ORIGINAL RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Antibacterial activity of synthesized 3-(2-(alkylthio) phenylazo)-2,4-pentanedione and its Cu(II) derivatives

Mrinal Kanti Paira[1]*, Mrinalini Parmar[1], Durbadal Ojha[2]

[1]Department of Chemistry, Raja N.L. Khan Women's College, Gope Palace, Midnapore, West Bengal, India
[2]Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.

Received for publication: August 30, 2015; **Revised**: September 21, 2015; **Accepted**: October 05, 2015

Abstract: 3-(2-(Alkylthio) phenylazo)-2,4-pentanedione (HL) O, N, S donor ligand, is used for the synthesis of Copper (II) derivatives. The composition of the complexes is supported by spectroscopic (IR, UV-Vis, NMR) data. The compounds are screened for *in vitro* anti-bacterial activity using Gram-positive and Gram-negative bacteria (*Bacillus subtilis UC564*, *Escherichia coli TG1*, *Staphylococcus aureus Bang25, Pseudomonas aeruginosa C/1/7, Salmonella typhi NCTC62, Salmonella paratyphi NCTC A2, Shigella dysenteriae 8NCTC599/52, Streptococcus faecalis S2, Vibrio cholerae DN7 and Mricococcus luteus AGD1).* The minimum inhibitory concentration is determined for the compounds. The effect of the structure of the investigated compounds on the antibacterial activity is discussed.

Key words: 3-(2-(Alkylthio) phenylazo)-2,4-pentanedione; its Cu(II) derivatives; Antibacterial activity.

INTRODUCTION

The coordination chemistry of transition metals with azo ligands is of interest due to the observation of several interesting properties [1-8]. The π-acidity and metal binding ability of azo nitrogen have drawn attention to the exploration of the chemistry of metal complexes incorporating azo ligands. Thus, the synthesis of ligands incorporating azo function in different backbone is an inspiring field of research. Notable examples of these ligands are arylazobenzene [9], arylazooxime [10], arylazophenol [11], arylazopyridine [7, 8, 12], arylazoimidazole [13], arylazopyrimidine [14], arylazoaniline [15].

Copper is second to iron in its usefulness in life and society. The metal and its compounds are used in every sphere of life. The potential role played by copper ions, present in the active sites of many metalloproteins having the CuN_2S_2 chromophore [16], has stimulated to design new ligand frame having N, S donor sets and their copper complexes as models for providing a better understanding of the biological system [17]. The copper(II)-N, S chelates have antineoplastic activities [18-20] and efficient photosensitizer to cleave DNA [21].

Acetylacetone and its derivatives have many applications. They are directly used in the complex synthesis or functionalized by condensation or coupling reaction with other functional groups [22-30]. Because of potential application of acetylacetonato metal chelates, the chemists are trying to functionalise this molecule to synthesise newer derivatives and their metal complexes. Wehave undertaken a programme to insert thioarylazo (R-S-C_6H_4-N=N-) function into acetylacetone to synthesise hitherto unknown, 3-((2-(alkylthio) phenylazo)-2,4-pentanedione.

Antibacterial activity of the ligands and the complexes are studied against standard bacteria *Bacillus subtilis UC564*, *Escherichia coli TG1*, *Staphylococcus aureus Bang25, Pseudomonas aeruginosa C/1/7, Salmonella typhi NCTC62, Salmonella paratyphi NCTC A2, Shigella dysenteriae 8NCTC599/52, Streptococcus faecalis S2, Vibrio cholerae DN7 and Mricococcus luteus AGD1* with a stander *gentamicine.* The antibacterial activity of the synthesized compounds are discussed in this work.

***Corresponding Author:**
Dr. Mrinal Kanti Paira,
Assistant Professor,
Department of Chemistry,
Raja N. L. Khan Women's College,
Midnapore-721102, Paschim Medinipur,
West Bengal, India.

