SYNTHESIS AND ANTIMICROBIAL ACTIVITY STUDIES OF TRIIODOIMIDAZOLE AND ITS TRANSITION METAL COMPLEXES

[1*]I. A. Duru, [2]C. E. Duru, [3]R. Ngochindo, and [1]L. Ukiwe
[1]Department of Chemistry, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria.
[2]Department of Chemistry, Imo State University, P.M.B. 2000, Owerri, Imo State Nigeria.
[3]Department of Pure and Industrial Chemistry, University of Port Harcourt, P.M.B. 5323, Port Harcourt Rivers State, Nigeria.
Accepted:31/03/2016
*Corresponding author:akunnaobiji@yahoo.com

Abstract
2,4,5-Triiodoimidazole (TII) was synthesized by reacting an aqueous solution of iodine and potassium iodide with imidazole. The Co (II), Cu (II), Cr (III) and Fe (II) complexes of the ligand were also synthesized. All the compounds prepared were purified by recrystalisation with suitable solvents and their purities checked by their melting point and thin layer chromatographic pattern. Structural elucidation of the synthesized compounds was done by Nuclear Magnetic Resonance (NMR), Infrared (IR) and Mass Spectroscopy (MS) analysis. Some absorption bands in the IR spectrum of the iodinated imidazoles were found to shift either to higher or lower wave numbers in the complexes, indicating the involvement of azomethine nitrogen in coordination to the metal ion. Appearances of medium bands at 540-620 cm^{-1} in some complexes were supportive in assignment of the proposed coordination sites. The synthesized TII and its metal complexes were screened for their antimicrobial activity against Proteus spp, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus, Aspergillus spp, Candida spp and Trichophyton spp. TII showed minimal activity against the selected bacterial strains when compared to Levofloxacin, a broad spectrum antibiotic. The synthesized complexes of the ligand did not give a substantial improvement on their bactericidal activity. Although the fungi strains showed little or no sensitivity to TII when compared with a standard antifungal agent such as Ketoconazole, its Co^{2+} complex was found to show geometric increase in antifungal activity, which surpassed the standard drug agent. This presents the Co^{2+} complex of TII as a lead in the search for medicinally active compounds against these organisms.

Keywords: Imidazole, triiodoimidazole, metal complexes, spectroscopy, antifungal

Introduction

Imidazoles are well known N-containing cyclic structures that have been reported to be associated with a wide range of biological activities [1,2]. Having structural similarity with histidine, imidazole compounds can bind with protein molecules with ease compared to other heterocyclic moieties [3]. Imidazoles offer better pharmacodynamic characteristics and also improves pharmacokinetic characteristics of lead molecules because of their polar and ionisable nature and as such are used as remedy to optimize solubility and bioavailability parameters of proposed poorly soluble lead molecules [4,5]. These high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents [5]. Synthetic Imidazoles are present in many antifungal, antiprotozoal and antihypertensive medications. Some imidazoles prepared as pharmacological agents are Azomycine, Clotrimazole, Miconazole, Ergothionine, Clonidine and Moxonidine [6,7].

Varieties of valuable pharmacological properties have been attributed to varying substitution patterns of the imidazole nucleus [5,8,9] and halogenated imidazoles are no exceptions as previous researches have revealed the biological activities of brominated and chlorinated imidazoles [10,11].

As organic halogen compounds continue to play an essential role in human health and well being chemists would continue to pursue the study of these fascinating chemicals. Aromatic iodination reactions are therefore important electrophilic substitution reactions since the resultant aromatic iodo-compounds are precursors for the synthesis of various pharmaceutical and bioactive compounds [12,13,14,15].

