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Synthesis, Characterization and Antimicrobial Screening of Mixed 5-(4-Chlorophenyl)-6-Ethyl- 2,4-Pyrimidinediamine-Aspirin Metal Drug Complexes

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ABSTRACT

A new series of complexes of 5-(4-chlorophenyl)-6-ethyl- 2,4-pyrimidinediamine-aspirin metal drug has been synthesized as potential antimalarial and analgesic activity which yielded excellent results. The synthesized complexes have been evaluated using some analytical techniques such as conductivity measurements, elemental analysis and spectroscopic analysis such as atomic absorption spectroscopy and FT-IR. The result of the conductivity measurement showed the compounds to be non-electrolyte and suggests a tetrahedral geometry. The ligands used are bidentate in nature. In pyrimethamine complexes, coordination occurred through the nitrogen of the N-H and C=N groups as seen in the results obtained from the FT-IR analysis. In aspirin, the coordination occurred through the oxygen of the C=O and O-H group. The novel complexes have been screened and investigated against some isolated organisms namely: *Escherichia coli, Bacillus megaterium, Proteus vulgarius, Pseudomonas aeruginosa, Euterobacter aerogenes, Staphylococcus aureus, Bacillus substillis and Serratia* marcesceus. Higher activity was observed due to ability to decrease the population of the bacterial species. This research work was carried out with the aim of improving the effectiveness and antibacterial activity of the parent ligands

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Keywords: Transition Metals, Pyrimethamine, Aspirin, Complexes, FT-IR, Anti-bacterial.

INTRODUCTION

In the past few years, numerous reports have appeared showing that the incorporation of transition metal salts into ligands with the aim of improving their effectiveness has been of great interest (Kathryn *et al.*, 2009). Interaction of metals with parent ligands to form complexes has been achieved in the design of new effective drugs (Micheal *et al.*, 2014). Some drugs exhibit their pharmacological and toxicological properties when taken in the complex form (Guangguo *et al.*, 2003, Brown *et al.*, 1980, Sousa *et al.*, 2012). Metals are very important in medicinal chemistry; this has been shown in the treatment of malaria as the causative agent malaria (*plasmodium falciparium*) has become resistant to major antimalarial drugs such as Quinolones, hence the need for modification of the drugs by addition of metals (Shazia *et al.*, 2010). It has been observed that the continuous research of inorganic compounds increases the effectiveness against some diseases for the treatment of cancer such as cisplatin (Ogunniran *et al.*, 2008, Paul *et al.*, 2006).

MATERIALS AND METHOD

The chelating agents and the metal salts used in this research work were obtained from Sigma-aldrich without any further purification. The micro-organisms: *Escherichia coli, Bacillus megaterium, Proteus vulgarius, Pseudomonas aeruginosa, Euterobacter aerogenes, Staphylococcus aureus, Bacillus substillis and Serratia marcesceus* are used for the biological screening of the synthesized complexes which were collected from Microbiology Department, University of Ilorin, Ilorin Nigeria. The method used for the synthesis of the complexes was as described by (Tella *et al.*, 2008).

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Synthesis of the complexes

Pyrimethamine (1 mmol) was dissolved in 20 ml of methanol and stirred for 20 minutes at room temperature. Aspirin (1 mmol) was also dissolved in 20 ml of ethanol. (1 mmol) of the metal salts used were each dissolved separately in 20 ml of their suitable solvents. The ligand solutions were mixed together with the solution of the metal salts. The mixed solution was refluxed for 5 hours at a temperature 70 oC. The resulting solutions were allowed to cool for 30 minutes after refluxing and then filtered to remove impurities. The solutions were left to stand for some days after which the compounds were precipitated. It was filtered, washed and stored in a desiccator.

Antimicrobial Screening

The procedure described by (Biradar et al., 2013) was adopted. 7g of nutrient agar was measured into 250 ml of sterilized water. The agar solution was heated for 15 minutes. It was then placed in an autoclave to sterilize at 121 °C. The heated nutrient agar was transferred into the sterilized Petri-dishes for it to set. Holes were drilled into the solid agar using a sterilized hole borer. Cotton wool swabs was soaked in the pure both culture of the organisms and was used to rub the surface of the solidified agar. The complexes solution was poured into the drilled holes. The plates were incubated at 37 °C for 24 hours. Clear zones around the holes showed the antibacterial activities of the complexes and their ligands on the isolated organisms.