MATERIALS AND METHODS

Materials and measurements

Acetylacetone (Hacac), 2-aminothiophenol, iodomethane (MeI), iodoethane (EtI), $CuCl_2$. $4H_2O$, NH_4SCN, NaN_3 were purchased from E. Merck, India. All other chemicals used of A.R. quality and were used as received. The organic solvents were purified and dried by standard methods [31]. 3-(2-(alkylthio) phenylazo)-2,4-pentanedione and their copper complexes {HL^1, HL^2, $Cu(L^1)$ Cl (**1a**), $Cu(L^2)$ Cl (**1b**), $Cu_2(L^1)_2(N_3)_2$ (**2a**), $Cu_2(L^2)_2(N_3)_2$ (**2b**), $Cu_2(L^1)_2(SCN)_2$ (**3a**), $Cu_2(L^2)_2(SCN)_2$ (**3b**)} (shown in **scheme-1**) were prepared and characterized by the reported procedure [32-34].

UV-VIS spectra were recorded using Perkin-Elmer Lambda 25 UV-VIS spectrophotometer (**Fig. 1**) and infrared spectra were obtained from Perkin-Elmer Spectrum RX1 instrument. Microanalyses were collected from Perkin-Elmer 2400 CHN elemental analyzer. ^{1}H-NMR spectra were recorded from Brucker 300 MHz FT-NMR. Molar conductance (Λ_M) were measured in a Systronics conductivity meter 304 model using *ca.*10^{-3} M solutions in acetonitrile.

Antibacterial Assays

Microorganisms: The microorganisms were used in this study included *Bacillus subtilis UC564*, *Escherichia coli TG1*, *Staphylococcus aureus Bang25, Pseudomonas aeruginosa C/1/7, Salmonella typhi NCTC62, Salmonella paratyphi NCTC A2, Shigella dysenteriae 8NCTC599/52, Streptococcus faecalis S2, Vibrio cholerae DN7 and Mricococcus luteus AGD1.* They were obtained from Division of Microbiology, Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata-

700 032, West Bengal. The bacterial strains were grown in blood agar or McConkey agar plates at 37°C and maintained on nutrient agar slants.

Preparation of Inoculums: Suspension of organism was prepared as per McFarland Nephelometer standard [35]. A 24-hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted such that it contained approximately $1.5x10^8$ cells/ml. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05ml of 1.175% of barium chloride and 9.95 ml of 1% sulphuric acid.

Drug solution: The compounds (10 mg) HL^1, HL^2, $Cu(L^1)$ Cl (**1a**), $Cu(L^2)$ Cl (**1b**), $Cu_2(L^1)_2(N_3)_2$ (**2a**), $Cu_2(L^2)_2(N_3)_2$ (**2b**), $Cu_2(L^1)_2(SCN)_2$ (**3a**), $Cu_2(L^2)_2(SCN)_2$ (**3b**) were screened for their antibacterial activity. All the drugs were dissolved in 4% of DMSO to get the concentration of 1mg/ml, which were used as stock solution. Evaluation of the activity was carried out by agar dilution technique using nutrient agar medium.

Assay: Sensitivity tests were performed by disc diffusion method, as per NCCLS [36] protocol. The Mueller Hinton agar plates, containing an inoculum size of 10^6cfu/ml of bacteria were used. Prepared drug solution impregnated discs at concentrations of 0–500µg/ml were placed aseptically on sensitivity plates with appropriate controls [37]. All the plates were then incubated either at 37°C overnight. The sensitivity was recorded by measuring the clear zone of growth inhibition on agar surface around the discs.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined by agar dilution methods [38]. Previously prepared drug dilutions (0–500 µg/ml) of the crude drug, with appropriate antibiotic control were prepared in Mueller Hinton Agar [39]. For agar dilution assay previously prepared sensitivity plates, using serial 2-fold dilutions of the drug and control antibiotics as above, were spot inoculated (2×10^6 cfu per spot). The inoculated plates were then incubated at 37°C for 24 h. The lowest concentration of plate which did not show any visible growth after macroscopic evaluation was considered as the MIC.

