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Synthesis and Biological Screening of Heterocyclic Ligands-Pyrazole Derivatives Metal Complexes

TASNEEM ULLAH SHEIKH[1,2], MUHAMMAD NAEEM KHAN[3,*], GHULAM HUSSAIN[4,5], MUHAMMAD MAKSHOOF ATHAR[4], MUHAMMAD ASHRAF[6], FAIZUL HASSAN NASIM[6], SHAFIA ARSHAD[1], KARAMAT MAHMOOD[1] and MISBAHUL AIN KHAN[1,4]

[1]Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[2]Department of Chemistry, Government College of Education, Afzalpur, Pakistan
[3]Applied Chemistry Research Center, PCSIR Laboratories Complex, Lahore, Pakistan
[4]Institute of Chemistry, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan
[5]Shafi Reso Chem, Ferozepur Road, Lahore, Pakistan
[6]Department of Biochemistry and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

*Corresponding author: changwani_1@yahoo.com

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The Cu(II), Ni(II) and Zn(II) complexes of 4-acyl-5-pyrazolones along with other novel complexes of 4-acyl-5-pyrazolones with the co-ligand 1,10-phenanthroline were prepared and screened for their antibacterial and antifungal activity. Zinc complexes **11**, **12** and **13** showed powerful antifungal activity while some of the nickel complexes **7-10** exhibited effective antibacterial activity against both Gram-positive and Gram-negative bacteria.

Keywords: Synthesis, Biological screening, Metal complexes, Pyrazole.

INTRODUCTION

Antipyrine, a pyrazolone, was the first synthetic compound used as an analgesic and antipyretic agent. Other pyrazolones have wider applications in pharmaceuticals, agrochemicals and act as dyes in photography and textile industry[1]. Phenylbutazone is another derivative having remarkable activity in various rheumatoid and arthritic conditions[2]. Appropriately substituted pyrazolones have also been used as starting materials to prepare other novel pyranopyrazol-4 and 6-ones[3].

Some complexes of 4-acetylpyrazolone have been mentioned in a recent literature review[4]. Our continuous interest in the chemistry of pyrazoles and the use of some of its derivatives as potential ligands for complexation with transition metals[5] has evoked our interest in exploring further studies. We would now like to report our present work on metal complexes of 4-acetyl-1-aryl-3-methylpyrazole-5-ones and their potential as antibacterial and antifungal agents.

EXPERIMENTAL

All chemicals were purchased from Fluka/Merck. Solvents were of Analytical grade. Melting points were determined using a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on FT-IR Perkin Elmer-Spectrum BX-II spectrometer and are reported as ν(cm^{-1}). Atomic absorptions were recorded on Perkin Elmer Analyst 800. Synergy HT (Bioteck, USA) 96-well plate reader was used for antimicrobial assays.

Preparation of ligands

Preparation of 4-acetyl-3-methyl-1-phenylpyrazole-5-one (Py) (1) and 4-acetyl-3-methyl-1-*p*-tolyl-5-one (TPy) (2): The ligands (Py, **1**) and (TPy, **2**) were prepared by the method reported by Jensen[6] with slight modifications[7] in solvent tetrahydrofuran and were re-crystallized from an acidified methanol-water mixture (1:1).

Synthesis of complexes

Method (A): Simple metal complexes: Py (**1**) and TPy (**2**) of 10 mM each were dissolved in 95 % methanol (25 mL) by warming in separate beakers. Prepared solutions of each ligand were added drop-wise with stirring to a solution of metal (II) chloride (10 mM of metal salt in 50 mL of 95 % methanol) in separate reaction vessel. Complexes precipitated out of solution after few minutes to 2 h of stirring. The precipitates were suction filtered, washed well with aqueous methanol (1:1), dried in air and stored in a desiccator over fused calcium chloride.

