African Journal of Biotechnology Vol. 11(39), pp. 9323-9329, 15 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.238
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Synthesis, characterization and antibacterial screening of 2,4-diaminopyrimidine pyrimethamine and trimethoprim silver complexes

Omoruyi G. Idemudia[1]*, Peter A. Ajibade[1] and Anthony I. Okoh[2]

[1]Department of Chemistry, University of Fort Hare, Alice 5700, South Africa.
[2]Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa.

Accepted 23 February, 2012

Air stable silver Ag(I) complexes of pyrimethamine and trimethoprim drugs have been synthesized and characterized by elemental analysis, Fourier transform infrared (FTIR) and ultraviolet visible (UV-Vis) spectroscopy, and conductivity measurement. The metal complexes formed a three and four coordinate geometry with the ligands acting as a monodentate molecule bonding to the silver ion in each case through the pyrimidine N (1) nitrogen. The complexes have non-electrolyte behaviour in dimethylformamide (DMF) solution with its low conductivity values. Silver complexes, their free ligands alongside the corresponding silver salts were screened against selected bacterial isolates. All the silver complexes showed enhanced antibacterial activities compared to their free ligands and potential antibacterial agents have been identified.

Key words: Pyrimethamine, trimethoprim, silver complexes, antibacterial screening, minimum inhibitory concentration.

INTRODUCTION

Activities of metal ions in biology have led to the coordination of metals with therapeutic agents in an effort to increase their reactivity and potency, hence the development of metal-based drugs.

Positively charged metal ions, are privileged to be attracted towards and bind to negatively charged bio-molecules to form metal complexes of these molecules. Li-june (2003) reported enhanced bleomycin, strep-tonigrin and bactracin compared to the original drug on coordination with metal ions.

Platinum group metals (PGM) on coordination with some pharmaceuticals have gained attention as anti cancer agents with enhanced efficacy and reduced toxicity (Timerbaev et al., 2006). Silver, either in its ionic, salt or complex form has been in the limelight of research which has been attributed mostly to its antimicrobial properties with minimal human toxicity (Melaiye et al., 2004; Legler et al., 2001; Gottschaldt et al., 2006; Sambhy et al., 2006; Nomiya et al., 2000). Biologically active silver compounds are capable of adversely altering bacterial cells by reacting with the thiol groups in protein synthesis, affecting the DNA and cell wall, as well as electron transportation interference (Russell and Hugo, 1994).

Amino pyrimidine and pyrimidine compounds play significant roles of great importance on biological systems (Saha and Kar, 1977). Pyrimethamine [5-(4-chlorophenyl)-6-ethyl-2,4-diaminopyrimidine] and trimethoprim [5-(3,4,5-trimethoxybenxyl)-2,4-diamino-pyrimidine] are clinically used amino pyrimidine compounds which have applications as antimicrobial and antiprotozoal agents (Hitchings, 1971). They are antifolate drugs (Zimmerman et al., 1987; Olliaro, 2001) that bind with great kinship to and inhibit the bacterial enzyme dihydrofolate reductase (DHFR) (Hitchings and Burchall 1965; Simo et al., 2000; Macreadie et al., 2000). Trimethoprim has been an antimicrobial agent used as an

*Corresponding author. E-mail: idemudiaog@yahoo.com. Tel: +27835991551. Fax: +27865818503.

Abbreviations: FTIR, Fourier transform infrared; **UV-Vis,** ultraviolet visible spectroscopy; **DMF,** dimethylformamide.

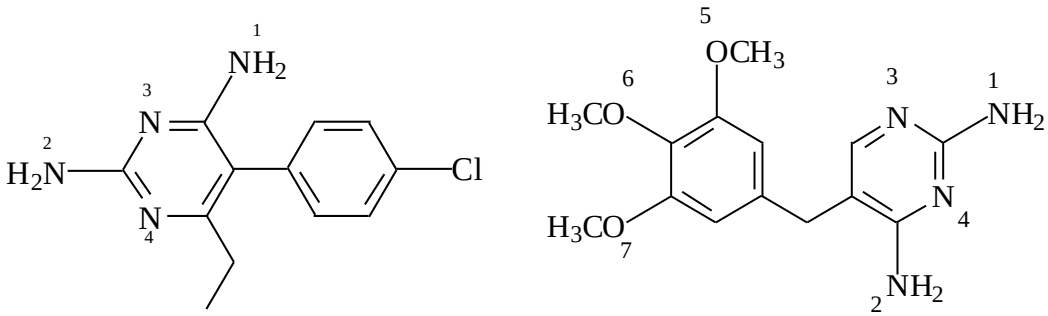

Figure 1. Structure of ligands showing potential coordination sites with metals.