Determination of minimal bactericidal concentration (MBC)

MBC was determined by broth dilution methods. Previously prepared drug dilutions (0–200 µg/ml) of the crude drug **2a** and **2b** in Mueller Hinton broth were used. The mixtures were then incubated at 37°C for 18 h with shaking on a platform shaker at 200 rpm. The drug concentration (10 µg/ml for *Shigella dysenteriae, Salmonella paratyphi* and *Micrococcus luteus* for **2b** but in **2a**, 25 µg/ml for *Bacillus subtilis* and *Mricococcus luteus*) was added to the mid-logarithmic phase of growth and aliquots of 1.0 ml were withdrawn at intervals for the determination of OD540 and colony count [38]. The lowest concentration of the tube which did not show any visible growth after colony count was considered as the MBC [40].

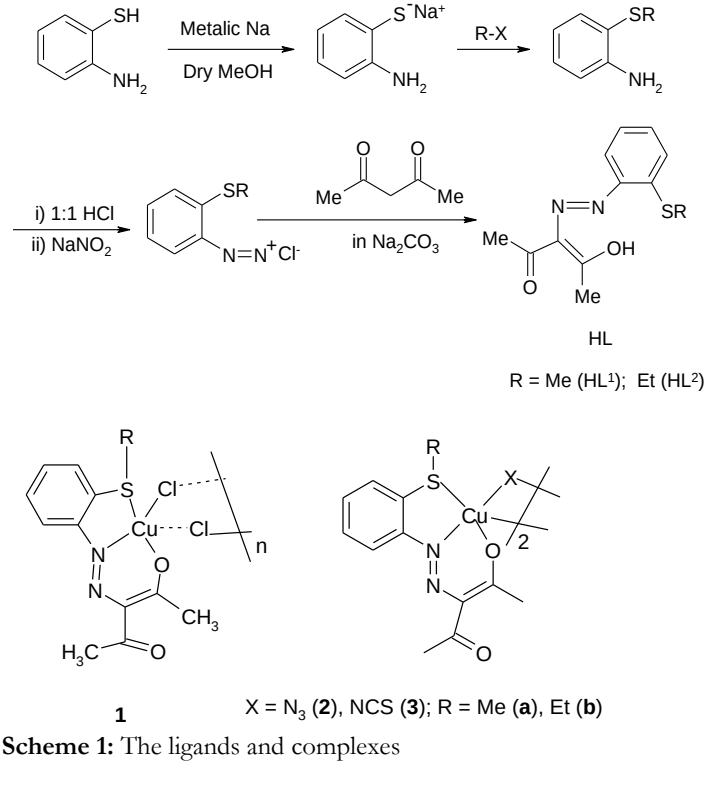
Determination of zone of inhibition

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121°C for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160°C for 1 ½ hours. 30 ml of sterile molten agar medium was seeded by organisms (about 2 ml according to Mc Farland's standard [41]), in semi hot conditions (40°C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on the medium using sterile borer and 0.1 ml of the diluted drugs (50 µg/ml and 100 µg/ml) were added to respective bore and 0.1ml of the standard Streptomycin at a concentration of 50µg/ml and 100µg/ml were taken as standard. The Petri plates seeded with organisms, containing extracts and the standard were kept in refrigerator at 4°C for 1 hour to facilitate the diffusion of the extracts and the standard in to the media. After diffusion the Petri plates were incubated at 37 ± 1°C for 24 hours in a incubator and zone of inhibition was observed and measured using a scale [40].