Method (B): Metal complexes with Co-ligands: Py (**1**) and TPy (**2**) of 10 mM each were dissolved in 25 mL of 95 % methanol by warming in separate beakers. Co-ligand (10 mM) was dissolved separately in 95 % methanol (25 mL). Prepared

solutions of ligand Py (**1**) and co-ligand-1,10-phenanthroline were added dropwise with stirring to a solution of the metal (II) chloride (10 mM of metal salt in 50 mL of 95 % methanol); after few minutes to 2 h stirring the complexes precipitated out of solution. The precipitates were suction filtered, washed well with aqueous methanol (1:1), dried in air and stored in a dessicator over fused calcium chloride. The same method was also adopted for ligand TPy (**2**).

Following complexes were thus prepared using methods A and B and their characteristics are presented in Table-1.

The complex $[Cu(Py)_2]$ **3** was prepared by following method A. After 0.5 h olive green product was obtained. Yield: (0.394 g; 80 %). The complex [Cu(Py)(Phen)] **4** was prepared by following method B. Green precipitates were obtained after 0.5 h. Yield: (0.354 g; 75 %). The complex $[Cu(TPy)_2]$ **5** was prepared by following method A. Green product obtained after 0.5 h. Yield: (0.406 g; 78 %). The complex [Cu(TPy) (Phen)] **6** was prepared by following method B. Green precipitates were obtained after 0.5 h. Yield: (0.365 g; 75 %). The complex $[Ni(Py)_2{\cdot}2H_2O]$ **7** was prepared by following method A. Light green precipitates were obtained after refluxing for 1 h. Yield: (0.351g; 67 %). The complex $[Ni(Py)(Phen){\cdot}2H_2O]$ **8** was prepared by following method B. White product obtained on refluxing for 1 h. Yield: (0.378 g; 75 %). The complex $[Ni(TPy)_2{\cdot}2H_2O]$ **9** was prepared by following method A. Light green product obtained after 1 h. Yield: (0.431 g; 78 %). The complex $[Ni(TPy)(Phen){\cdot}2H_2O]$ **10** was prepared by following method B. Light green precipitates obtained after 1 h. Yield: (0.389 g; 75 %). The complex $[Zn(Py)_2{\cdot}2H_2O]$ **11** was prepared by following method A. White product obtained after 2 h reflux. Yield: (0.424 g; 80 %). The complex $[Zn(Py)(Phen){\cdot}2H_2O]$ **12** was prepared by following method B. White product obtained after 2 h reflux. Yield: (0.397 g; 78 %). The complex $[Zn(TPy)_2{\cdot}2H_2O]$ **13** was prepared by following method A. Light pink product obtained after 2 h reflux. Yield: (0.457 g; 82 %). The complex $[Zn(TPy)(Phen){\cdot}2H_2O]$ **14** was prepared by following method B. White product obtained after 2 h reflux. Yield: (0.398 g; 76 %).

Antibacterial Activity: The antibacterial activity was performed in sterile 96-wells micro plates under aseptic environments after standardization of the methods[8,9]. Clinical isolates obtained from Pathology Department, Quaid-i-Azam Medical College, Bahawalpur were tested. Four Gram-negative (*Shigella sonnei*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and two Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) were included in the study. The organisms were maintained on stock nutrient broth culture. The test compounds and reference drugs were dissolved in DMSO. Overnight maintained fresh bacterial nutrient broth culture (180 µL) was poured into wells containing 20 µg/well of the test compound to a total volume of 200 µL/well. The initial absorbance of the added culture per well was strictly maintained between 0.12-0.19 at 540 nm which gave 10^6 CFU/mL. Incubation was done at 37 °C for 16-24 h. Absorbance was measured at 540 nm using micro plate reader, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = 100\ (X - Y)/X$$

where X is absorbance of control with bacterial culture and Y is absorbance of test sample. Results are mean of triplicate (n = 3, mean ± s.d.). Ciprofloxacin, gentamycin and ampicillin were used as positive and solvent as negative controls. MIC was measured from the data obtained after suitable dilutions (5-40 µg/well) of drugs and standards and results were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software and data expressed as MIC_{50} and MIC_{90}.