antibiotic against β-lactam resistant strain pathogens (Frester et al., 1996). Pyrimethamine is mostly administered in conjunction with other drugs while maintaining its desired delivery properties (Sirawaraporn and Yuthavong, 1986; Chulay et al., 1984), for the treatment of resistant *Plasmodium falciparum* malaria strain in the form of a combinational therapeutic agent and in some cases in combating opportunistic infections in patients with AIDS (Tanaka et al., 2004). As shown in Figure 1, pyrimethamine can bind to metal ion through one of its amine nitrogen or via the nitrogen of the pyrimidine ring, while trimethoprim binds through any of the three methoxy oxygens in addition with the two amine groups and the pyrimidine nitrogen; however, it is rarely coordinated via the methoxy. Crystal structures of rhodium complexes of trimethoprim and pyrimethamine with coordination occurring via the pyrimidinic nitrogen have been reported (Zoroddu et al., 1987). Complexes of trimethoprim with Ag^{+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Ni^{2+} whereby the metal ion is coordinated to the monodentate ligand through the -NH_2 group based on infrared data have been synthesized and characterized (Seekhon et al., 1999). Cu (II) complex of pyrimethamine with enhanced biological activity and reduced toxicity, generally less than the standard anti-malaria chloroquine, has being reported (Ajibade and Kolawole, 2008). Bearing in mind therapeutic agents ability to produce desirable effect upon coordination with metals and in continuation of our ongoing search for alternative therapeutic agents (Idemudia and Ajibade, 2010) we present herein, the synthesis, characterization and antibacterial screening of Ag(I) complexes of pyrimethamine and trimethoprim. Evaluation of the antibacterial activity of the metal salts of silver was compared to the activities of the synthesized complexes.

MATERIALS AND METHODS

Materials

All reagents and solvents were procured as analytical grade and used without further purification. Elemental analyses for C, H and N were carried out on a Fison elemental analyzer. Melting point determination was obtained with the GallenKamp melting point apparatus. Molar conductivity measurement (10^{-3}M solution in dimethylformamide) was obtained on the CON 6/TDS 6 Hand-held conductivity/TDS Meter. IR spectra of the complexes were recorded as KBr pellets on a Perkin-Elmer Model System 2000 Fourier transform infrared (FTIR) spectrophotometer in the range 4000 to 370 cm^{-1}, ensuring that the pellets are moisture free. Electronic spectra of complexes were recorded on a Perkin-Elmer Lambda 25 ultraviolet visible (UV-Vis) spectrophotometer in dimethylformamide (DMF). Gram (+) *Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), and Gram (-), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 19582), and American type culture collection obtained from the Biochemistry and Microbiology Department, University of Fort Hare were used.

Synthesis of silver complexes

A previously reported procedure was employed in the synthesis of the silver complexes but with some modification (Idemudia and Ajibade, 2010). To a 1 mmol (0.17 g) of $AgNO_3$ or CH_3COOAg in methanol slightly heated to dissolve completely, a solution of pyrimethamine (pyrm) (1 mmol, 0.25 g) in methanol was added in drops. After reflux for 2 h a solution of triphenylphosphine PPh_3 (1 mmol 0.26 g) in methanol was added and reflux was continued for another 4 h. The resultant solution was evaporated under reduced pressure, filtered to collect the precipitate, washed with methanol, diethylether and finally dried over silica gel in a dessicator. The same procedure was carefully carried out using trimethoprim (tmp) (1 mmol, 0.29 g) and the substitution of triphenylphosphine with diphenylphosphinoethane (DPPE) in the case of Ag(tmp)(NO_3) (DPPE), to obtain the silver trimethoprim complexes.