RESULTS AND DISCUSSION

Synthesis and formulation

2-(Alkylthio) phenyldiazonium chloride is coupled with acetylacetone in Na_2CO_3 solution and orange-yellow precipitate so obtained is purified by repeated crystalisation from aqueous ethanol (1:1, v/v) mixture. The purity of the product is checked by elemental analyses and Mass spectrometry. The ligands are abbreviated 3-(2-(alkylthio)-2-phenylazo)-2,4-pentanedione, (R = Me, HL^1; Et, HL^2) (**Scheme 1**)



Scheme 1: The ligands and complexes

The reaction between methanolic solution of $CuCl_2.2H_2O$ and HL in 1:1 mole ratio has isolated shining dark green crystals of $[Cu(L)Cl]_n$ (**1**). The reaction of $CuCl_2.2H_2O$, HL, NaN_3 or NH_4CNS in 1:1:2 mole ratio has isolated $[Cu(L)(\mu\text{-}X)]_2$ (X = N_3 (**2**); CNS (**3**)). The composition of the complexes is supported by microanalytical and mass spectrometric data. The complexes are sufficiently soluble in chloroform, dichloromethane, acetonitrile, DMF, DMSO but insoluble in hexane, benzene, toluene. They are non-conducting in solution of acetonitrile, DMF etc. At room temperature the effective magnetic moments are 1.73 (**1a**), 1.80 (**1b**), 1.55 (**2a**), 1.64 (**2b**), 1.60 (**3a**) and 1.55 BM (**3b**) per copper centre. The subnormal moment data may be due to magnetic exchange between Cu(II) (d^9) centres *via* bridging Cl or pseudohalide (N_3, NCS).

Antibacterial activity

Antibacterial screening test of the ligands HL^1/HL^2 and their copper(II) complexes against the microorganisms has been carried out initially by employing disc diffusion method [29]. Drug **2a** show prominent inhibitory activity against 5 bacterial strains. The results of the antimicrobial activity of all drugs as shown in **Table 1**. revealed that in **2a** the growth of two organisms (*Micrococcus luteus and Staphylococcus aureus)* had the MIC of 10 µg/ml. But here we had seen that 6% of DMSO had no antimicrobial activity against of these 10 bacterial strains. Further study to determine the action of the drug **2a** on two susceptible bacterial species at different concentrations showed that the growth of these organisms were decreased by increasing concentration of the drugs and were completely inhibited at their MIC values (**Fig. 2**). The minimum bactericidal concentration (MBC) was always found to be 4- to 8-fold higher than MIC values. The results also revealed that the drugs exhibited bacteriostatic but bacteriocidal at higher concentrations, probably due to interference by the active principle(s) of the drugs. Different concentration (50µg/ml and100µg/ml) of drug **2a** exhibited different zone of inhibition in different bacterial stains with respect to different concentration of streptomycin (50µg/ml and100µg/ml) in T**able 2**.

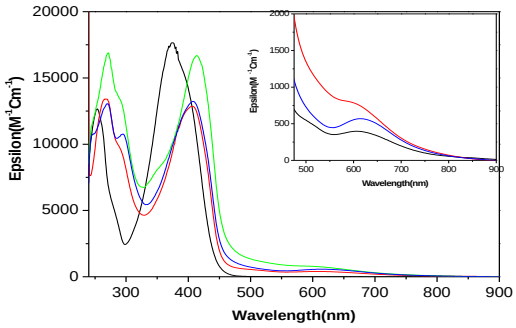


Figure 1: UV-Vis spectra of HL^2(——), $Cu(L^2)Cl$(——), $Cu_2(L^2)_2(SCN)_2$(——), $Cu_2(L^2)_2(N_3)_2$(——) in $CHCl_3$. The insert picture show the higher wavelength spectra of $Cu(L^2)Cl$(– – –), $Cu_2(L^2)_2(SCN)_2$(– – –), $Cu_2(L^2)(N_3)_2$(– – –)for showing d-d transition.

