Bioscience Research Communications
Vol. 13, No. 4, October 31, 2001
Printed in Nigeria

0795-8072/2001 $3.00 + 0.00

BRC 200075/13408

The Effect of 3-Chloro-Substitution on the Antimicrobial Activities of Some Cobalt (II) β-Ketoamines and Their Adducts

A. A. Osowole[1], J. A. O. Woods[1], O. E. Fagade[2] and A. O. Odunola[3]

[1]Department of Chemistry, University of Ibadan, Ibadan, Nigeria

[2]Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria

[3]Department of Chemistry, University of the North, Sovenga, South Africa

(Received July 17, 2000)

ABSTRACT: Four cobalt (II) 3-chloro-ß-ketoamines, seven cobalt (II) ß-ketoamines and fifteen 2,2'-bipyridine and 1,10-Phenanthrdine adducts were screened against thirteen organisms namely *Bacillus subtilis Bacillus cereus, Staphylococcus aureus, Pseudomonas flourescens, Pseudomonas auriginosa, Escherichia coli, Aeromonas* spp, *Flavobacterium* spp, *Salmonella* spp *and Streptococcus pyogens.* The cobalt (II) ß-ketoamines were more effective than their cobalt (II) 3-chloro-ß-ketoamines analogs. The adducts all showed less antimicrobial activities than the parent, cobalt(II) ß-ketoamines and cobalt (II) 3-chloro ß-ketoamines, even though the cobalt (II) ß-ketoamines adducts, were more effective than their corresponding cobalt (II) 3-chloro-ß-ketoamine adducts. Antimicrobial activities in all cases, were related to the electron density on the coordinated cobalt atom. Thus, the lower the electron density on the coordinated cobalt atom in these ß-ketoamines, 3-chloro-ß-ketoamines and adducts the lower the antimicrobial activity.

The minimum inhibitory concentrations for cobalt (II) ß-ketoamines and adducts were between 1.25-3.95 mg/ml while that of cobalt(II) 3-chloro-ß-ketoamines and adducts were between 1.29-5.20mg/ml.

Key Words: Antimicrobial activities, ß-ketoamines, cobalt (II) complexes, inductive effect; mesomeric effect.

Introduction

The antimicrobial activities of some ketones and amines have been reported [1-4] Divalent ions, Zn, Fe, Ni, Ru and Os of tris-1,10-Phenanthroline, tris-2,2'-bipyridine and bis-2,2',2"-terpyridine, are known to inhibit acetylcholine-sterase in mice. [5].

Antimicrobial activities of β-aminoketones and its phenyl substituted analogs were found to be functions of their structures and lipophobity (6,7). Furthermore, halogens (e.g Cl_2) are strong disinfectants which kill bacteria through destruction of their cytoplasmic membrane (8).

The objective of this work is to investigate the effect of chlorine substitution on the antimicrobial activities of the cobalt (II) β-ketoamines and its 2,2'-bipyridine and 1,10-Phenanthroline adducts.

Materials and Methods

The complexes were prepared by dissolving 0.02moles of cobalt(II) acetate in 40% ethanolic solution, 0.08 moles of aliphatic primary or 0.04moles of aliphatic diamines in ethanol was added dropwisely, followed by the addition of acetylacetone (AA-H) and 3-chloroacetylacetone (3ClAAH).

