Int. J. Chem. Sci.: 12(1), 2014, 136-144
ISSN 0972-768X
www.sadgurupublications.com

SYNTHESIS, CHARACTERIZATION AND PRELIMINARY ANTIMICROBIAL SCREENING OF (2Z, 4Z)-4-(PYRIMIDIN-2-YLIMINO) PENT-2-EN-2-OL (PIP) AND ITS COPPER (II) AND CHROMIUM (III) COMPLEXES

U. S. ORUMA[*], J. N. ASEGBELOYIN, F. U. EZE and K. F. CHAH[a]

Department of Pure and Industrial Chemistry, University of Nigeria, NSUKKA, NIGERIA
[a]Department of Veterinary Pathology and Microbiology, University of Nigeria,
NSUKKA ENUGU STATE, NIGERIA

ABSTRACT

The condensation of 2-aminopyrimidine with pentan-2, 4-dione yielded (2Z, 4Z)-4-(pyrimidin-2-ylimino) pent-2-en-2-ol (PIP). Its Cu (II) and Cr (III) complexes were synthesized and characterized via stoichiometric ratio of combination, conductivity measurement, UV/Visible and infrared spectroscopic studies. The ligand was further characterized by elemental analysis, ^{1}H and ^{13}C NMR. Monobasic bidentate coordination of the ligand in the metal complexes was established on the basis of analytical and spectra data. The ligand and complexes were screened for *in vitro* antimicrobial activity against multi-resistant bacterial strains: *Escherichia coli* (ECO 14 and ECO 6), *Pseudomonas aeruginosa* (Ps 34) and *Staphylococcus aureus* (G101 and G72b). The ligand and Cr (III) complex showed activity only on *Pseudomonas aeruginosa* while the Cu (II) complex was active against the strains of *Escherichia coli* (ECO6 and ECO14) and *Staphylococcus aureus* (G101).

Key words: 2-aminopyrimidine, pentan-2, 4-dione, complexes, antimicrobial activity.

INTRODUCTION

Heterocyclic compounds are known to possess variety of physiological activities and are important class of compounds in medicinal chemistry[1]. Heterocyclic rings are found in several compounds, such as drugs, dye stuffs, enzymes, genetic materials DNA & RNA, vitamin B complex etc. Pyrimidine is the parent substance of a large group of heterocyclic compounds and plays a vital role in many biological processes as found in nucleic acids, several vitamins, co-enzymes and purines[2]. Pyrimidine itself is not found in nature but substituted pyrimidines and compounds containing the pyrimidine ring system are widely

[*]Author for correspondence; E-mail: orumau@yahoo.com, susan.oruma@unn.edu.ng

distributed in nature[3]. A variety of natural products such as alkaloids also contain pyrimidine ring system, these include hypoxanthine, xanthine, theophyline and theobromine[3]. Several heterocyclic derivatives with pyrimidine moiety have been prepared and reported in literature with appreciable biological activities[4-10]. However, metal complexation of these synthesized compounds were relatively less studied. It has been established that interaction of metal ions with organic antibiotics enhances or suppresses antimicrobial activity[11]. Based on these, we synthesized (2z, 4z)-4-(pyrimidin-2-ylimino)pent-2-en-2-ol (PIP), derived from 2-aminopyrimidine and pentan-2, 4-dione and its Cu (II) and Cr (III) complexes. These compounds were screened for possible antimicrobial activity on multi-resistant bacteria strains.

EXPERIMENTAL

All the reagents and solvents used were analytical reagent grade and were used as supplied unless otherwise stated. The melting point of the ligand and complexes were determined using Fisher-Johns melting point apparatus. Electronic spectra were recorded on a UV-2500 PC series spectrophotometer. FTIR spectra of the compounds were run as Nujol mulls on FTIR-8400S spectrophotometer (Shimadzu) in the range 4000-200 cm^{-1}. The ^{1}H NMR and ^{13}C NMR of the ligand were recorded in DMSO-d_6 on JEOL AS 400 (400 MHz) NMR spectrometer using TMS as internal standard at the Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Scotland, UK. Elemental analysis of the ligand was recorded on Perkin Elmer 2400 CHN analyser. The molar conductance in DMSO was carried out using conductivity meter model WTWLS 90 digital.