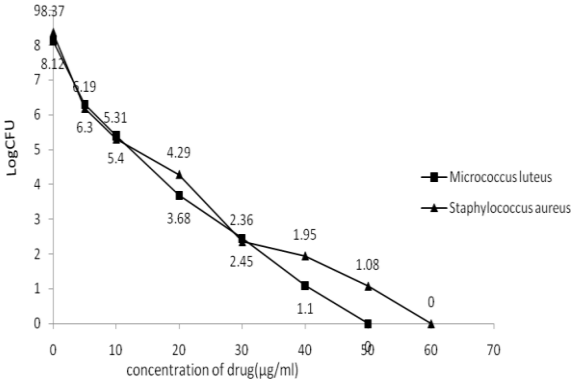


Figure 2: Effect of drug **2a** on two bacteria at different concentrations

Table 1: Antimicrobial activities of chemically synthetic compound

Name of bacteria	Minimum Inhibitory Concentration (µg/ml)								
	HL^1 a	HL^2 b	$Cu(L^1)Cl$ 1a	$Cu(L^2)Cl$ 1b	$Cu_2(L^1)_2(N_3)_2$ 2a	$Cu_2(L^2)_2(N_3)_2$ 2b	$Cu_2(L^1)_2(SCN_3)_2$ 3a	$Cu_2(L^2)_2(SCN_3)_2$ 3b	Gentamicin
Shigella dysenteriae	500	–	200	100	25	300	–	300	2
Escherichia coli	–	500	–	–	–	300	–	–	1
Salmonella typhi	–	–	–	–	–	–	–	–	1
Salmonella paratyphi	–	500	–	300	50	100	–	300	1
Bacillus subtilis	–	–	–	50	25	100	–	300	1
Pseudomonas aeruginosa	–	–	–	–	–	–	–	–	64
Streptococcus faecalis	–	–	–	–	–	–	–	–	1
Micrococcus luteus	–	300	–	–	10	300	–	50	4
Staphylococcus aureus	–	–	100	100	10	200	500	50	1
Vibrio cholera	–	–	–	300	100	300	–	100	1

*no inhibition was found upto 6% DMSO used as control.
* '–' shows no antimicrobial activity upto 500 µg/ml

Table 2: Different concentration of drug **2a** exhibit different zone of inhibition in different bacterial strains with respect to streptomycin

Name of Organisms	Diameter of the inhibition zone (in mm) of drug in different concentration		Diameter of the inhibition zone (in mm) of streptomycin in different concentration	
	50µg/ml	100µg/ml	50µg/ml	100µg/ml
Micrococcus luteus	15.4±0.28	21±0.88	21	26±0.16
Staphylococcus aureus	13±0.33	16.5±0.76	18±0.44	23±0.33
Shigella dysenteriae	8.5±0.28	10±0.16	12±0.16	16±0.44
Bacillus subtilis	9±0.16	10.5±0.28	17±0.33	20.5±0.28

*Values are in terms of Mean ± SEM of results done in triplicate.

CONCLUSION

Drugs **2a** exhibited better antibacterial activity as compared to other five drugs. Among the organisms tested *Micrococcus luteus* and *Staphylococcus aureus* were more susceptible to the above drug. After comparison with stander *Gentamicin*, *Micrococcus luteus* was more susceptible. Further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of the drug **2a** in treating various infections and skin diseases.

ACKNOWLEDGEMENTS

Financial support from the University Grants Commission New Delhi, are gratefully acknowledged.

REFERENCES

1. Wong W Y, Cheung S H, Lee S M, Leung S Y. Synthesis, characterization and crystal structures of some tungsten and triosmium carbonyl complexes with monopyridyl and dipyridyl azo ligands. *J. Organomet. Chem.* 596 (2000): 36-45.

2. Carlucci L, Ciani G, Proserpio D M, Rizzato S. Silver(I) polymeric coordination frameworks assembled with the new multimodal. *New. J. Chem.* 27 (2003): 483-489.