The adducts were prepared from these complexes by mixing 0.01 moles of the dry solid complex with o.o2 moles of the organic base. This was then introduced into warm chloroform while stirring for thirty minutes. The compounds were filtered and dried over silica gel (9-10). The compounds were analysed complexometrically with EDTA and their stoichiometric molecular formulae were given as follow, $[Co(AA)_2]$, $[Co(AA)_2(MA)_2(H_2O)_2]$, $[Co(3ClAA)_2(MA)_2]$, $[Co(AA)_2(ETOHNH_2)_2(H_2O)]$, $[Co(3ClAA)_2(ETOHNH_2)]_2$, $[Co(AA)_2(EDA)_2(H_2O)]$, $[Co(3ClAA)_2(EDA)_2]$, $[Co(AA)_2(BA)_2]$, $[Co(3ClAA)_2(BA)_2]$, $[Co(AA)_2(1,6)]$, $[Co(AA)_2(1,8)(H_2O)_2]$, $[Co(AA)_2(MA)_2(bipy)_3)]$, $[Co(AA)_2(MA)_2(Phen)_3]$, $[Co(AA)_2(ETOHNH_2)_2(bipy)_4]$, $[Co(3ClAA)_2(ETOHN_2)_2(bipy)_2]$, $[Co(AA)_2(ETOHNH_2)_2(Phen)_3]$, $[Co(3ClAA)_2(ETOHNH_2)_2(Phen)_4]$, $[Co(AA)_2(EDA)_2(Phen)_3]$, $[Co(AA)_2(BA)_2(bipy)_2]$, $[Co(3ClAA)_2(bipy)_3]$, $[Co(3ClAA)_2(BA)_2(Phen)_2]$, $[Co(AA)_2(1,6)(bipy)]$, $[Co(AA)_2(1,6)_2(Phen)_2]$, $[Co(AA)_2(1,8)(bipy)_3]$, $[Co(AA)_2(1,8)(Phen)]$ and $[Co(AA)_2(EDA)_2(bipy)_6]$.
A solution 10mg/ml each of the compound in methanol was used for the screening.

Abbreviations used

AA- Acetylacetonate anion; 1,6 = 1,6-diaminoHexane; Phen = 1,10-Phenanthroline; MA= Methylamine; EDA = Ethylenediamine; Bipy = 2,2'-bipyridine; 3Cl AA- = 3-chloroacetylacetonate anion; $EtOHNH_2$ = Ethanolamine; EDTA = Ethylenediaminetetraacetic acid; BA = Butylamine; 1,8 = 1,8-diaminoOctane; Co = Cobalt

Antimicrobial Assay:

The organisms used were identified laboratory strains of *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Pseudomonas flourescens, Pseudomonas aeuriginosa, Escherichia coli, Aeromonas spp, Flavobacterium spp, Samonella spp* and *Streptococcus pyogenes* cultures supplied by Dr. O.E. Fagade of Botany and Microbiology. Department, University of Ibadan, Ibadan, Nigeria.

Antimicrobial susceptibility tests were performed using the agar diffusion technique. The surface of the Muller Hinton's agar in a petri dish was uniformly innoculated with 0.3ml of 18 hours old test bacteria cultures. 0.06ml of 10mg/ml concentration of each compound were added to 10mm well bore unto the agar. The plates were incubated at 37°C for 24 hours after which inhibitory zones were observed in millimetres (mm) as a measure of the antimicrobial activity.

The lowest concentration of each metal complex that inhibited growth of test bacteria was taken as the minimum inhibitory concentration (MIC).

Results and Discussion

All the cobalt (II) β-ketoamines derived from monoamines namely $[Co(AA)_2(MA)_2(H_2O)_2]$, $[Co(AA)_2(EDA)_2(H_2O)]$, $[Co(AA)_2(ETOHNH_2)_2(H_2O)_2]$ and $[Co(AA)_2(BA)]$ were effective against the thirteen organisms used with inhibitory zone diameters, 16-26mm, 16-24mm, 12-27mm and 12-30mm respectively. On the introduction of chloro substituent to these complexes, forming, $[Co(3ClAA)_2(MA)_2]$, $[Co(3ClAA)_2(EDA)_2]$, $[Co(3ClAA)_2(ETOHNH_2)_2]$ and $[Co(3ClAA)_2(BA)_2]$. The antimicrobial activities were reduced, with activity against eleven-twelve organisms with inhibitory zone diametres 9-30mm, 11-28mm, 12-25mm and 15-31mm (Table 1).

Table 1: Zones of inhibition (mm) of 10mg/ml of cobalt (II) b-ketoamines and adducts against some bacteria isolates.