In recent decades, problems of multidrug-resistant microorganisms have reached an alarming level in many countries around the world and consequently there has been intense drive towards search for novel molecules which inhibit their activity [16,17]. Among these novel drugs are metal complexes of organic compounds due to the dual possibility of both ligands plus metal ion interacting with different steps of the pathogen life cycle. In exploring the role of metal complexes in relation to antibacterial activity, it has been observed that metal ions have considerable effect

on the antimicrobial activity of antibiotics and ligands/drugs may become more bacteriostatic on complexation when compared to unchelated ones [18,19].

Iodination of imidazole was reported once since 1993 by Katritzky and his colleagues [20] but the biological activity of Co(II), Cu(II), Cr(III), and Fe(II) metal complexes of iodinated imidazole (TII) have not been studied earlier. In the present work, biological activity of the above mentioned metal chelates against some selected bacteria and fungi strains were studied.

Methodology

Synthesis of 2,4,5-Triiodoimidazole

2,4,5-Triiodoimidazole (TII) was synthesized using the procedure according to Katritzky *et al*. [20]. An aqueous solution (150 ml) of iodine (20.32 g, 0.08 M) and potassium iodide (26.56 g, 0.16 M) was added dropwise with stirring to a solution of imidazole (1.36 g, 0.02 M) in aqueous sodium hydroxide (2 M, 200 ml) at room temperature. The solution was stirred for 4 hours and left to stand overnight. Addition of 25 % aqueous acetic acid until the solution became neutral gave a creamy precipitate which was filtered washed and air dried. These crystals were further recrystallized with redistilled ethanol.

Preparation of 2,4,5-Triiodoimidazole Metal Complexes

All the complexes were prepared using the same procedure. Each hydrated metal salt (1 mmole) was dissolved in 2 ml of distilled water and added a dropwise with continuous stirring to 2 mmoles of the TII dissolved in 10 ml of acetone. The mixture was stirred at room temperature for half an hour and allowed to stand. The crystals formed were filtered through sintered glass crucible, washed with cold water and dried under vacuum at 50 ^{o}C. The products were recrystallized with redistilled ethanol.

Characterization of Products

FTIR, Mass Spectral (MS) and Nuclear Magnetic Resonance (NMR) characterization of the synthesized TII and its transition metal complexes were done at Asclepia Outsourcing Solutions, Damvalleistraat 49, B-9070 Destelbergen, Belgium. FT-IR spectra were obtained using KBr discs and Nujol mull techniques on IR Spectrophotometer FT-IR 8400S Shimadzu in the 4000–450 cm^{-1} range. Mass spectra were obtained from Shimadzu LCMS-2010EV Liquid Chromatography Mass Spectrometer, ESI. NMR spectra were recorded in DMSO–d_6, on a Varian 400-MR Magnetic Resonance Spectrometer.

Antimicrobial Activity of Products

Collection of Microbial Cultures

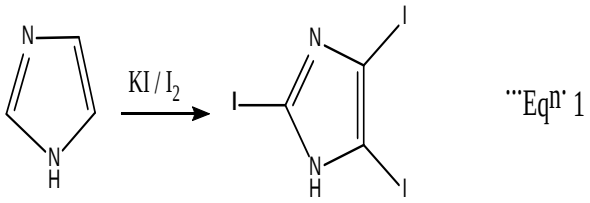
Pure clinical isolates of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus spp*, *Aspergillus spp*, *Candida spp* and *Trichophyton spp* were collected from the Microbiology Department of the Federal Medical Center Owerri, Imo State Nigeria. A microbial loop was used to remove a colony of each organism from the pure culture and transferred into liquid broth (Nutrient broth) then incubated for 24 h at 37 ^{o}C. These were maintained in sterile conditions.

Determination of Antimicrobial Activity of Product and its Complexes

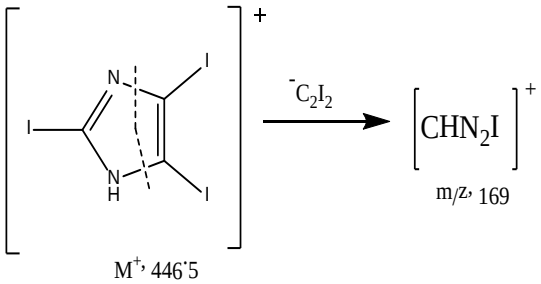
Paper disc diffusion method [21] was used for the determination of the antibacterial activity of the ligand and its complexes.

