 (N-CH_2), 113.4, 121.5-136.2, 141.8, 146.2, 158.3 (phenyl ring C), 151.1 (C=N), 168.0 (C=O); MS: *m/z* 630 (M^+), 632 (M+1). Anal. Calcd for $C_{34}H_{34}BrClN_4O$: C, 64.71; H, 5.39; N, 8.88. Found: C, 64.43; H, 5.21; N, 8.76%.

1-(3-(4-(3-(Benzyl(methyl)amino)-1-(4-bromophenyl)propylamino)phenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 4i: m.p. 135-37°C. Yield: 68%. R_f value 0.31 (8.5:1.5,

Hexane: Ethyl acetate); IR (KBr): 3427 (NH), 2886 (CH), 1656 (C=O), 1635 (C=N), 1455 (C=C, str), 1258 (C-O-C), 578 cm^{-1} (C-Cl); ^1H NMR (400MHz, $CDCl_3$): δ 1.86-1.93 (m, 2H, 2-CH_2), 2.17 (s, 3H, $COCH_3$), 2.36 (m, 3H, N-CH_3), 2.37 (m, 2H, 3-CH_2), 3.37 (m, 2H, CH_2-benzyl), 3.75 (dd, 1H, CHa of CH_2), 3.82 (s, 3H, OCH_3), 3.90-3.95 (m, 1H, 1-CH), 4.02-4.24 (dd, 1H, CHb of CH_2), 4.35-4.40 (dd, 1H, CH-Ar), 4.93 (s, 1H, NH), 6.68-6.72 (d, 2H, 2′6′ NH-ArH, J = 16 Hz), 6.85-6.90 (d, 2H, 2′,6′, MeO-ArH, J = 20 Hz), 7.11-7.99 (m, 13H, ArH); ^{13}C NMR (100MHz, $CDCl_3$): δ 26.2 (CH_3), 37.5 (2-CH_2), 39.2 (CH_2, pyrazoline ring), 50.9 (N-CH_3), 51.6 (3-CH_2), 54.7 (OCH_3), 56.3 (1-CH), 58.8 (CH, pyrazoline ring), 62.2 (N-CH_2), 113.6,124.6-133.6, 143.6, 148.9, 156.4 (phenyl ring C), 152.04 (C=N), 168.1 (C=O); MS: *m/z* 624 (M^+), 626 (M+1), 609, 579, 525, 510, 502, 490, 425, 316, 220, 195, 194, 132, 120. Anal. Calcd for $C_{35}H_{37}BrN_4O_2$: C, 67.21; H, 5.92; N, 8.97. Found: C, 68.19; H, 6.12; N, 8.17%.

In vitro antimicrobial assay/studies

Zone of inhibition of the synthesized compounds was determined by disc diffusion method[30,31] and Minimal Inhibitory Concentration (MIC) by two fold serial dilution method[32-34].

Conclusion

It is interesting to point out that all the compounds exhibiting better biological activities bear a benzyl(methyl)amino group in their molecular structure. Pyrazoline derivatives bearing nitro and methoxy substituents were found more active than other compounds. Compounds having morphilino propylamino function in their structure were found to exhibit little or no activity. The increased antimicrobial activity of pyrazoline may be due to its structure leading to a better fit at the receptor site. It is expected that compounds **4d**, **4g** and **4i** can be transformed into more effective compounds with the antimicrobial activity point of view by substituting some electron withdrawing groups in the molecular structure of pyrazoline.

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Conflict of Interest

None of the authors of the above manuscript has declared any conflict of interest which may arise from being named as an author in the manuscript.

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