3. Akasaka T, Mutai T, Otsuki J, Araki K. Photo-induced energy transfer and its switching in dyad and triad chromophore systems composed of coumarin, Ru(II) and Os(II) terpyridine-type complexes. *J. Chem. Soc. Dalton Trans.* (2003): 1537-1544.

4. Casalbani F, Mulazzani Q G, Clark C D, Hoffman M Z, Orizondo P L, Perkovic M W, Rillema D P. Acid−Base and Electrochemical Properties of the MLCT Excited States and One-Electron-Reduced Forms of Ruthenium(II) Complexes Containing 2-(2‘-Pyridyl) pyrimidine and 2,2‘-Bipyridine in Aqueous Solution. *Inorg. Chem.* 36.11 (1997): 2252–2257.

5. Yan V W-W, Lau V C –Y, Cheung K –K. Synthesis, photophysics and photochemistry of novel luminescent rhenium(I) photoswitchable materials, J. Chem. Soc. *Chem. Commun.* (1995): 259-261

6. Frantz S, Fiedler J, Hartenbach I, Schleid T, Kaim W. A complete series of tricarbonylhalidorhenium(I) complexes (abpy)Re(CO)$_3$(Hal), Hal = F, Cl, Br, I; abpy = 2-2‘-azobispyridine: Structures, spectroelectrochemistry and EPR of reduced forms. *J. Organomet. Chem.* 689 (2004): 3031-3039.

7. Ghosh B K, Chakravorty A. Electrochemical studies of ruthenium compounds part-I. Ligand oxidation levels. *Coord. Chem. Rev.* 95 (1989): 239-294.

8. Santra B K, Thakur G A, Ghosh P, Pramanik A, Lahiri G K. A Novel Example of Metal-Mediated Aromatic Thiolation in a Ruthenium Complex. Crystal Structure of $Ru^{II}(SC_6H_4N{=}NC_6H_4N)_2$‡ . *Inorg. Chem.* 35 (1996): 3050-3052.

9. Cope A C, Siekman R W. Formation of Covalent Bonds from Platinum or Palladium to Carbon by Direct Substitution. *J. Am. Chem. Soc.* 87 (1965): 3272.

10. Ganguly S, Chattopadhyay S, Sinha C, Chakravorty A. Synthesis and structure of dimeric silver azooximates. Hydrogen bonding and nonbonded Ag...Ag interaction. *Inorg. Chem.* 39 (2000): 2954-2956 and references therein.

11. Aharyya R, Basuli F, Wang R Z, Mak T C W, Bhattacharya S. Iridium(III) Complexes Formed by O−H and/Or C−H Activation of 2-(Arylazo)phenols. *Inorg. Chem.* 43 (2004): 704-711 and references therein.

12. Velders A H, van der Schilden K, Hotze A C G, Reedijk J, Kooijman H, Spek A L. Dichlorobis(2-phenylazopyridine) ruthenium (II) complexes: characterisation, spectroscopic and structural properties of four isomers. *J. Chem. Soc., Dalton Trans.* (2004): 448-455.

13. Byabartta P, Dinda J, Santra P K, Sinha C, Panneerselvam K, Liao F L, Lu T-H. Heteroleptic tris-chelates of ruthenium(II)-bipyridine-arylazoimidazoles: Synthesis, spectral characterisation and electrochemical properties. Single crystal X-ray structure of [(2,2/-bipyridine)-bis-{1-methyl-2-(p-tolylazo) imidazole} ruthenium(II)] perchlorate. *J. Chem. Soc., Dalton Trans.* (2001): 2825-2832.

14. Senapati S, Ray U S, Santra P K, Sinha C, Woolins J D, Slawin A M Z. Osmium–azopyrimidine chemistry. Part VII: synthesis, structural characterisation and electrochemistry. *Polyhedron* 21 (2002): 753-762.

15. Maiti N, Pal S, Chattopadhyay S. Reaction of 2-(Phenylazo) aniline with Na_2PdCl_4: Formation of a 2-(Phenylazo)imino Complex of Bivalent Palladium. *Inorg. Chem.* 40 (2001): 2204-2205 and references therein.