Characterization

The melting point of the complexes was determined using a Gallen kamp melting point apparatus WRS-1B (China). The molar conductivity was determined using HANNAH instrument EC 214 conductivity meter. The elemental analysis was carried out using a Perkin – Elmer CHN 2400 analyzer (Germany). The AAS was carried out and reported on alpha 4 – atomic absorption spectrophotometer (United Kingdom) at the central laboratory, Obafemi Awolowo University Ile-Ife Osun state Nigeria. The IR was carried out and reported in solid state of KBr pellet within 400 – 4000cm-1 using Thermo Scientific Nicolet FT-IR Spectrometer (United State) at Redeemer's University, Mowe Ogun state, Nigeria. The antimicrobial activities of the synthesized complexes and their parent ligands were evaluated against some isolated organisms: Escherichia coli, Bacillus megaterium, Proteus vulgarius, Pseudomonas aeruginosa, Euterobacter aerogenes, Staphylococcus aureus, Bacillus substillis and Serratia marcesceus. The organisms were obtained from the Microbiology Department, University of Ilorin, Ilorin Kwara state Nigeria.

RESULTS AND DISCUSSION

Table1: Physicochemical properties of mixed Pyrimethamine-Aspirin metal drug complexes.

Ligands/ Complexes	Melting point(^oC)	Colour	Elemental Analysis (Cal/Expt)			Conductivity $\Omega^{-1}Cm^2\ Mol^{-1}$	Metal Content($^o/_o$) (Cal/Expt)
			$^o/_o$ C	$^o/_o$ H	$^o/_o$ N		
Aspirin	135-136	White					
Pyrimethamine	238-240	White					
$[Zn(Asp)(Py)SO_4]$	259-261	White	42.78 (42.01)	3.57 (3.34)	9.51 (9.00)	2.24	11.04 (11.32)
$[Ni(Asp)(Py)SO_4]$	278-279	Green	43.22 (43.19)	3.60 (3.59)	9.61 (9.52)	3.28	10.12 (9.99)
$[Cu(Asp)(Py)SO_4]$	251-252	Blue	42.86 (42.90)	3.57 (3.40)	9.52 (9.41)	3.16	10.88 (10.00)
$[Mn(Asp)(Py)SO_4]$	273-275	Pink	43.52 (43.57)	3.63 (3.59)	9.67 (9.65)	2.47	9.50 (9.43)

*The Calculated and Experimental value in percentage signify upper values and lower values respectively

TABLE 2: Infrared Data of mixed Pyrimethamine-Aspirin metal drug Complexes.

Ligands/ Complexes	√(C=O) (cm^{-1})	√(O-H) (cm^{-1})	√(NH_2) (cm^{-1})	√(C=N) (cm^{-1})	√(M-N) (cm^{-1})
Aspirin	1698	3218	-	-	-
Pyrimethamine	-	-	3339	2225	750
[Zn(Asp)(Py)SO_4]	1725	3284	3364	2234	754
[Ni(Asp)(Py)SO_4]	1701	3300	3351	2256	754
[Cu(Asp)(Py)SO_4]	1727	3258	3389	2236	793
[Mn(Asp)(Py)SO_4]	1738	3237	3468	2248	754

Table 3: Antimicrobial screening of mixed Pyrimethamine-Aspirin metal drug complexes.