Results and Discussion

The reaction equation for the synthesis of the ligand (TII) is as represented in Equation 1. The product obtained had a mass of 8.15 g, which is equivalent to a yield of 91 % with a melting point ranging between 190-192 ^{o}C.



The mass spectrum of TII gave a molecular ion peak of 446.7 and a fragment ion peak of 169. The molecular ion peak corresponds to the mass of the compound with formula $C_3HN_2I_3$ (M^+ = 446.5). The fragment ion peak of 169 corresponds to the mass of the fragment CHN_2I with the assumption that one of the nitrogen atoms is of M+1 isotope. The assignment of peaks to fragments is shown in Scheme 1. The remaining fragment (C_2I_2) not accounted for is considered as a fragment lost from the heterocycle as neutral species [22].



Scheme 1: Fragmentation Pattern of 2,4,5-Triiodoimidazole

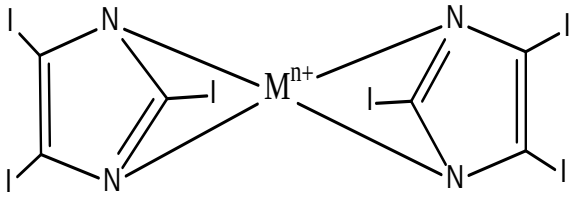
The ^{1}H-NMR gave a peak at 13.356 ppm which is assigned to N-H hydrogen. A peak at 89.646 ppm on the

^{13}C-NMR represents the C-4/C-5 peaks (lit. 89.0) [22]. The expected peak at 91.1 ppm for C-2 is considered to have been embedded in the observed peak since the chemical shift axis values seems to be too close for values ranging from 89-91 to be overlapped, possibly accounting for the difference between the literature value and the observed value. The colours of the products and their characteristic bands in the IR spectrum are shown in Table 1.

Table 1: Colour and IR Spectral Results of the Ligands and their Complexes

S/N	Compound Code	Colour	IR Wave numbers cm^{-1}
1	TII	Cream	3029 (N–H) m, 432(C–I) m, 1379(C=N) v, 1246 (C–N) s, 1493(C=C) w
2	[Co(TII)]	Pink	3399 (N–H) m, 596(C–I) w, 1491(C=N) m, 1380 (C–N) s, 1634(C=C)w
3	[Cr(TII)]	Lemon	3412 (N–H) m, 572(C–I) w, 1522(C=N) m, 1400(C–N) s, 1634(C=C) w
4	[Cu(TII)]	Dark brown	3444 (N–H) m, 460(C–I) w, 1499(C=N) m, 1384 (C–N) s, 1627(C=C) w
5	[Fe(TII)]	Light brown	3395(N–H) m, 622(C–I) w, 1494(C=N) m, 1381 (C–N) s, 1642(C=C) w

The main frequencies observed in IR scan of TII were 3029 cm^{-1}, 1493 cm^{-1}, 1379 cm^{-1}, 1266 cm^{-1} and 432 cm^{-1} representing the main functional groups as N–H, C=C, C=N and C–Cl respectively. The IR characteristics bands of the ligand when compared to that of the complexes to ascertain the coordination sites that may be involved in chelation were observed to shift to either higher or lower wave numbers indicating the involvement of the imidazole N–H bond in coordination to the metal ion (M-N). The proposed structure of the complexes is as shown in Figure 1.



Where M represents any of Cu,Zn,Ni,Co

Fig. 1: Proposed Structure of the Complexes

The activities of TII and its Co^{2+}, Cr^{3+}, Cu^{2+} and Fe^{2+} complexes on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus spp*, *Aspergillus spp*, *Candida spp* and *Trichophyton spp* are shown in Table 2 and Table 3.