	COMPOUNDS	BI1 2	BD2	BD4	BD53	BP34	BK4	12	Ose mb9	Aerq	Sal1	P31	P3y	Staph
1	Co(AA)2	25	16	25	20	22	20	20	18	21	14	-	17	26
2	Co(AA)2(MA)2(H2O)2	25	16	24	17	22	19	25	17	20	21	16	17	23
3	Co(3ClAA)2(MA)2	12	9	25	15	30	15	30	25	20	20	-	17	15
4	Co(AA)2(EDA)2(H2O)2	23	17	24	17	22	20	21	16	20	18	17	16	24
5	Co(3ClAA)2(DA)2	15	11	18	20	22	20	28	24	20	20	-	17	11
6	Co(AA)2(ETOHNH2)2(H2O)2	27	12	22	25	20	22	16	17	18	20	15	17	25
7	Co(3ClAA)2(ETOHN)2	12	12	24	13	18	15	25	23	12	20	-	17	-
8	Co(AA)2(BA)2	20	19	12	25	21	18	18	20	18	30	21	15	22
9	Co(3ClAA)2(BA)2	20	20	29	28	31	20	25	28	18	31	-	15	-
10	Co(AA)2(1,6)	23	24	20	20	16	24	19	20	21	16	-	-	27
11	Co(AA)2(1,8)(H2O)2	25	18	17	18	22	17	15	-	15	20	12	-	17
12	Co(AA)2(MA)2(bipy)3	17	-	20	-	11	18	17	-	13	-	-	10	25
13	Co(AA)2(MA)2(Phen)3	24	21	19	17	20	17	21	20	12	17	-	15	17
14	Co(AA)2(EDA)(bipy)6	23	-	20	17	11	19	21	17	20	13	-	10	23
15	Co(AA)2(EDA)2(Phen)3	21	17	17	17	15	16	20	18	15	17	-	17	17
16	Co(AA)2(ETOHNH2)2(bipy)	23	-	26	12	15	17	-	14	13	12	11	22	-
17	Co(3ClAA)2(ETOHNH)2(bipy)2	19	19	15	11	17	10	-	-	-	-	11	-	-
18	Co(AA)2(ETOHN2)(Phen)3	24	12	20	17	13	16	21	18	-	-	15	15	25
19	Co(3ClAA)2(ETOHN2)(Phen)4	18	12	14	12	17	14	-	13	-	16	-	-	-
20	Co(AA)2(BA)2(bipy)2	19	13	20	13	20	16	18	15	11	-	-	-	15
21	Co(3ClAA)2(BA2)(bipy)3	23	19	15	20	16	13	10	14	13	16	10	-	-
22	Co(3ClAA)2(BA)2(Phen2)3	19	13	14	-	17	-	-	-	-	-	-	-	-
23	Co(AA)2(1,6)(bipy)	-	21	-	-	19	-	-	13	-	-	-	-	12
24	Co(AA)(1,6)22(Phen)22	17	-	23	17	18	17	20	16	-	20	13	13	28
25	Co(AA)2(1,8)2)(bipy)2	30	-	20	30	12	16	12	26	20	25	12	-	25
26	Co(AA)2(1,8)(Phen)	29	21	19	17	17	18	17	20	15	23	15	-	25
27	Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-

Key:-- Not effective; BI1 = *Bacillus subtilis*; BP3 = *Streptococcus pyogenes*; BK4,Osemb9 = *Escherichia coli*; Staph = *Staphylococcus aureus*; P3 = *Pseudomonas aeuriginosa*; BD2 = *Bacillus cereus*; BD4,BD5 = *Flavobacterium* spp; Sal = *Salmonella* spp; P1= *Pseudomonas flourescens*; 12,AERQ = *Aeromonas* spp

Table 2 Minimum inhibitory concentrations (mg/ml) of the cobalt (II) β-ketoamines and their adducts against Streptococcus pyogenes