Antibacterial screening

Screening of metal complexes, the ligands, and the silver salts for their bioactivity was carried out using the zone of inhibition technique (agar well diffusion method) (Russell and Furr, 1977, Chaudhary et al., 2003; Shahzadi et al., 2006). Agar wells of 0.6 mm in diameter were bored in the solidified mixture of Mueller Hilton agar in agar plates that has been carefully prepared by standard procedures and having a suspension of a specific bacterial isolate with optical density (OD) of 0.1 minimum in it. Solution of complexes were prepared using DMF and distilled water in the ratio 1:10 respectively to minimize any effect that may be due

Table 1. Analytical data and some physical properties of complexes.

Complex	Molecular formulae	Colour	Analytical data (%)			Yield (%)	M.P (°C)	Cond. (µS)
			C found (calc.)	H found (calc.)	N found (calc.)			
Ag(pyrm)(NO_3)(PPh_3)	$C_{30}H_{28}N_5ClO_3PAg$	Light Brown	52.81 (52.92)	4.23 (4.14)	9.72 (10.29)	97	98−99	16.30
Ag(pyrm)(CH_3COO)(PPh_3)	$C_{32}H_{31}N_4ClO_2PAg$	Off white	57.01 (56.70)	5.08 (4.61)	8.42 (8.26)	89	236−239	4.19
Ag(tmp)(NO_3)(DPPE)	$C_{40}H_{42}N_5O_6P_2Ag$	Light Brown	55.39 (55.95)	4.80 (4.93)	8.53 (8.16)	90	190-193	13.62
Ag(tmp)(NO_3)(PPh_3)	$C_{32}H_{33}N_5O_6PAg$	Light Brown	53.68 (53.20)	4.35 (4.60)	9.40 (9.69)	92	160-162	12.20

M.P (°C), Melting point; Cond. (µS), conductivity.

to DMF. Prepared solutions of complexes, the free ligands, silver salts, as well as a mixture of DMF and distilled water in the right proportion were dispensed into these wells. The agar plates were carefully placed in the incubator to avoid spilling of the well content, within a temperature range of 37 to 39°C for 24 h and then observed for possible growth of bacterial.

Minimum inhibition concentration (MIC) determination

The MIC of the complexes was determined by the macro-broth dilution technique (Ibrahim et al., 1997; Akinpelu and Kolawole, 2004) with little modifications. 2 ml (1:10 of DMF and distilled water, respectively) of the prepared complexes were diluted in sterile distilled water to achieve a final concentration of 2.50, 1.25, 0.63, 0.31, 0.16, 0.08, 0.04 and 0.02 mg/ml. Prepared complexes of different concentration were introduced one after the other into pre sterilized molten nutrient agar. The mixture was poured in an agar plate and allowed to set. To the solidified nutrient agar mixture, the bacterial isolate with standardized inoculums was streaked on it. The test plates were incubated at 37°C for 24 h. The MIC was taken as the lowest concentration of the tested antibacterial agent that shows no visible bacterial growth (Nishizawa et al., 1988).

Minimum bactericidal concentration (MBC) determination

After determination of the MIC, samples of organisms were taken from plates which were used for the MIC test that were with no visible growth and sub cultured onto a freshly prepared nutrient agar medium by streaking. These diluted cultures were then incubated at 35 to 37°C for 24 h. MBC was carried out with the method of (Olorundare et al., 1992) and was taken as the lowest concentration of the antibacterial agent at which all bacteria in the diluted culture are killed.

RESULTS AND DISCUSSION

Ag(I) complexes of pyrimethamine and trimethoprim were successfully synthesized from the reaction of the nitrate or acetate salt of silver with the appropriate ligands in methanol according to the reaction scheme below.

$$MX + L + D \xrightarrow[\text{Heat}]{\text{Methanol}} M(X)(L)(D)$$

Where, L = pyrimethamine or trimethoprim, X = NO_3 or CH_3COO, D = DPPE or PPh_3 and M = Ag^+.