Table 2: Activity of the Ligands and their Complexes against some Strains of Fungi

Samples	Concentrations (mg/ml)	Diameter of Zone of Inhibition (mm)		
		Aspergillus spp	*Candida spp*	*Trichophyton spp*
TII	200	8	–	6
	100	4	–	2
	50	–	–	–
	25	–	–	–
TII-Co	200	12	14	12
	100	8	10	10
	50	6	8	6
	25	2	4	6
TII-Cr	200	10	10	12
	100	6	4	4
	50	4	–	2
	25	–	–	–
TII-Cu	200	8	–	6
	100	2	–	4
	50	–	–	–
	25	–	–	–
TII-Fe	200	10	6	8
	100	4	2	4
	50	2	–	–
	25	–	–	–
Ketoconazole	200	–	–	2

The antibacterial activity of the TII when compared to the standard antibiotics was found to be poor except for its activity against *Streptococcus pyogene*. Complexation with transition metals gave marginal increase in its activity except for *Streptococcus pyogene*, which showed reduced sensitivity to Cr^{3+}, Cu^{2+} and Fe^{2+} complexes.

The fungi strains used in this study were found to show little sensitivity to TII when compared to ketoconazole which is a standard antifungal agent. Upon complexation with the transition metals, the complexes gave improved activity against the selected fungi strains especially the Co (II) complex which proved to be highly potent against all the fungi strains even at a concentration as low as 25 mg/ml

Table 3: Activity of the Ligands and their Complexes against some Strains of Bacteria

Samples	Concentrations (mg/ml)	Diameter of Zone of Inhibition (mm)			
		Streptococcus pyogenes	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus spp*
TII	200	18	8	6	6
	100	14	4	4	2
	50	10	–	2	–
	25	6	–	–	–
TII-Co	200	20	16	12	12
	100	14	10	8	6
	50	10	8	4	4
	25	8	6	4	2
TII-Cr	200	16	10	14	10
	100	12	4	8	4
	50	8	2	4	–
	25	4	–	4	–
TII-Cu	200	16	12	14	10
	100	10	6	6	6
	50	8	8	4	4
	25	4	6	4	2
TII-Fe	200	16	10	10	12
	100	10	4	6	4
	50	6	–	4	–
	25	4	–	–	
Levofloxacin	2	14	14	10	8

Conclusion

Triiodoimidazole has been shown to be a good bioactive agent with its activity being enhanced by chelation especially with Co^{2+} ions. Complexation with transition metals was found to enhance the antifungal activity of this compound much more than what was achieved with a standard antifungal agent used in the study. The complexes of TII can therefore serve as potent drugs to combat ailments caused by stubborn pathogens like the *Candida spp*. and related diseases.

Acknowledgements

We are grateful to the Education Trust Fund of Nigeria, for research sponsorship to I.A.D. through the Federal Universsity of Technology Owerri, Nigeria. Our gratitude also goes to Dr. Frederik Deroose of Asclepia Outsourcing Solutions Destelbergen Belgium, for his technical assistance.