Organisms (µg/ml)	*E. coli*	*B. megaterium*	*P. Vulgaris*	*P. aeruginosa*	*E. aerogenes*	*S. aureus*	*B. substillis*	*S. marcesceus*
Ligands/ Complexes	Zone of inhibition (mm)							
Aspirin	14.85	16.66	22.05	14.02	16.75	28.00	24.89	20.67
Pyrimethamine	15.05	19.03	-	15.04	15.56	-	-	-
[Zn(Asp)(Py)SO_4]	21.97	18.74	21.82	20.07	25.12	19.35	-	-
[Ni(Asp)(Py)SO_4]	24.43	11.29	22.40	23.18	-	17.04	19.37	18.42
[Cu(Asp)(Py)SO_4]	21.00	22.79	10.59.	-	-	-	20.14	-
[Mn(Asp)(Py)SO_4]	14.95	19.87	14.67	19.59	-	-	18.06	21.24

Table 4: Minimum inhibitory concentration (MIC) of mixed Pyrimethamine -Aspirin metal drug complexes.

Ligands/ Complexes	*E. aerogenes*	*E. coli*	*S. aureus*	*S. marcesceus*	*B.megaterium*	*B. subtilis*
	Zone of inhibition					
Aspirin	0	1	0	0	0	0
Pyrimethamine	0	2	6	0	0	0
[Zn(Asp)(Py)SO_4]	30	31	17	21	32	20
[Ni(Asp)(Py)SO_4]	27	22	23	37	27	11
[Cu(Asp)(Py)SO_4]	12	20	16	12	21	29
[Mn(Asp)(Py)SO_4]	11	17	12	18	24	0

Table 5: Minimum bacteria concentration (MBC) of mixed Pyrimethamine-Aspirin metal drug complexes.

Ligands/Complexes	*Euterobacter aerogenes*	*Escherichia coli*	*Staphylococcus aureus*	*Serratia marcesceus*	*Bacillus megaterium*	*Bacillus Subtilis*
	Zone of inhibition (mm)					
Aspirin	0	0	0	0	0	0
Pyrimethamine	0	0	2	0	5	1
[Zn(Asp)(Py)SO_4]	32	36	27	37	12	25
[Ni(Asp)(Py)SO_4]	29	31	16	27	33	35
[Cu(Asp)(Py)SO_4]	32	34	34	16	39	11
[Mn(Asp)(Py)SO_4]	0	19	26	12	0	0

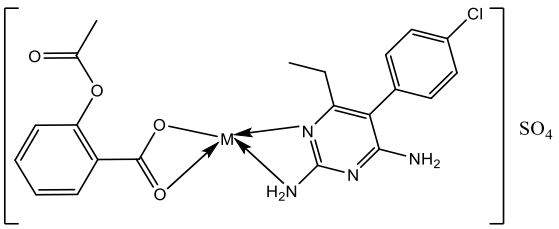
Co-ordination occured through the oxygen of the carbonyl and the ester groups in Aspirin complexes. The relationship of the ligands with their metal ions led to the formation of some new alternative

complexes in ratio 1:1. The physicochemical properties of mixed Aspirin - pyrimethamine complexes are presented in Table 1. It was observed that the melting point of the new products were higher than their ligands indicating formation of complexes. The novel complexes were found to be stable in air. From the elemental analysis of the complexes, it was observed that the data are in good agreement with the proposed structure (Ogunniran *et al.*,2012). The result of the molar conductivity measurements of the free ligands and the complexes in DMSO are presented in Table 1. The data obtained, was within the range of 2.24 – 3.28 Ω^{-1} Cm^2 Mol^{-1} indicating that the complexes are non-electrolytes Ogunniran *et al.* (2012), Sadeek *et al.* (2011). The IR spectra analyses of the ligands and their complexes are presented in Table 2. It has been discovered from previous work, that coordination occurs in pyrimethamine through the nitrogen of the amine group and nitrogen of the cyanide group (Sandrine *et al.,* 2008). In aspirin, coordination occurs through the oxygen of the carboxylic group and oxygen of the hydroxyl group (Ajibade *et al.*, 2013). The intense band at 1701 cm^{-1} has been assigned to √(C=O). This shows at a high frequency confirming that the carboxylic group is not deprotonated (Obaleye *et al.,* 2007). From the results obtained, the IR spectrum for Pyrimethamine and Aspirin showed characteristics absorption bands at (3339cm^{-1}, 2225cm^{-1}) and (1698 cm^{-1}, 3218 cm^{-1}). These were attributed to (NH_2, C=N) and (C=O, O-H) respectively. The (NH_2, C=N) at 3339 cm^{-1} and 2225 cm^{-1} are shifted to high frequency in all the complexes. The complexes undergo hypsochromic shift (Dhahir *et al.*, 2012), (Ian *et al.,* 2004). Based on the data given, it was observed that the complexes are all in tetrahedral geometry. Test for sulphate ion were carried out on all the complexes. It was observed that the test was positive due to coordination of the ion to the metal.