	Compounds	Concentration (mg/ml)
1	$Co(AA)_2$	2.68
2	$Co(AA)_2(MA)_2(H_2O)_2$	1.26
3	$Co(3ClAA)_2(MA)_2$	2.53
4	$Co(AA)_2(EDA)_2(H_2O)$	1.30
5	$Co(3ClAA)_2(EDA)_2$	2.80
6	$Co(AA)_2(ETOHNH)_2(H2O)_2$	1.28
7	$Co(3ClAA)_2(ETONH2)_2$	2.50
8	$Co(AA)_2(BA)_2$	1.26
9	$Co(3ClAA)_2(BA)_2$	1.50
10	$Co(AA)_2(1,6)$	3.95
11	$Co(AA)_2(1,8)(H_2O)_2$	1.31
12	$Co(AA)_2(MA)_2(bipy)_3$	1.26
13.	$Co(AA)_2(MA)_2(phen)_3$	1.25
14	$Co(AA)_2(EDA)_2(bipy)_6$	2.85
15	$Co(AA)_2(EDA)_2(Phen)_3$	1.25
16	$Co(AA)_2(ETOHNH_2)_2(bipy)_4$	1.29
17	$Co(3ClAA)_2(ETOHNH_2)_2(bipy)_2$	5.20
18	$Co(AA)_2(ETOHNH_2)_2(Phen)_3$	1.31
19	$Co(3ClAA)_2(ETOHNH_2)_2(Phen)_4$	1.68
20	$Co(AA)_2(BA)_2(bipy)_2$	2.50
21	$Co(3ClAA)_2(BA)_2(bipy)_3$	5.00
22	$Co(3ClAA)_2(BA)_2(Phen)_2$	1.29
23	$Co(AA)_2(1,6)(bipy)$	2.80
24	$Co(AA)_2(1,6)_2(Phen)_2$	2.50
25	$Co(AA)_2(1,8)(bipy)_2$	1.25
26	$Co(AA)_2(1,8)(Phen)$	1.65

KEY: BP_3 = *Streptococcus pyogenes*; AA- = Acetylacetonate anion; 3ClAAA- = 3-Cloroacetylacetonate anion; $EtOHNH_2$ = Ethanolamine; MA = Methylamine; EDA = Ethylene diamine; BA = Buthylamine; 1,6 = 1,6-diaminoHexane; 1,8 = 1,8-diaminoOctane; bipy = 2,2'-bipyridine; Phen = 1,10-Phenanthroline

The complexes $[Co(AA)_2(MA)_2(H_2O)_2]$ and $[Co(3ClAA)_2(MA)_2]$ had the same activity against *Aeromonas* spp and *Pseudomonas aeuriginosa* comparative activity against *Flavobacterium* spp and *Samonella* spp but different activity against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Aeromonas* spp and *Pseudomonas fluorescens* (Table 1). Similarly, the complexes $[Co(3ClAA)_2(AA)(EDA)_2]$ and $[Co(AA)_2(EDA)_2(H_2O)]$ had the same activity against *Escherichia coli*, *Aeromonas* spp and *Streptococcus pyogenes*, comparative activity against *Samonella* spp, *Pseudomonas aeruginosa*, but different activity against the remaining organisms. (Table 1). The complexes $[Co(AA)_2(ETOHNH_2)_2(H_2O)_2$ and $[Co(3ClAA)_2(ETOHNH_2)_2]$ also had same activity against *Bacillus cereus*, *Salmonella* spp and *Pseudomonas aeruginosa*, comparative activity against *Flavobacterium* spp and *Streptococcus pyogenes* but different activity against the rest of the organisms (Table 1). Furthermore the complexes $[Co(AA)_2(BA)_2]$ and $[Co(3ClAA)_2(BA)]$ both had the same activity against *Bacillus subtilis*, *Aeromonas* spp and *Pseudomonas aeuriginosa*, comparative activity against *Bacillus cereus*, *Salmonella* spp and *Escherichia coli* but different activity towards the remaining organisms *Flavobacterium* spp, *Aeromonas* spp, *Escherichia coli*, *Pseudomonas flourescens* and *Staphylococcus aureus* (Table 1). This was attributed to the +1 inductive effect of the Chlorine which reduces the net electron on the coordinated Cobalt (12). Thus, the greater the net electron, the greater the antimicrobial activity. The introduction of strong Lewis base 2,2'-bipy and 1,10-Phen to parents, Cobalt(II) β-ketoamine and Cobalt(II)-3-Chloro-β-ketoamine, resulted in adducts with lower antimicrobial activities. This was attributed to the +1 inductive effect of the bi-phenyl rings of the 2,2'-bipyridine molecules and the tri-phenyl rings of the 1,10-phenanthroline molecules respectively which outweighs the +M mesomeric effect of the lone pair of electrons on their coordinated Nitrogen atoms (-12). Thus, the net electron on their coordinated cobalt atom was lower than that of the parents.