Synthesis of (2Z, 4Z)-4-(pyrimidin-2-ylimino) pent-2-en-2-ol (PIP)

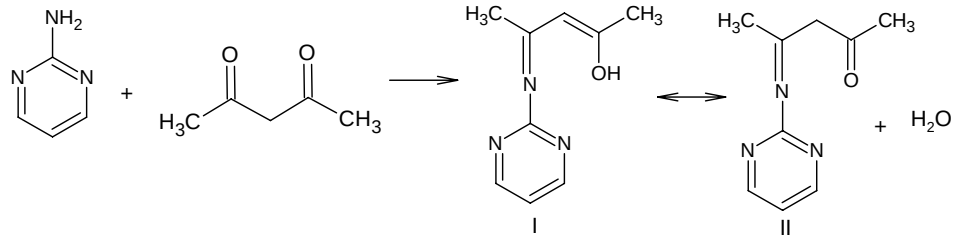
Ethanolic solution of 2-aminopyrimidine (1.90 g; 0.02 mol) was refluxed with ethanolic solution of pentan-2, 4-dione (1 g; 0.01 mol) for 6 hr. The resultant pale yellow solution was cooled and yellowish product was obtained, filtered and recrystallized from hot ethanol[12].

Anal. Calcd. for $C_9H_{11}N_3O$: C = 61.02, H = 6.21, N = 23.73%. Found: C = 60.80, H = 6.11, N = 23.50%

Syntheses of Cu (II) and Cr (III) metal complexes of PIP

Ethanolic solution of copper (II) sulphate pentahydrate (0.50 g; 0.002 mol) was added to (0.71 g 0.004 mol) of PIP in 50 cm^3 of ethanol. The mixture was refluxed for 2 hr. It was concentrated to half its volume and kept in a dessicator for two days over anhydrous $CaCl_2$. The green precipitates formed were filtered off, washed with ethanol and dried. In the

same manner, chromium (III) nitrate nanohydrate (0.80 g; 0.002 mol) was added to PIP (0.71 g; 0.004 mol) to prepare the Cr (III) complex.



Scheme 1: Synthesis of PIP

Mole ratio determination of Cu (II) and Cr (III) complexes by Job's continuous variation method

A 0.002 M solution of the metal salts $CuSO_4.5H_2O$ and $Cr(NO_3)_3.5H_2O$ were prepared by suitable dilution of the standard solution. A 0.002 M solution of PIP was prepared in absolute ethanol. Varying volumes of the metal salt solution were mixed with various volumes of the ligand in such a way that the total volume of the mixture at each run equals 10 mL as shown below[13].

Volume of metal solution (V_m) : 0 1 2 3 4 5 6 7 8 9 10

Volume of the ligand solution (V_l) : 10 9 8 7 6 5 4 3 2 1 0

The mixture was subjected to heat to attain a similar complexation condition. The absorbance was taken at 318 and 314 nm for Cr (III) and Cu (II) complexes, respectively. The absorbance was then plotted against the volume fraction, which is equal to the mole ratio of the reactants.

$$\text{Volume fraction} = V_m/V_m + V_l$$

Antimicrobial activity

The antibacterial activity of the newly synthesized ligand and complexes were evaluated by the Agar well diffusion method in DMSO[14,15]. The bacteria strains used were multi-resistant namely: *Escherichia coli* (ECO 14 and ECO 6), *Pseudomonas aeruginosa* (Ps 34) and *Staphylococcus aureus* (G101 and G72b), isolated under clinical conditions. The test organisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5 mm in diameter were made on the nutrient agar using a sterile cork borer. To each well, 0.2 mL of each test extract was introduced. The plates were incubated at 37^oC for 24 hours. The zones of inhibition were then recorded.

RESULTS AND DISCUSSION

The ligand and complexes are air stable, coloured solid with high melting points. The molar conductivities of the complexes (10^{-3} M) in DMSO suggest that the complexes are non-electrolytes, since their values are low.[16,17] Job's continuous variation gave 1:2 metal to ligand stoichiometry for both complexes. The complexes were soluble in dimethyl sulphoxide and dimethyl formamide but insoluble in hexane and carbon tetrachloride. The result of some physical properties of PIP and its Cu (II) and Cr (III) is shown in Table 1.

Table 1: Physical properties of PIP and its Cu (II) and Cr (III) Complexes

Compound	Yield (%)	Colour	Physical state	Melting point (^oC)	Ohm^{-1} cm^2 mol^{-1}
PIP	71	Yellow	Crystalline	170	-
$Cu(H_2O)_2(PIP)_2$	55.7	Green	Powdery	264	29.8
$Cr(NO_3)(H_2O)(PIP)_2$	64	Reddish-brown	Powdery	272	18.4

The elemental analysis of the ligand shows that the amount of carbon, hydrogen and nitrogen are close to the experimentally determined values.