Antifungal activity: Antifungal activity was performed in sterile 96-wells micro plates as described[10]. *Candida albicans* was isolated from clinical samples in the Department of Pathology Quaid-i-Azam Medical College, Bahawalpur. The organism was maintained on stock Saboraud dextrose agar culture medium. Overnight maintained fungal culture (180 µL) was poured into wells containing 20 µg/well test compound. The initial absorbance of the culture was strictly maintained between 0.54-0.56 at 405 nm. The total volume in each well was kept to 200 µL. The incubation was done at 23 °C for 24 h. Absorbance was measured at 405 nm micro plate reader before and after incubation and the difference was noted as an

TABLE-1
CHARACTERISTICS OF SYNTHESIZED COMPLEXES (**1-14**)

S. No.	Ligand/complexes	Colour	m.p (°C)*	IR(C=O) ν cm^{-1}	Metal (%)		Formula**
					Calculated	Found	
1	**1**	Yellow	58	1621	-	-	$C_{12}H_{12}N_2O_2$
2	**2**	Yellow brown	90-92	1624	-	-	$C_{13}H_{14}N_2O_2$
3	**3**	Olive green	300	1606	12.82	12.76	$[Cu(Py)_2]$
4	**4**	Green	234	1606	13.33	13.48	[Cu(Py)(Phen)]
5	**5**	Green	292	1592	12.17	12.31	$[Cu(TPy)_2]$
6	**6**	Green	268	1593	12.95	12.70	[Cu(TPy)(Phen)]
7	**7**	Light green	190	1614	11.19	10.83	$[Ni(Py)_2.2H_2O]$
8	**8**	White	216	1612	11.56	11.40	$[Ni(Py)(Phen).2H_2O]$
9	**9**	Light green	276 d	1623	10.10	10.04	$[Ni(TPy)_2.2H_2O]$
10	**10**	Light green	220	1600	11.24	11.08	$[Ni(TPy)(Phen).\ 2H_2O]$
11	**11**	White	196	1613	12.29	12.24	$[Zn(Py)_2.2H_2O]$
12	**12**	White	252	1615	12.72	12.65	$[Zn(Py)(Phen).2H_2O]$
13	**13**	Light Pink	194	1621	11.68	11.86	$[Zn(TPy)_2.2H_2O]$
14	**14**	White	310	1603	12.36	12.20	$[Zn(TPy)(Phen).2H_2O]$

Phen = 1,10-phenanthroline; d* = Decomposition Point; Py** = 4-acetyl-3-methyl-1-phenylpyrazole-5-one; TPy = 4-acetyl-3-methyl-1-p-tolyl-5-one

index of fungal growth. The percent inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = 100\ (X - Y)/X$$

where X is the absorbance of control with fungal culture and Y is the absorbance of test sample. Results are mean of triplicate (n = 3, mean ± s.d.). Fluconazole and clotrimazole were taken as standards. Minimum inhibitory concentration to kill 50 or 90 % microbes (MIC_{50} and MIC_{90}) was measured with suitable dilutions (5-50 μg/well) and results were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software.

RESULTS AND DISCUSSION

Some metal salts like $CuCl_2$ have been reported to possess antibacterial activity. This activity is also known to be preserved/ enhanced on complex formation with various ligands[4]. Ligand (**1**) is, inherently a virtual β-diketo compound and thus a suitable candidate for complexation with metals. It is known to exist in both enolic and ketonic tautomeric forms[11]. Presence of an acetyl at 4-position helps stabilize the hydroxyl form through hydrogen bonding. All the metal complexes of ligand (Py) (**1**) and (TPy) (**2**) together with the co-ligand 1,10-phenanthroline show a displacement of carbonyl frequency confirming complexation through the participation of 5-hydroxy and 4-acetyl functional groups (Fig. 1).