In all cases, the phenanthroline adducts were more effective than the bipyridine adducts due to hyperconjugation (12) with the exception of $[Co(3ClAA)_2(BA)_2(Phen)_2$ whose very low activity was probably due to resistance from the organisms used, which pumps out the compound as soon as it entered their all membrane. (11).

Conclusion

This work showed that the introduction of 3-chloro-substituent on cobalt (II) β-ketoamine and adducts, reduced their antimicrobial activities drastically, due to the ability of this group to reduce the net electron on the coordinated cobalt atom. through +1 inductive effect. Thus, the higher the net electron on the coordinated cobalt in cobalt(II) β-ketoamines and its adducts, the higher the antimicrobial activity.

The minimum inhibitory concentrations of the cobalt (II)β-ketoamine and adducts were between 1.25-3.95mg/ml while that of cobalt (II) 3-chloro-β-ketoamines and adducts were between 1.29-5.20mg/ml (Table 2).

References

(1) Erciyas, E, Erkaleli H.I and Cosor G (1994 'Antimicrobial Evaluation of some styrylketone derivatives and related thiol adducts' J.Pharm. Sci 83(4), 573-8

(2) Saitkulova-, F.G. (1989) 'Antimicrobial activity aliphatic-aromatic ketones, β-glycols and α-ketols 'Khim Farm. Zh 23(2), 591-2

(3) Koelzer .P and Buechi .J. (1971) 'Relations between Physicochemical properties and antimicrobial activity of homologous aliphatic amino-II' Arzneim-Forsch, 21(11), 1721-7.

(4) Barkely G.W., Mast. G.F., Grail L.E., Tenenbarm F.E (1956) 'Relation of chemical structure to antimicribial activity in diamines and related compounds in vitroactivity-fungal and bacterial. Antibiotics and Chemotheraphy 6, 554-60.

(5) Kosh. J.N., Gyanfms, E.C. and Dioyer, F.P (1956) "Biological activity of complex ions - mechanism of inhibition of acetylcholinesterase.Aust.J. Biol. Sci, 9, 371-81

(6) Schoenenberger. H. Bastug. T and Adam D (1969) 'Cytostatics VIII Relations between chemical structure and antimicrobial activity in β-aminoketones. Arzneim- Forsch, (19(7) 1082-91

(7) Schroetter, E and Weuffen W (1976) 'Bacteriostatic efficiency of β-aminoketones Pharmazie, 31(5), 314-16

(8) Thomas Nogrady (1988) Medicinal Chemistry, A biochemical approach and Edition. New York Oxford University Press Pg8-50.

(9) Curtis N.F.(1986)'Marcrocyclic coordination compounds formed by condensation of metal-amine complexes with aliphatic carbonyl compounds' Coord. Chem. Rev. 3,3-47

(10) Patel K.S and Woods J.A.O. (1990) ;Preparation and Physico-chemical studies of some 3-substituted-2,4-pentanedionato copper (II) complexes and their adducts. Synth. React. Inorg. Met. Org. Chem. 20(1), 97-109

(11) Prescott L.M. Harley J.P and Klein D.A (1996) Microbiology, 3rd Edition WMC. Brown Publishers, Pgs 138-142

(12) Murray, P.R.S(1978) 'Principles of Organic chemistry, 2nd Edition Heinemann Educational Books London pgs 201-204.