16. Solomon E I, Szilagyi R K, George S D, Basumallick L. Electronic Structures of Metal Sites in Proteins and Models: Contributions to Function in Blue Copper Proteins. *Chem. Rev.*, 104 (2004): 419-458.

17. Robb D A. In "Copper Proteins and Copper Enzymes" R Lontie (Ed.), Bocaraton, vol. 2. (1984): pp 207. Karlin K D, Tyeklar Z, Bioinorganic Chemistry of Copper. *Chapmann & Hall, New York,* (1993).

18. Balamurugan R, Palaniandavar M, Gopalan S R, Kulkarni G U. Copper(II) complexes of new pentadentate bis(benzimidazolyl)-dithioether ligands: synthesis, structure, spectra and redox properties. *Inorg. Chim. Acta.*, 357 (2004): 919-930.

19. Westerby B C, Juntunen K L, Leggett G H, Pett V B, Koenigbauer M J, Purgett M D, Taschner M J, Ochrymowycz L A, Rorabacher D B. Macrocyclic polyamino polythiaether ligands with NxS4-x and NxS5-x donor sets: protonation constants, stability constants, and kinetics of complex formation with the aquocopper(II) ion. *Inorg. Chem.*, 30 (1991): 2109-2120.

20. Ambundo E A, Ochrymowycz L A, Rorabacher D B. Electron-Transfer Kinetics of Tris (2-(methylthioethyl)) aminecopper (II/I). A Tripodal Ligand Complex Exhibiting Virtual C_{3v} Symmetry. *Inorg. Chem*. 40 (2001): 5133-5138.

21. Dhar S, Senapati D, Das P K, Chattopadhyay P, Nethaji M, Chakravarty A R, Ternary Copper Complexes for Photocleavage of DNA by Red Light: Direct Evidence for Sulfur-to-Copper Charge Transfer and d−d Band Involvement. *J. Am. Chem. Soc.*125 (2003): 12118-12124.

22. Mishra L, Yadaw A K, Srivastava S, Patel A B. Synthesis, spectroscopic, electrochemical and antibacterial studies of new Ru(II) 1,10-phenanthroline complexes containing aryldiazopentane-2,4-dione as co-ligand. *New J. Chem.*24(7) (2000): 505-510.

23. Mishra L, Yadaw A. K, Phadke R. S, Choi C. S, Araki K. Studies on some new Ru(II) complexes using aryl-azo pentane-2,4-dione and 2,6-bis (2'-benzimidazoyl) pyridine as ligands: Synthesis, spectroscopic, luminescent, electrochemical and biological activities. *Metal-Based Drugs* 8(2) (2001): 65-71.

24. Sharma P, Kumar A, Pandey P, Rane N, A comprehensive electrochemical studies on some synthesized substituted 3-arylazo-2,4,6-trimethylquinolines. *Indian J. Chem*. 43B (2004): 1320-328.

25. Gadzhieva S R, Mursalov T M, Makhmudov K T, Chyragov F M. Quantum-chemical calculations of the tautomeric forms of 3-phenylazopentane-2,4-dione and the thermodynamic parameters of complexation between its isomers and some metals in aqueous ethanol. *Russian J. Coord. Chem*. 32.4 (2006): 304-308.

26. Marten J, Seichter W, Weber E, Bohme U. Synthesis and structural study of 2'- and 2',6'-positioned methyl- and nitro-substituted 3-(arylhydrazono) pentane-2,4-diones. *J. Phys. Org. Chem*. 20(10) (2007): 716-731.

27. Krishnankutty K, Sayudevi P, Ummathur M B. Schiff bases of 3-(2-thiazolylazo)-2,4-pentanedione with aliphatic diamines and their metal complexes. *J. Indian Chem. Soc.*, 85 (2008): 48-52.