Antibacterial and antifungal activities of compounds (1-14): MIC_{50} and MIC_{90} values (minimum inhibitory concentration to kill 50 and 90 % microbial population) of compounds against two Gram-positive and four Gram-negative bacteria are given in Table-2a & 2b. Lower the MIC value better is the antimicrobial agent. Data indicates that compounds **1**, **7**, **9** possess effective antibacterial activity as the standard drugs against Gram-positive bacteria (*S. aureus* and *B. subtilis*). MIC_{50} values of these compounds against *S. aureus* are 10.33 ± 0.11, 10.44 ± 0.11 and 8.73 ± 0.23 μg/mL, respectively; whilst for ciprofloxacin, gentamycin and ampicilin, MIC_{50} values are 9.42 ± 0.11, 8.42 ± 0.12 and 10.69 ± 0.06 μg/mL, respectively. Similar profiles are exhibited against *B. subtilis*. Other compounds showed moderate antibacterial activity with comparatively higher MIC values. However, compounds **2**, **3** showed no activity whatsoever.

TABLE-2A
ANTIBACTERIAL ACTIVITY OF COMPOUNDS (**1-14**)

No.	Compound	*Staphylococcus aureus* Gram-positive		*Bacillus subtilis* Gram-positive		*Shigella sonnei* Gram-negative	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
1	1	10.33 ± 0.11	29.39 ± 0.11	10.32 ± 0.04	34.29 ± 0.16	8.89 ± 0.17	46.24 ± 0.18
2	**2**	13.62 ± 0.18	32.19 ± 0.26	18.17 ± 0.25	51.86 ± 0.26	13.74 ± 0.26	54.00 ± 1.01
3	**3**	13.96 ± 0.11	28.94 ± 0.12	>20	>40	17.75 ± 0.13	48.68 ± 0.26
4	**4**	13.27 ± 0.23	29.36 ± 0.23	12.66 ± 0.10	29.33 ± 0.29	9.89 ± 0.23	29.65 ± 0.29
5	**5**	13.78 ± 0.19	27.47 ± 0.22	18.92 ± 0.29	31.46 ± 0.18	10.66 ± 0.24	29.23 ± 0.23
6	**6**	13.94 ± 0.23	28.09 ± 0.19	19.00 ± 0.22	37.47 ± 0.15	7.70 ± 0.22	33.17 ± 0.03
7	**7**	10.44 ± 0.11	48.48 ± 0.19	7.08 ± 0.01	35.09 ± 0.38	8.73 ± 0.02	31.46 ± 0.17
8	**8**	13.91 ± 0.14	29.5 ± 0.27	18.17 ± 0.19	51.86 ± 0.01	8.92 ± 0.01	49.22 ± 0.28
9	**9**	8.73 ± 0.23	24.19 ± 0.19	11.17 ± 0.18	27.81 ± 0.18	19.89 ± 0.12	48.04 ± 0.28
10	**10**	13.15 ± 0.28	27.27 ± 0.11	14.69 ± 0.17	29.73 ± 0.11	15.54 ± 0.34	29.15 ± 0.11
11	**11**	13.16 ± 0.22	28.10 ± 0.34	13.61 ± 0.11	47.71 ± 0.14	13.92 ± 0.34	35.49 ± 0.04
12	**12**	12.36 ± 0.03	29.65 ± 0.15	19.10 ± 0.26	47.37 ± 0.20	13.92 ± 0.25	48.67 ± 0.63
13	**13**	14.66 ± 0.17	27.68 ± 0.28	19.89 ± 0.25	48.04 ± 0.18	7.65 ± 0.27	49.22 ± 0.11
14	**14**	17.00 ± 0.01	38.68 ± 0.17	20.89 ± 0.25	47.04 ± 0.18	13.58 ± 0.25	29.47 ± 0.44
Ciprofloxacin		9.42 ± 0.11	23.49 ± 0.26	8.36 ± 0.13	20.07 ± 0.24	7.31 ± 0.08	23.95 ± 0.19
Gentamycin		8.42 ± 0.12	23.94 ± 0.25	10.36 ± 0.13	22.07 ± 0.22	9.31 ± 0.18	28.87 ± 0.21
Ampicilin		10.69 ± 0.06	24.61 ± 0.06	11.66 ± 0.14	31.98 ± 0.21	11.98 ± 0.13	34.34 ± 0.14