Ag(pyrm)(NO_3)(PPh_3), Ag(pyrm)(CH_3COO)(PPh_3) and Ag (tmp)(NO_3)(PPh_3) formed a three coordinate geometry, while Ag(tmp)(NO_3)(DPPE) gave a four coordinate geometry.Each complex contain a molecule of pyrimethamine or trimethoprim acting as a monodentate ligand through the pyrimidinyl N(1) nitrogen atom. The geometry around the metal ion is completed by its coordination to the nitrate ions, acetate ions, triphenyl-phosphine or bidentate DPPE in the case of Ag(tmp) (NO_3)(DPPE). Analytical data and some physical properties of the synthesized complexes are presented in Table 1. The solid, air stable, non-hygroscopic silver complexes have different colours varying from off-white to light brown, with a minimum of 89% yield in each case. Their melting point is between 98 and 239°C. The results obtained from elemental analytical measurements agree with calculated values and confirm the stoichiometry of the complexes, therefore their proposed structures (Figure 2). The complexes are generally insoluble in water and in non-coordinating solvents but soluble in strong donor polar DMF and dimethyl sulfoxide (DMSO). The complexes were non-electrolytes in DMF with conductivity values between 4.19 to 16.30 µS (Kettle, 1975). To ascertain the bonding of the ligands to the metal ion, a careful comparison of the infrared spectroscopy of ligands and complexes was done. Presented in Table 2, are the relevant frequency bands assigned accordingly. A sharp frequency band at 1630 in pyrimethamine and 1635 in trimethoprim assigned to the pyrimidine nitrogen *v*(C=N) (Contini et al., 1997) shifted to 1620, 1619, 1607 and 1611 cm^{-1} in Ag(pyrm)(NO_3) (PPh_3), Ag(pyrm)(CH_3COO)(PPh_3), Ag(tmp)(NO_3)(DPPE) and Ag(tmp)(NO_3)(PPh_3), respectively. The significant shifts to a lower frequency band suggest the coordination of the metal ion to the ligand through the pyrimidine nitrogen of the ligands. The symmetrical and asymmetrical stretching bands of the $–NH_2$ group in the higher frequency region (Nakamato, 1997) are displaced

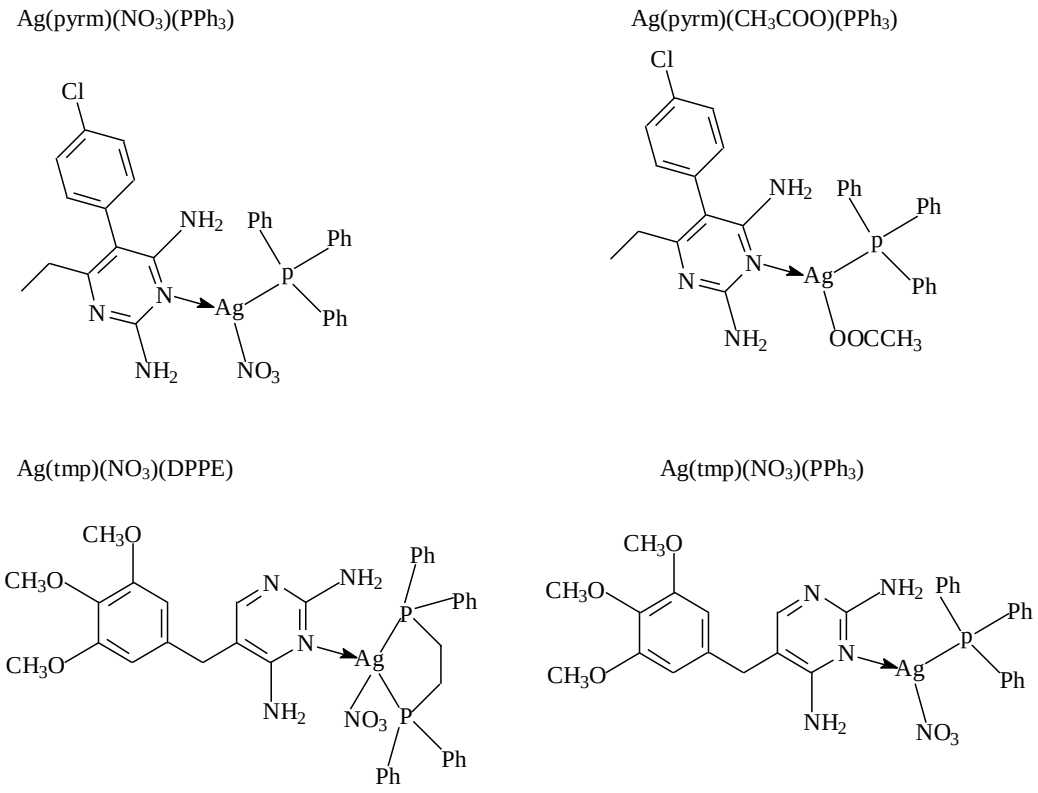

Figure 2. Proposed structures of silver complexes.