Electronic spectra

The relevant electronic spectra data of the ligand and metal complexes are presented in Table 2. The bands around 33, 003 and 36, 101 cm^{-1} in the spectrum of the ligand have been assigned to n-π* and π-π* transitions, respectively.

Table 2: Electronic spectra data of PIP and metal complexes

Compound	Bands (nm)	Bands (cm^{-1})	Assigned transitions
PIP	277	36101	π-π*
	303	33003	n-π*
$Cr(NO_3)(H_2O)(PIP)_2$	729	13717	d-d
	893	11198	d-d
$Cu(H_2O)_2(PIP)_2$	729	13717	$^2B_{1g} \rightarrow {}^2B_{2g}$
	897	11148	$^2B_{1g} \rightarrow {}^2A_{1g}$

In the electronic spectrum of a six coordinate Cu (II) complex, three spin allowed transitions are expected in the visible and near IR region. The three bands assignable to

$^2B_{1g} \rightarrow {}^2A_{1g}$, $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2E_g$ have very low energy difference and are often difficult to resolve. The two bands observed around 11, 148 and 13717 cm^{-1} in the spectrum of $Cu(H_2O)_2(PIP)_2$ have been assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$, and $^2B_{1g} \rightarrow {}^2B_{2g}$, respectively. No band was observed below 10,000 cm^{-1}, which ruled out the possibility of the complex being tetrahedral[18-20]. Bands assignable to d-d transitions were observed around 13717 and 11198 cm^{-1} in the Cr (III) complex.

FT-Infrared spectra

The important infrared vibrational frequencies of the ligand and complexes are displayed in Table 3. The IR spectra of the ligand exhibits a strong band at 1650 cm^{-1}, which has been assigned to ν(C=N)[21]. This band shifted to higher (Cu (II)) and lower (Cr (III)) frequencies in the metal complexes, which indicates the participation of –C= N- nitrogen in coordination with the metal ions[22,23]. This is further supported by the appearance of new weak bands at 524 and 550 cm^{-1} assignable to ν (M-N)[24]. A broad band in the region 3345-3134 cm^{-1}, due to the presence of hydroxyl group was observed in the IR spectra of the ligand. This suggest that the ligand was isolated in the enol form. The medium band at 1368cm^{-1} assigned to enolic ν(C-O) in the ligand, shifted to higher frequencies in the metal complexes, which indicates the ligation through the enolic oxygen[25,26]. Also, in the spectra of the complexes, there exist weak bands at 613 and 653 cm^{-1} assignable to ν(M-O) vibration[27,28]. The presence of coordinated water in the complexes was revealed by the appearance of broad bands in the region 3408 cm^{-1} for Cu (II) complex and 3482 cm^{-1} in the spectra of the Cr (III) complex[21,25].

Table 3: IR spectra of PIP and its Cu (II) and Cr (III) complexes

Compound	O-H	C=N	C-O	M-O	M-N
Ligand	3345 br	1650 s	1368 m	-	-
$Cu(H_2O)_2(PIP)_2$	3408 br	1657 s	1370 m	653 w	524 w
$Cr(NO_3)(H_2O)(PIP)_2$	3482 br	1630 s	1378 m	613 w	550 w

^{1}H and ^{13}C NMR spectra of PIP

The ^{1}H NMR spectrum of PIP showed strong signals at δ 2.50 (3H, s), 3.44 (3H, s), 6.52-6.55 (1H, t), 6.62 (1H, s), 8.16-8.24 (2H, d) and 12.50 (1H, s) ppm. These have been assigned to -CH_3 protons (5, 9), pyrimidine proton (2), pentan-2, 4-dione proton (7), pyrimidine protons (1 & 3)[29,30] and -OH proton[21,28,29], respectively. The broad singlet peak at 12.50 ppm confirms the existence of PIP in its enol form (**Scheme 1**).

The ^{13}C NMR spectrum of PIP showed signals at 40.02, 110.69, 158.58 and 164.13 ppm assigned to -CH_3 carbons (5, 9), pentan-2, 4-dione carbons (7,8), pyrimidine carbons (1-4), and –C= N carbon (6), respectively[22].