MIC_{50} and MIC_{90} values (μg/mL) are mean of three independent determination (mean ± s.d., n = 3)

TABLE-2B
ANTIBACTERIAL ACTIVITY OF COMPOUNDS (**1-14**)

No.	Comp.	*Pseudomonas aeruginosa* Gram-negative		*Salmonella typhi* Gram-negative		*Escherichia coli* Gram-negative	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
1	**1**	12.97 ±0.22	28.17±0.90	12.11±0.12	26.73±0.14	17.93±0.31	28.02±0.50
2	**2**	>20	>40	>20	>40	12.08±0.42	29.55±0.51
3	**3**	>20	>40	14.52±0.21	36.72±0.21	13.24±0.33	33.92±0.71
4	**4**	12.43 ±0.29	26.43±0.28	12.16±0.43	26.26±0.44	20.8 ±0.23	31.35±0.63
5	**5**	14.21 ±0.21	29.02±0.31	12.73±0.23	26.81±0.18	12.73±0.02	31.32±0.65
6	**6**	12.35 ±0.33	26.14±0.28	11.84±0.13	26.59±0.71	12.62±0.35	32.36±0.41
7	**7**	11.73 ±0.32	28.03±0.45	10.39±0.30	24.07±0.31	16.04±0.11	48.30±0.71
8	**8**	10.99 ±0.61	26.14±0.25	11.54±0.14	27.00±0.13	11.83±0.45	49.14±0.36
9	**9**	12.27 ±0.20	26.15±0.34	11.46±0.24	26.37±0.22	16.04±0.07	38.15±0.19
10	**10**	10.83 ±0.27	24.22±0.03	10.65±0.14	24.12±0.02	12.91±0.22	53.00±0.99
11	**11**	12.63 ±0.15	34.89±0.20	11.13±0.24	26.33±0.11	12.51±0.40	49.28±0.10
12	**12**	11.47 ±0.35	26.73±0.21	13.36±0.21	34.96±0.09	10.87±0.52	29.12±0.53
13	**13**	12.36 ±0.21	26.19±0.24	10.42±0.20	26.77±0.23	14.06±0.12	48.11±0.31
14	**14**	13.43 ±0.77	34.42±0.13	12.61±0.33	28.98±0.57	10.79±0.70	23.86±0.70
Ciprofloxacin		10.33 ± 0.21	24.91±0.29	11.66±0.13	23.59±0.18	8.21 ±0.02	24.09±0.19
Gentamycin		10.89 ± 0.11	23.15±0.18	11.21±0.31	23.81±0.26	9.29 ± 0.02	23.77±0.19
Ampicilin		12.33 ± 0.15	32.91±0.11	10.85±0.16	26.45±0.22	11.32±0.13	31.09±0.23

MIC_{50} and MIC_{90} values (μg/mL) are mean of three independent determination (mean ± s.d., n = 3)