Table 2. Relevant infrared and electronic spectra of the ligands and complexes.

Complex	π→π*arom ring	*v*(C=N)	*v*(N-H)as	*v*(N-H)a	*v*(COO)	*v*(M-N)
Pyrimethamine	–	1630	3468	3313	–	–
Trimethoprim	–	1635	3471	3319	–	–
Ag(pyrm)(NO_3)(PPh_3)	275	1620	3470	3327	–	468-490
Ag(pyrm)(CH_3COO)(PPh_3)	270	1619	3473	3324	1480	460-485
Ag(tmp)(NO_3)(DPPE)	272	1607	3420	–	–	465-495
Ag(tmp)(NO_3)(PPh_3)	274	1611	3423	3341	–	467-493

v(C=N), Pyrimidine nitrogen.

slightly to a higher frequency after coordination, which may be due to inter and intra molecular hydrogen bonding, an effect caused by the interactions of the metal with the ligand (Raso et al., 2000, Toyssie and Charette, 1963). The presence of the acetate ion in Ag(pyrm)(CH_3COO)(PPh_3) is observed as a medium frequency band at 1480 cm^{-1} which is absent in the free ligand (Simo et al., 2000). Frequency vibrations due to Ag-P from DPPE and PPh_3 can be seen at the region of 600 cm^{-1} and below which are completely absent in the ligands. The band observed in the complexes in the region 542 to 502 cm^{-1} was attributed to *v*(Ag-N) (Liu et al., 2007) The electronic spectra of the synthesized silver complexes show intense absorptions in the UV-region, at around 270 to 275 nm which is a characteristics of a metal charge 4d to 4s transition at relatively high energies (Kunkely and Vogler, 2006) and assigned to charge transfer from π orbital of the donor ligand to the d orbitals of the metal, d→π* and interligand n→π* transitions (Naeimi and Moradian, 2009). Moreover, the charge transfer transitions may interfere and prevent the observation of all the expected transitions in the spectra (West et al., 1993). The electronic configurations of Ag(I) complexes are d^{10} which confirms the absence of any (d−d) transitions and absence of visible region absorptions in their electronic spectra. The *in vitro* antibacterial activity of the silver complexes, the two ligands, and the corresponding silver salts against the selected bacteria *S. aureus*, *S. faecalis*, *P. aeruginosa*, and *E. coli* as zone of growth inhibition using the agar

Table 3. Zone of inhibition exhibited by compounds on selected pathogens 5 mg/ml (mm).

Pathogen	*S. aureus* (ATCC 6538)	*S. faecalis* (ATCC 29212)	*E. coli* (ATCC 8739)	*P. aeruginosa* (ATCC 19582)
Pyrimethamine	–	–	–	–
Trimethoprim	20	–	–	15
Ag(pyrm)(NO_3)(PPh_3)	21	19	18	20
Ag(pyrm)(CH_3COO)(PPh_3)	22	20	17	20
Ag(tmp)(NO_3)(DPPE)	22	26	19	25
Ag(tmp)(NO_3)(PPh_3)	40	22	23	22
$AgNO_3$	18	17	15	14
$AgOOCCH_3$	16	14	12	15

Table 4. MIC values on selected bacteria (mg/ml).

Pathogens	*S. aureus* (ATCC 6538)	*S. faecalis* (ATCC 29212)	*E. coli* (ATCC 8739)	*P. aeruginosa* (ATCC 19582)
Trimethoprim	2.5	–	–	2.5
Ag(pyrm)(NO_3)(PPh_3)	0.31	0.63	0.63	0.63
Ag(pyrm)(CH_3COO)(PPh_3)	0.63	0.63	1.25	1.25
Ag(tmp)(NO_3)(DPPE)	0.16	0.31	0.16	0.16
Ag(tmp)(NO_3)(PPh_3)	0.02	0.08	0.08	0.04
$AgNO_3$	1.25	1.25	>2.50	2.50
$AgOOCCH_3$	>2.50	>2.50	>2.50	2.50

MIC, Minimum inhibition concentration.