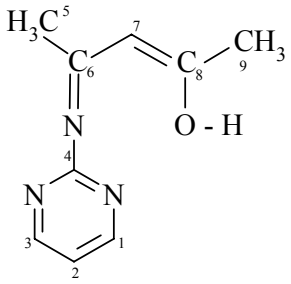


Fig. 1: Structure of PIP showing carbon numbering

Antimicrobial activity

The antimicrobial activities of the ligand and its metal complexes are recorded in Table 4. The antimicrobial activities of the ligand and its complexes were tested *in vitro* against multi-resistant bacterial strains isolated under clinical conditions. The bacteria strains used namely: *Escherichia coli* (ECO 14 and ECO 6), *Pseudomonas aeruginosa* (Ps 34) and *Staphylococcus aureus* (G101 and G72b) have been found to be resistant to oxacillin, cefoxitin, mampicillin, erythromycin, clindamycin, kanamycin, tetracycline, tobramycin, septrin and chloramphenicol[15,31].

Table 4: Antimicrobial activity of PIP and its Cu (II) and Cr (III) complexes. (zone of inhibition in mm)

Compound	ECO 6	ECO 14	PS34	G101	G72b
PIP	-	-	13	-	-
$Cu(H_2O)_2(PIP)_2$	12	9	-	10	-
$Cr(NO_3)(H_2O)(PIP)_2$	-	10	10	-	-

ECO = *Escherichia coli*, PS = *Pseudomonas aeruginosa*, G = *Staphylococcus aureus*

The ligand showed negligible activity against *Escherichia coli* (ECO 6 and ECO 14) and *staphylococcus aureus* (G101 and G72b) but moderate activity on *Pseudomonas aeruginosa* (Ps 34). The Cu (II) complex showed moderate activity on ECO 6, ECO 14 and G101. Bearing in mind that these micro-organisms are multi-resistant, $Cu(H_2O)_2(PIP)_2$ can serve as a chemotherapeutic agent.

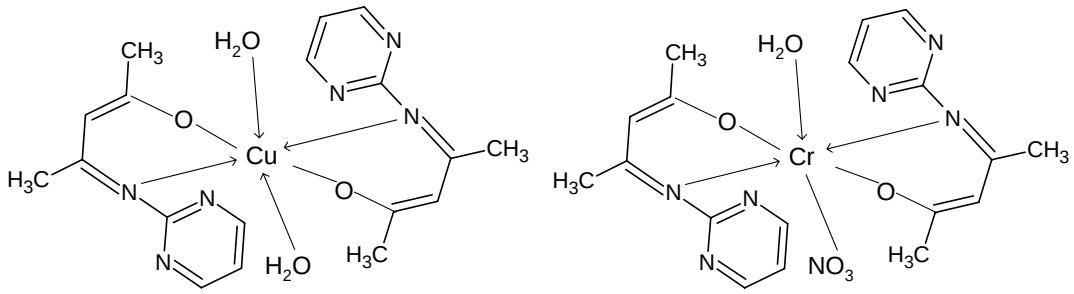


Fig. 2: Proposed structures of the metal complexes

CONCLUSION

(2Z, 4Z)-4-(pyrimidin-2-ylimino) pent-2-en-2-ol (PIP) derived from 2-amino-pyrimidine and Pentane-2, 4-dione and its Cu (II) and Cr (III) complexes were synthesized. Spectral data indicated that PIP exist in its enol form and coordinates as a monobasic bidentate ON donor via the -C=N nitrogen and enolic oxygen atom. Octahedral geometry (Fig. 2) was assigned to the metal complexes. Of all the compounds studied, Cu $(H_2O)_2$ PIP exhibited the highest antimicrobial activity against the multi-resistant bacterial strains used.

ACKNOWLEDGEMENT

U. S. Oruma is grateful to Dr. John Igoli for running the ^{1}H and ^{13}C NMR spectra and elemental analysis of the ligand at Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Scotland, UK.