References

1. J.R. Kumar (2010). "Review of Imidazole heterocyclic ring containing compounds with their biological activity", *Pharmacophore*, 1, (3), pp 167-177.
2. D. Lednicer and L.A. Mitscher (1997). "In Organic Chemistry of Drug Synthesis", *Wiley Interscience, NewYork*, 1, pp 226.
3. T.L. Gilchrist (1985). "Heterocyclic Chemistry", *Pitman, London*, 11-25, pp 185-217.
4. M.R. Grimmett (1997). "Imidazole and Benzimidazole Synthesis", *Academic Press, New York*, pp 15-188.
5. S. Kumari, K.S.Pramod and K. Nitin (2010). "Imidazole and its biological activities: A review", *Der Chemica Sinica*, 1, (3), pp 36-47.
6. E.G. Brown (1998). "Ring Nitrogen and Key Biomolecules", *Kluwer, Academic Press, Boston*, pp 45-78.
7. A.F. Pozharskii, A.T. Soldatenkov and A.R. Katritzky (1997). "Heterocycles in Life and Society", *John Wiley & Sons, Chichester, U.K.*, pp 27-198.
8. R.I. Ngochindo (1992). "Novel Imidazole Derivative as Potential Agrochemicals", *Proceeding of Indian Academy of Science (Chem. Sci.)*, 104, pp 21-22.
9. N.T.Manisha, S.Y. Gabhe, D.V. Urmila, B.P. Rima and P.S. Charmi (2011). "Synthesis of some 2-methyl-5-nitroimidazole derivatives as potential antimicrobial agents", *J. Chem. Pharm. Res*., 3(1), pp 313-319.
10. I.A. Duru, R.I. Ngochindo and C.E. Duru (2014). "Enhancement of the Bioactivity of 4,5-dibromoimidazole by its Cobalt (II) Complex", *Journal of Applied Chem.* Vol. 7, Issue 9, Ver. I, pp 28-32.
11. I.A. Duru, R.I. Ngochindo, C.E. Duru, C.E. Ogukwe and I. Orji (2014). "Chlorinated Imidazole Complexes as Potent Antifungal Agents", *Journal of Applied Chem.*, Vol. 7, Issue 9, Ver. II, pp 20-23.
12. R. Dahiya, and R. Mourya (2012). "Synthesis and Antimicrobial Screening of Some Novel Halogenated Phenoxyacetyl Amino Acid and Peptide Analogs", *Bull. Pharm. Res.*, 2(2) pp 56-65.
13. C.S.Vairappan, T. Kawamoto, H. Miwa and M. Suzuki (2004). "Potent antibacterial activity of halogenated compounds against antibiotic-resistant bacteria", *Planta Med*. 70(11), pp1087-1090.
14. P.V.Randhavane, Narwade S.K. George S., and Karale B.K. (2010). "Synthesis and biological screening of some halogenated thiazoles and triazoles", *Indian Journal of Chemistry*, 49B, pp 89-95.
15. K.Kasumbwe, K.N.Venugopala, V. Mohanlall and B. Odhav (2014). "Antimicrobial and antioxidant activities of substituted halogenated coumarins", *J. Med. Plants Res.*,Vol. 8(5), pp 274-281.
16. M.L. Cohen (1992). "Epidemiology of drug resistance: implications for a postantimicrobial era", *Sci.* 257, pp 1050-1055.

17. H.C. Neu (1992). "The crisis in antibiotic resistance", *Sci*, 257, pp 1064-1073.
18. Saeed-ur-Rehman, M. Ikram, S. Rehman, A. Faiz and Shahnawaz (2010). "Synthesis, Characterization and Antimicrobial Studies of Transition Metal Complexes of Imidazole Derivative", *Bull. Chem. Soc. Ethiop*., 24(2), pp 201-207.
19. D.G. Desai, A.K.Seth, K.I. Molvi, M.M. Mansuri and B.R.Prajapati (2012). "Synthesis and Antibacterial Activity of Nickel Complex of Some Novel Nitroimidazole", *Pharma Science Monitor (IJPS)* 3:2, pp 133-141.
20. A.R. Katritzky, D.J. Cundy and J. Chen (1993). "Polyiodoimidazoles and their nitration products", *Journal of Energetic Materials*, 11, pp 345-352.
21. A.W.Bauer, W. M. Kirby, J. C. Sheriss, M. Turck (1966). "Antibiotic susceptibility testing by a standardized single disk method", *Am. J. Clin. Pathol*. 45, 493-496.
22. D.B. Mistry (2009). "A handbook of spectroscopic data chemistry (UV, IR, PMR, CNMR and Mass spectroscopy)", *Oxford Book Company, Jaipur, India*, pp 27-233.