Table 5. MBC values on selected bacteria (mg/ml).

Pathogens	*S. aureus* (ATCC 6538)	*S. faecalis* (ATCC 29212)	*E. coli* (ATCC 8739)	*P. aeruginosa* (ATCC 19582)
Trimethoprim	>2.50	–	–	>2.50
Ag(pyrm)(NO_3)(PPh_3)	>0.31	0.63	>0.63	0.63
Ag(pyrm)(CH_3COO)(PPh_3)	0.63	0.63	>1.25	1.25
Ag(tmp)(NO_3)(DPPE)	0.16	0.31	>0.16	0.16
Ag(tmp)(NO_3)(PPh_3)	0.02	0.08	>0.08	>0.04
$AgNO_3$	>2.50	>2.50	>2.50	>2.50
$AgOOCCH_3$	>2.50	>2.50	>2.50	>2.50

MBC, Minimum bactericidal concentration.

well diffusion technique is presented in Table 3. The MIC and MBC of the active compounds was evaluated by the macro dilution method, and their values are presented in Tables 4 and 5, respectively. Ag(I) complexes exhibited a broad spectrum antimicrobial activity as they are active against all selected bacterial isolates. They have also shown a stronger antibacterial activity than the free ligands as well as the metal salts. Ag(tmp)(NO_3)(PPh_3) appears to be with the strongest antibacterial activity showing a highest zone of growth inhibition of 40 mm against *S. aureus.* Although this may seem so, the high zone of inhibition however do not reveal for certain, that Ag(tmp)(NO_3)(PPh_3) is a stronger antibacterial agent, as this may be attributed, amidst many factors, to the diffusing strength (diffusability) of the antibacterial agent and also to the ratio of the amount of bacterial isolate present in a certain amount of agar solution (Rios et al., 1988). However, the MIC values support the good activity of Ag(tmp)(NO_3)(PPh_3). Trimethoprim showed activity against *S. aureus* and *P. aeruginosa* with 20 and 15 mm zones of inhibition respectively, while pyrimethamine did not exhibit any activity, which suggests that trimethoprim is more active than pyrimethamine. Furthermore, silver complexes of trimethoprim tend to have stronger

antibacterial activities. The generally lower MIC values of the metal complexes as compared to their free ligands once again show the enhancement in their activity against the reported bacterial strain. Ag(tmp)(NO_3)(PPh_3) lowest MIC and MBC value of 0.02 mg/ml against *S. aureus* again emphasizes the high level of activity.

Conclusion

Four mononuclear Ag(I) complexes of pyrimethamine and trimethoprim have been synthesized and charac-terized based on elemental analysis and spectroscopic analysis. The monodentate trimethoprim and pyri-methamine coordinates to the metal ion through the pyrimidine nitrogen. All silver complexes show stronger antibacterial activity than the free drugs, thus potential metal based bactericidal are identified.

REFERENCES

Ajibade PA, Kolawole GA (2008). Synthesis Characterization and antiprotozoal studies of some metal complexes of antimalarial drugs, Transition Met Chem. 33: 493-497.

Akinpelu DA, Kolawole DO (2004). Phytochemistry and antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum). Sci. Focus, 7: 64-70.

Chaudhary A, Bansal N, Gajraj A, Singh RV (2003). Antifertility, Antibacterial, Antifungal and Percent Disease Incidence Aspects of Macrocyclic Complexes of Manganese (II). J. Inorg. Biochem. 96. 393-400

Chulay JD, Watkins WM, Sixsmith DG (1984). Synergistic antimalarial activity of pyrimethamine and sulfadoxine against Plasmodium falciparum *in vitro*. Am. J. Trop. Med. Hyg. 33: 325-330.

Contini G, Ciccioli A, Cozza C, Barbaro M, Marabini M (1997). Infrared study of 2-mercaptobenzothiazole and two of its derivatives adsorbed on PbS. Int. J. Miner. Process, 51(1-4). 283-289

Frester M, Furneri PM, Mezzasalma E, Nicolosi VM, Pugeisi G (1996). Correlation of trimethoprim and brodimoprim physicochemical and lipid membrane interaction properties with their accumulation in human neutrophils. Antimicrob. Agents Chemother. 40(12): p. 2865.