REFERENCES

1. S. J. Vaghasia and V. H. Shah, J. Serb. Chem. Soc., **72**, 109 (2007).
2. S. K. Hadjikakou, M. A. Demertzis, M. Kubicki and D. Kovala-Demertzis, Appl. Organomet. Chem., **14**, 727 (2000).
3. D. N. Dhas, The Chemistry of Chalcones and Related Compounds, Wiley Interscience (1981).
4. G. Chattopadhyay, D. Saha, P. S. Ray, S. Naskar and S. Sarkar, Indian J. Chem., **49B**, 1229 (2010).
5. M. S. Baseer, G. K. Shan Karaiah, S. C. Santosh, G. M. Gajanan, S. Y. Omprakash and S. D. Bhaskar, J. Chem. Pharm. Res., **3(2)**, 435 (2011).
6. H. M. Hassan and A. A. Farrag, J. Chem. Pharm. Res., **3(2)**, 776 (2011).

7. M. Seki, K. H Matsumoto Kazuo, J. Org. Chem., **58(23)**, 6354 (1993).

8. M. Suresh, P. Lavanya, K. Vasu, D. Sudhakar and Venkata Rao C., J. Chem. Pharm. Res., **2(2)**, 82 (2010).

9. P. Lavanya, V. K. Veena, K. Vasu, M. Suresh, R. V. Jhansi and C. R. Venkata, J. Chem. Res., **2(3)**, 25 (2010).

10. V. M. Ravi, A. Mujahid and R. A. Srinivas, Synlett, **1**, 67 (2003).

11. M. S. Igbal, A. R. Ahmed, M. Sabir and B. M. Asaed, J. Pharm. Pharmacol, **51**, 371 (1999).

12. N. Raman, V. Muthuraj, S. Ravichandran and A. Kulandiasamy, Indian Acad. Sci (Chem. Sci.), **115**, 161 (2003).

13. G. R. Jani, K. B. Vyas, K. S. Nimavat and J. Franco, Der Pharma Chemica, **1(2)**, 304 (2009).

14. C. H. Collins, P. M. Lynes and J. M. Grange, Microbiological Methods, 7th Edition, Butterwort-Heineman Ltd, Britian (1995) pp. 175-190.

15. K. F. Chah, I. C. Agbo, S. Somalo, V. Estepa and C. Torrez, J. Basic Micribiology, **50**, 1 (2010).

16. R. Sreekala and K. M. Yusuf, Synth. React. Inorg. Met. Org. Chem., **24(10)**, 1773 (1994).

17. N. Pooja and R. V. Singh, Appl. Organomet. Chem., **18**, 221 (2004).

18. S. Chandra and A. Kumar, Spectrochimica Acta, part A, **66**, 1347 (2007).

19. U. L. Kala, S. Suma, M. R. P. Kurup, S. Krishnan and P. J. Rohith, Polyhedron, **26**, 1427 (2007).

20. V. V. Pavlishchuk, S. P. Koloyilov, A. W. Addisou, R. J. Butche and E. Sinu, J. Chem. Soc., Dalton Trans., 335 (2000).

21. N. Raman, Y. P. Raja and A. Kulandaisamy, Proc. Indian Acad. Sci. (Chem. Sci.) **113(3)**, 183 (2001).

22. U. S. Oruma and P. O. Ukoha, J. Chem. Soc. Nig., **37(2)**, 4 (2012).

23. M. U. Hassan, Z. H. Chochan and C. T. Supuran, Main Group Metal Chemistry, **25**, 291 (2002).

24. B. U. Muhammed, P. Sayudevi and K. Krishnankutty, Argent. Chem. Soc., **97(2)**, 31 (2009).

25. K. Rathore, R. Singh and H. B. Singh, E-J. Chem., **7(S1)**, S566 (2010).

26. S. S. Konstantinovic, B. C. Radovanovic, Z. Cakic and V. Vasic, J. Serb. Chem. Soc., **68(8-9),** 641 (2003).

27. C. S. Andotra, J. Khajuria, B. S. Singh and S. Singh, J. Indian Chem. Soc., **70(3)**, 266 (1993).

28. M. M. Mashaly, Z. H. Abd-Elwahab and A. A. Faheim, J. Chinese Chem. Soc., **51**, 905 (2004).

29. M. O. Abd El-Fatah, S. A. Mayada, S. S. Mamdouh, M. E. Ahmed and I. M. Sahar, J. Korean Chem. Soc., **54(4)**, 406 (2010).

30. H. M. Hassan and A. A. Farrag, J. Chem. Pharm. Res., **3(2)**, 778 (2011).

31. K. F. Chah, Gomez-Sauz, J. A. Nwanta, B. Asadu, I. C. Agbo, C. Lozano and C. Torrez, 2nd ASM-ESCMID Conference on MILSA, **31** (2011).

Accepted : 22.11.2013