28. Tayyari S F, Sammelson R E, Tayyari F R, Rahemi H, Ebrahimi M. Conformational analysis, tautomerization, IR, Raman, and NMR studies of 3-phenylazo-2,4-pentanedione. *J. Mol. Str.* 920(1-3) (2009): 301-309.

29. Metz S, Burschka C, Tacke R. Neutral Hexacoordinate Silicon(iv) Complexes with an SiSO$_3$NC Skeleton and a Neutral Pentacoordinate Silicon(iv) Complex Containing a Trianionic Tetradentate *O*, *N*, *O*, *O* Ligand. *Organomet*. 28 (2009): 2311-2317.

30. West N M, White P S, Templeton J L, Nixon J F. Cycloaddition Reactions of Terminal Alkynes and Phosphaalkynes with an Isolable 5-Coordinate β-Diiminate Platinum(IV) Dihydrosilyl Complex. *Organomet*. 28 (2009): 1425-1434.

31. Vogel A I. A Text Book of Practical Organic Chemistry, 2nd Ed, Longman, London, 1959.

32. Chattopadhyay P, Sinha C. Regioselective mercuration of 2-(methylthio)-N-(benzylidene)anilines. *Polyhedron* 13 (1994): 2689-2693.

33. Paira M K, Mondal T K, Torres E L, Ribas J, Sinha C. Copper(II) complexes of thioarylazo-pentanedione: Structures, magnetism, redox properties and correlation with DFT calculations. *Polyhedron* 29 (2010): 3147-3156.

34. Paira M K, Mondal T K, Ojha D, Slawin A M Z, Woollins J D, Tiekink E, Samanta A, Sinha C. Structures, redox behavior, antibacterial activity and correlation with electronic structure of the complexes of nickel triad with 3-(2-(alkylthio) phenylazo)-2,4-pentanedione. *Inorganica Chimica Acta* 370 (2011): 175-186.

35. Chattopadhyay D, Dastidar S G, Chakrabarty A N, Bioactive Heterocycles VI: Flavonoids and Anthocyanins in Plants. *Arzneim Forsch (II)* 38 (1988): 869-872.

36. Chattopadhyay D, Mukherjee T, Pal P, Saha B, Bhadra R. Altered membrane permeability as the basis of bactericidal action of methdilazine. *J. Antimicrobial Chemotherapy* 42 (1998): 83-86.

37. Chattopadhyay D, Sinha B K, Vaid, L K. Antibacterial activity of Syzigium sp. *Fitoterapia* 69(4) (1998): 365.

38. Chattopadhyay D, Maiti K, Kundu AP, Chakraborty MS, Bhadra R, Mandal SC, Mandal AB. Antimicrobial activity of *Alstonia macrophylla*: a folklore of bay islands. *J. Ethnopharmacology* 77 (2001): 49-51.

39. http://en.wikipedia.org/wiki/McFarland_standards.

40. Roopashree T S, Dang R, Shobha Rani R H, Narendra C. Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. *Intl. J. Appl. Res. Nat. Pdts.*1 (2008): 20-28.

41. Kahn O, Sikarov S, Gouteron J, Jeannin S, Jeannin Y. Crystal structure and magnetic properties of (.mu.-azido) - (.mu.-hydroxo) bis [(N,N,N'N' tetramethylethylenediamine) copper(II)] perchlorate, a copper(II) dinuclear complex with a large ferromagnetic interaction. *Inorg. Chem.* 22 (1983): 2877-2883.

CITE THIS ARTICLE AS:
Mrinal Kanti Paira, Mrinalini Parmar, Durbadal Ojha. Antibacterial activity of synthesized 3-((2-(alkylthio) phenylazo)-2,4-pentanedione and its Cu(II) derivatives. *International Journal of Bioassays* 4.12 (2015): 4679-4684.

Source of support: University Grants Commission New Delhi, India
Conflict of interest: None