Gottschaldt M, , Pfeifer A, Koth D, Görls H-M, Dahse H, Möllmann U, Obata M, Yano S (2006). Silver(I) complexes based on novel tripodal thioglycosides: synthesis, structure and antimicrobial activity. Tetrahedron, 62(48): 11073-11080

Hitchings GH (1971). Folate antagonists as antibacterial and antiprotozoal agents. Ann. New York Acad. Sci. 186: 444-451.

Hitchings GH, Burchall JJ (1965). Inhibition of folate biosynthesis and function as a basis for chemotherapy. Adv. Enzymol. Relat. Areas Mol. Biol. 27: 417-468.

Ibrahim MB, Owonubi MO, Onaolapo JA (1997). Antimicrobial effects of extracts of leaf, stem, and root-bark of *Anogiessus leicarpus* on *Staphylococcus aureus* NCTC 8190, *Escherichia coli* NCTC 10418 and *Proteus vulgaris* NCTC 4636. J. Pharmaceut. Res. Dev. 2: 20-26.

Idemudia OG, Ajibade PA (2010) Antibacterial activity of metal complexes of antifolate drug pyrimethamine. Afr. J. Biotechnol. 9(31): 4885-4889.

Kettle SFA (1975). Coordination Compounds, Thomas Nelson and Sons. London, p.165

Kunkely H, Vogler A (2006). Photoproperties of silver sulfadiazine. Inorganic Chem. Commun. (10): 226-228.

Legler AV, Kazachenko AS, Kazbanov VI, Per'yanova VO, Veselova OF (2001). Synthesis and antimicrobial activity of silver complexes with arginine and glutamic acid. Pharmaceut. Chem. J. 35(9): 35-36.

Li-june M (2003) Structure and function of metallo-antibiotics. Med. Res. Rev. 6(23): 697-762.

Liu J, Zhang B, Wu B, Zhang K, Hu S (2007). The Direct Electrochemical Synthesis of Ti(II), Fe(II), Cd(II), Sn(II), and Pb(II) Complexes with N, N-Bis(Salicylidene)-o-Phenylenediamine. Turk. J. Chem. 31: 623-629.

Macreadie I, Ginsburg H, Sirawaraporn W, Tilley L (2000). Antimalarial Drug Development and New Targets, Parasit. Today, 16: 10-15

Melaiye A, Simons RS, Milsted A, Pingitore F, Wesdemiotis C, Tessier CA, Youngs WJ (2004). Formation of Water-Soluble Pincer Silver(I)-Carbene Complexes: A Novel Antimicrobial Agent. J. Med. Chem. 47: 973-977

Naeimi H, Moradian M (2009). Synthesis and characterization of nitro-Schiff bases derived from 5-nitro-salicylaldehyde and various diamines and their complexes of Co(II). J. Coord. Chem. 62: 156-160

Nakamato K (1997). Infrared and Raman Spectra of Inorganic and Coordination Compounds, fifth ed., Wiley-Interscience, New York. p. 7.

Nishizawa K, Hirano M, Kimura A, Mochizuki T, Yamamoto Y, Yamamura S, Momose Y (1998). Evaluation of the antimicrobial activity of carbapenem and cephem antibiotics against *Pseudomonas aeruginosa* isolated from hospitalized patients. J. Infect. Chemother. 4: 174-176.

Nomiya K, Takahashi S, Noguchi R, Nemoto S, Takayama T, Oda M (2000). Synthesis and characterization of water-soluble silver(I) complexes with L-histidine (H_2his) and (S)-(-)-2-pyrrolidone-5-carboxylic acid (H_2pyrrld) showing a wide spectrum of effective antibacterial and antifungal activities. Crystal structures of chiral helical polymers [Ag(Hhis)](n) and {[Ag(Hpyrrld)$]_2$}(n) in the solid state. Inorg. Chem. 39: 3301-3311.

Olliaro, P (2001). Mode of action and mechanisms of resistance for anti-malarial drugs. Pharmacol. Ther. 89(2): 207-219

Olorundare EE, Emudianughe TS, Khasar GS, Koteyi SA, Irobi N (1992). Antibacterial properties of leave extract of Cassia alata. Biol. Res. Chem. 4: 113-117.