The antimicrobial activity of the complexes and their parent ligands were evaluated as presented in Table 4. This was determined using Nutrient Agar diffusion method and Minimum inhibitory concentration (MIC). Based on the antimicrobial activities data, shown in Table 4, it was observed that the complexes show high activity and compared favorably than their parent free ligands against the isolated organisms under the same conditions. This indicated that the complexes have the ability to reduce the population of the bacterial species (Obaleye *et al.,* 2007). In Table 3, the zones of inhibition exhibited by metal complexes on some selected bacterial species showed that *P. aeruginosa* exhibited the least value of zone of inhibition followed by *E. Coli* on Aspirin complexes but the least value of zone of inhibition using Pyrimethamine is on *P. aeruginosa* and *E. Coli* respectively. [Zn(Asp)(Py)SO_4, [Ni(Asp)(Py)SO_4, [Cu(Asp)(Py)SO_4 and [Mn(Asp)(Py)SO_4, shows their least value of zone of inhibition follows by *Bacillus megaterium* and *P. Vulgaris* respectively. The Aspirin complexes on *Euterobacter aerogenes, Staphylococcus aureus, Serratia marcesceus, Bacillus megaterium* and *Bacillus substilis* showed no growth indicating MIC, whereas Pyrimethamine complexes show MIC on *Euterobacter aerogenes, Serratia marcesceus, Bacillus megaterium* and *Bacillus substilis*. But [Mn(Asp)(Py)SO_4] show MIC on *Bacillus substilis.* In MBC, the Aspirin complexes showed no growth on *Euterobacter aerogenes, E. Coli, Staphylococcus aureus, Serratia marcesceus, Bacillus megaterium* and *Bacillus substilis*. In Pyrimethamine complexes, the MBC showed no growth is on *Euterobacter aerogenes* and *Serratia marcesceus* and for [Mn(Asp)(Py)SO_4, the MBC shows no growth on *Euterobacter aerogenes, Bacillus megaterium* and *Bacillus substilis*.

CONCLUSION

Mixed complexes of mixed Aspirin – Pyrimethamine were synthesized and characterized using melting point, Conductivity measurement, IR, AAS and elemental analysis. Antimicrobial screening of the prepared complexes were done and reported. From the results, it could be observed that the complexes had higher activity against the bacterial species than their parent free ligands. It is very necessary to put into consideration, the metal based drugs as potential therapeutic agent for resistance. Based on the analytical techniques, it has been observed that the complexes exhibited Tetrahedral geometry. From this work, it has been shown and confirmed that the complexes could be used to treat diseases caused by bacteria. They act as a potential antibacterial agents.



M= Zn, Ni, Cu, Mn

Fig 1: Proposed structure of mixed Pyrimethamine-Aspirin metal drug complexes.

REFERENCES

Ajibade, P.A., Idemudia, O.G. (2013). Synthesis, characterization, and antibacterial studies of Pd(II) and Pt(II) complexes of some diaminopyrimidine. derivatives. *Bioinorganic Chemistry and Applications*. 10:1155- 1159

Biradar, V.D., Mruthyunjayaswamy, B.H. (2013). Synthesis characterization and antimicrobial activity studies of some transition metal complexes derived from 3-chloro-N'-[(1E)-(2-hydroxy phenyl)methylene]-6-methoxy-1-benzothiophene-2-carbohydrazide. ScientificWorld Journal. 2013:451629. doi: 10.1155/2013/451629.

Brown DH, Lewis AJ, Smith WE, Teape JW (1980). Anti-inflammatory effects of some copper complexes. *J. Med. Chem.* 23:729-734.