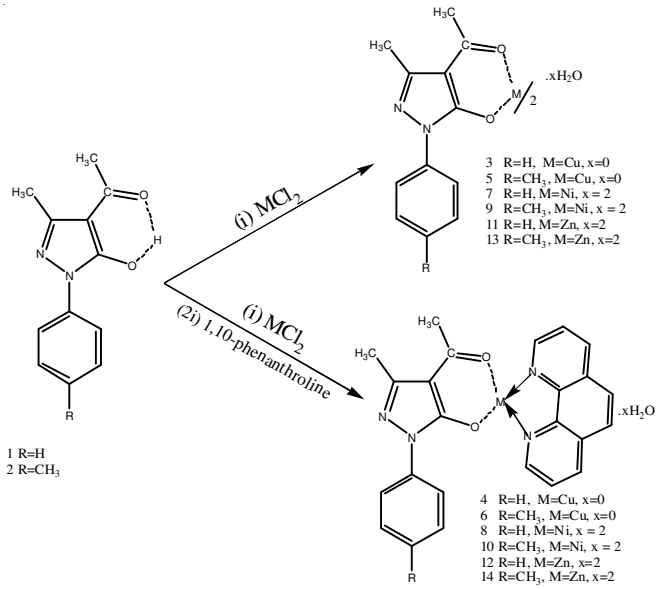

Fig. 1. General scheme for the metal complexation with ligands and co-ligands

When data was analyzed against Gram-negative bacteria: compounds **1**, **6**, **7**, **8** and **13** inhibited *S. sonnei* growth as effectively as did the standard drugs. Compounds **7**, **8**, **10** and **12** exhibited MIC values close to the standards for *P. aeruginosa*. Compounds **6-11**, **13** were effective to inhibit growth of *S. typhi* and compounds **8**, **12**, **14** had the lowest MIC_{50} values against *E. coli*. These results show that MIC values of most of these compounds are close to the MIC values (MIC_{50} and MIC_{90}) of standard antibiotics and therefore these molecules have potential role as antibacterial agents.

When antifungal activity was measured against *C. albicans*, compounds **11**, **12** and **13** were the most potent antifungal agents with MIC_{50} values of 10.86 ± 0.10, 10.44 ± 0.01, 10.47 ± 0.09 μg/mL, respectively, compared with the standard drugs fluconazole (11.69 ± 0.02 μg/mL) and clotrimazole (9.63 ± 0.23 μg/mL). Other compounds **4**, **7**, **8** also exhibited the lowest MIC values against *C. albicans* (Table-3).

From the foregoing it is concluded that the zinc complexes **11-13** were very good antifungal agents while for the antibacterial activity against Gram-positive and Gram-negative bacteria. The nickel complexes **7-10** seem to be more effective than copper and zinc complexes. Although copper complexes have been reported as good antibacterials.

Conclusion

Two 4-acetyl-5-pyrazolones and twelve novel complexes were synthesized. Most of these complexes were found to possess effective antibacterial activity against Gram-positive and Gram-negative bacteria when compared with the standard drugs. The antifungal activity of the complexes was also very close to the standard antifungal drugs.

TABLE-3
ANTIFUNGAL ACTIVITY OF COMPOUNDS (**1-14**);
MIC_{50} AND MIC_{90} VALUES (μg/mL) ARE MEAN OF THREE INDEPENDENT DETERMINATIONS (MEAN ± s.d, n = 3)

No	Compound	*Candida albicans*	
		MIC_{50}	MIC_{90}
1	**1**	12.22 ± 0.42	31.97 ± 0.26
2	**2**	16.04 ± 0.28	44.97 ± 0.31
3	**3**	16.47 ± 0.10	38.74 ± 0.23
4	**4**	11.64 ± 0.11	26.66 ± 0.18
5	**5**	>20	>40
6	**6**	19.42 ± 0.02	48.74 ± 0.01
7	**7**	11.66 ± 0.11	26.54 ± 0.28
8	**8**	11.26 ± 0.62	26.69 ± 0.84
9	**9**	15.29 ± 0.28	34.33 ± 0.27
10	**10**	12.52 ± 0.24	27.41 ± 0.29
11	**11**	10.86 ± 0.10	26.52 ± 0.23
12	**12**	10.44 ± 0.01	29.76 ± 0.17
13	**13**	10.47 ± 0.09	27.77 ± 0.19
14	**14**	>20	>40
15	Fluconazole	11.69 ± 0.02	26.55 ± 0.17
	Clotrimazole	9.63 ± 0.23	24.39 ± 0.33

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