Raso AG, Fiol JJ, Rigo S, Lopez AL, Molins E, Espinosa E, Borrass E, Castineiras A (2000). Coordination behaviour of sulfanilamide derivatives. Crystal structures of [Hg(sulfamethoxypyridazinato)2], [Cd(sulfadimidinato)2(H2O)]·2H2O and [Zn(sulfamethoxazolato)2-(pyridine)2(H2O)2]. Polyhedron, 19: p. 991.

Rios JL, Recio MC, Villar A (1988). Screening methods for natural Products with antimicrobial activity: A review of the literature. J. Ethnopharmacol. 23: 127-149.

Russell AD, Furr JR (1977). The antimicrobial activity of a new chloroxylenol preparation containing ethylenediamine tetraacetic acid. J. Appl. Bacteriol. 43: 253-260.

Russell AD, Hugo WB (1994). Antimicrobial activity and action of silver. Prog. Med. Chem. 31: 351-370

Saha N, Kar Sk (1977) Metal complexes of pyrimidine-derived ligands-I. Nickel(II) complexes of 2-hydrazino-4,6-dimethyl pyrimidine, J. Inorg. Nucleic Chem. 39(1): 195-200.

Sambhy V, MacBride MM, Peterson BR, Sen A (2006). Silver bromide nanoparticle/polymer composites: Dual action tunable antimicrobial materials. J. Am. Chem. Soc. 128(30): 9798-9808.

Seekhon BS, Randhawa HS, Sahai HK (1999) On the coordinating behaviour of trimethoprim towards some metal ions. Synth. React. Inorg. Met.–Org. Chem. 29(2): 309-407.

Shahzadi S, Ali S, Bhatti MH, Fettouhi M, Athar M (2006). Chloro-diorganotin(IV) complexes of 4-methyl-1-piperidine carbodithioic acid: Synthesis, X-ray crystal structures, spectral properties and antimicrobial studies J. Organometallic Chem. 691(8): 1797-1802

Simo B, Porello L, Ortiz R, Castineiras A, Latorre J, Canton E (2000). Interactions of metal ions with a 2,4-diaminopyrimidine derivative (trimethoprim) Antimicrobial studies. J. Inorganic. Biochem. 81. 275-283.

Sirawaraporn W, Yuthavong (1986). Potentiating Effect of Pyrimethamine and Sulfadoxine against Dihydrofolate Reductase from Pyrimethamine-Sensitive and Pyrimethamine-Resistant *Plasmodium chabaudi*. Antimicrob. Agent Chemother. 29(5): 899-905.

Tanaka R, Arai T, Hirayama N (2004). Structure of Pyrimethamine Hydrochloride. Anal. Sci. 20: 175-176.

Timerbaev AR, Hartinger CG, Keppler BK (2006) Metallodrug research

and analysis using capillary electrophoresis. TrAC Trends Anal. Chem. 25(9): 868-875.
Toyssie P, Charette JJ (1963). Physico-chemical properties of co-ordinating compounds-III Infra-red spectra of N-salicyclidenealkylamines and their chelates. Spectrochim. Acta. 19: 1407-1423.
West DX, Lockwood MA, Liberta AE, Chen X, Willett RD (1993). Spectral nature, antifungal activity and molecular structure of metal complexes of acetylpyrazine 4N-substituted thiosemicarbazones. Transition Met. Chem. 18: p. 221.
Zimmerman J, Selhub J, Rosenberg IH (1987). Competitive inhibition of folate absorption by dihydrofolate reductase inhibitors, trimethoprim and pyrimethamine11-3. J. Clin. Nuir. 46(5): 18-22.
Zoroddu MA, Naldini L, Demartin F, Masciocchi N (1987). Metal complexes of 2,4-diamino-5-(3′,4′,5′-trimethoxybenzyl)pyrimidine (trimethoprim) and 2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine (pyrimethamine). Part III. Syntheses and x-ray structures of $[Rh_2(O_2CCH_3)_4(trimethoprim)_2]\cdot 2C_6H_6\cdot CH_3OH$ and $[Rh_2(O_2CCH_3)_4(pyrimethamine)_2]$. Inorg. Chim. Acta. 128(2): 179-183.