Dhahir, S.A., Aziz, N.M., Bakir, S.R. (2012). Synthesis, Characterization and Antimicrobial Studies of complexes of Some Metal Ions with 2-[2-Amino-5- (3,4,5-Trimethoxy-benzyl)-Pyrimidinyl-4-azo]-4- Bromo-Phenol. *International Journal of Basic and Applied Sciences.* 12(6): 58-67.

Guangguo, W., Guoping, W., Xuchun, F., Longguan, Z. (2003). Synthesis, Crystal Structure, Stacking Effect and Antibacterial Studies of a Novel Quaternary Copper (II) Complex with Quinolone. **Molecules**. 8(2): 287-296.

Ian, M.H. (2004). The origins of antimalarial drug resistance. *Trends in Parasitology (Elsevier)* 20 (11): 512-518.

Kathryn, L.H., Katherin, J.F. (2009). Application of Metal Coordination Chemistry to Explore and Manipulate Cell Biology. Chem Rev. 109(10): 4921–4960.

Micheal, G.B., Drew, J.M., Fayz, M.D. (2004). Antimalarial Drugs based on Artemisin DFT Calculations on the Principal on the principal reaction. *J. Mol.Struc. (Theochem).* 711(95).

Obaleye, J.A., Akinremi, C.A., Balogun, E.A., Adebayo, J.O. (2007). Toxicological studies and antimicrobial properties of some Iron (III) complexes of Ciproflxacin. *African Journal of Biotechnology*. 6(24): 2826-2832.

Ogunniran, K.O., Ajanaku, K.O., James, O.O., Ajani, O.O, Adekoya, J.A., Nwinyi, O. (2008). *Synthesis, characterization, antimicrobial activity and toxicology study of some metal complexes of mixed antibiotics. African Journal of Pure and Applied Chemistry*. 2(7). 69-74.

Ogunniran, K.O., Ajani, O.O., Ehi-Eromosele, C.O., Obaleye, J.A., Adekoya, J.A., Ajanaku, C.O. (2012). Cu(II) and Fe(III) complexes of sulphadoxine mixed with pyramethamine: Synthesis, characterization, antimicrobial and toxicology study. *International Journal of Physical Sciences.* 7(13):1998 – 2005.

Paul, J.D., Giann, S. (2006). Metal-based antitumour drugs in the past genomic era. *Dalton trans.* 10:1929-1933.

Prasad, S., Agarwal, R. (2007). Cobalt (II) complexes of various thiosemicarbazones of 4-aminoantipyrine: syntheses, spectral, thermal and antimicrobial studies," *Transition Metal Chemistry*, 32(2) :143–149.

Sadeek, S.A.H., El-Shwiniy, W., Zordok, W.A., Kotb, E. (2011). Spectroscopic studies, thermal analyses and biological evaluation of new V(IV), Zr(IV) and U(VI) moxifloxacin complexes. *Journal of Molecular Structure*. 19(2):100- 106.

Sandrine, D., Emilia, P., Louis, M., Elisabeth, M., Christian, S., Philippe, G., Patricia, M. (2008). Synthesis and antimalarial activity of new analogues of amodiaquine. *European Journal of Medicinal Chemistry*. 43(2): 252-260.

Shazia, R., Muhammad, I., Anwar, N., Haji, A., Amin, A. (2010). Transition metal complexes as potential therapeutic agents. *Biotechnology and Molecular Biology*. 5(2):38-45.

Sousa, E.H., Basso, L.A., Santos, D.S, Diógenes, I.C., Longhinotti, E., Lopes, L.G., Moreira, S. (2012). Isoniazid metal complex reactivity and insights for a novel anti-tuberculosis drug design. *J Biol Inorg Chem.*17(2):275-83.

Tella, A.C., Obaleye, J.A (2010). Synthesis and biological studies of Co(II) and Cd(II) 5-(3,4,5-trimethoxybenzyl) pyrimidine-2,4-diamine (Trimethoprim) complexes. *Int. J. Biol. Chem. Sci.* 4(6):2181-2191.