In vitro antimicrobial studies of new Zn (II) complexes of N- hydroxymethylsaccharin (Sac-CH_2OH) and amine ligands

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Abstract

A new five Zn(II) complexes of the type [Zn(Sac-$CH_2O)_2$(N^N)] {where N^N = Bipy, Phen, en, dmen} were prepared from the reaction of diamine ligands N^N with [Zn(Sac-$CH_2O)_2$] in equivalent molar ratio. The prepared complexes have been characterized by IR, ^{1}HNMR, elemental analysis, and molar conductivity.

The antimicrobial studies of the Zn(II) complexes were also tested against *Escherichia coli, and Pseudomonas aeruginosa*, as Gram-negative; *Staphylococcus aureus*, *Salmonella typhi*, and *Bacillus subtilis*, as Gram-positive bacteria . and results suggested that Zn(II) complexes have significant antimicrobial activity.

Key words: Antimicrobial, Zinc, anime, N-hydroxymethylsaccharin, complex.

دراسة مختبرية للمعقدات الزنك الثنائي مع ليكاند N–هيدروكسي مثيل سكارين والامينات كمضادات بكتيرية

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المستخلص

حُضرت خمس معقدات جديد للزنك الثنائي ذات الصيغة [Zn(Sac–$CH_2O)_2$(N^N)$_2$] حيث ان (N^N هي : بايبريدين ، فينانثرولين ، اثيلين ثنائي امين و N,N–ثنائي ميثيل اثيلين امين من تفاعل ليكاندات الامينات الثنائية (N^N) مع نفس النسبة المولية من معقد [Zn(Sac–$CH_2O)_2$] وقد شخصت المعقدات المحضرة بالتوصيلية المولارية، التحليل الدقيق للعناصر، مطيافية الأشعة تحت الحمراء، ومطيافية الرنين النووي المغناطيسي للبروتون ^{1}H–nmr .

اجريت دراسة مختبرية للمعقدات كمضادات ميكروبية ضد عدد من البكتيريا منها *Escherichia coli* و *Pseudomonas aeruginosa* كبكتيريا سالبة كرام وضد كلا من *Salmonella typhi* و

Staphylococcus aureus , *Bacillus subtilis* و كبكتيريا موجبة كرام، وقد اظهرت المعقدات المحضرة فعالية ضد البكتيريا .

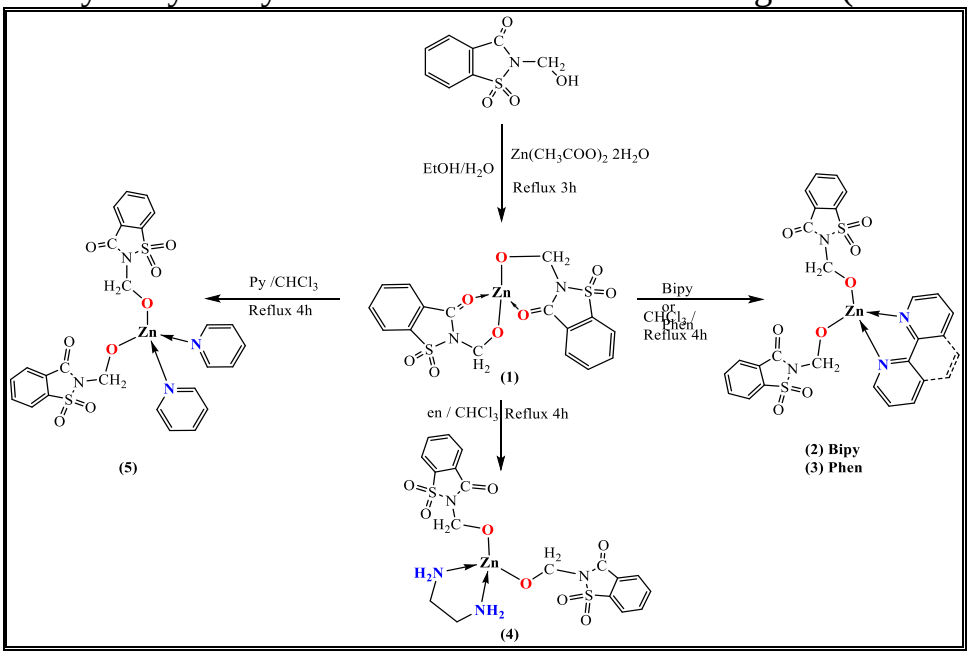
كلمات المفتاحية : الزنك، مضادات بكتيرية، امين، معقد، N–هيدروكسي مثيل سكارين

1. Introduction

Saccharin,1,2-benzisothiazole-3-one-1,1-dioxide, its widely used as an artificial sweetening agentsince 1885. And its derivatives such as, meloxicam, piroxicam, ampiroxicam, droxicam, cinoxicam, and sudoxicam, exhibit a range of biological activities, including anti-tumour, anti-inflammatory, anti-angiogenic, anti-glaucoma, cytotoxic and antimicrobial properties[2,8,9,12-14,18-22]

The importance of metal ions in biological systems is well known. One of the most interesting features of metal coordinated systems is the concerted spatial arrangement of the biological ligands around the ion. Among metal ions of biological importance, Zn(II), Fe(II) and Mn(II) ions are presents a high number of complexes with distortion [1].

In recent years, several saccharin derivatives and their complexes have been synthesized and their biological activities have been explored [3,4], but few experimental data about their antimicrobial and toxicological activities have been reported. Yet, nothing new on the complexation N-hydroxymethyl saccharin and amines with Zn(II) is reported. The present research, we study the antimicrobial of new zinc(II) complexes with N-hydroxymethylsaccharin and amines as a co-ligands(see Scheme 1).



Scheme 1: preparation of Zn(II) complexes (**1-5**)

2. Experimental

2.1. Methods and Materials:

All starting materials , reagent , and solvents used in this research were commercial products , and were used without further purification. The N-

hydroxymethylsaccharin was synthesized according to the literature procedure [19] . The ^{1}H-NMR spectra were recorded on Varian unity 400 spectrometer, with DMSO-d6 as solvent, and Me_4Si as internal reference. Infrared spectra of prepared compounds were recorded in the 4000 – 200 cm^{-1} range, on Bruker Tensor 28 spectrometer with a Pt- ATR unit. Melting points were measured on an electrothermal 9300 melting point apparatus. Elemental analysis was carried out on a CHN analyzer type 1106 Carlo-Erba. The NMR spectra and element analysis were determined in Institut fur Chemie, Martin-Luther-Universita¨t, Halle-Wittenberg, Halle, Germany. N-hydroxymethylsaccharin (Sac-CH_2OH) was fully characterized prior use .

2.2 Preparation of N-hydroxymethylsaccharin (Sac-CH_2OH)

A mixture of saccharin (5g, 27mmol) and formalin 37% (5ml, 67 mmol) in water (20ml) was heated under reflux for 1 hr., cooled at room temperature and filtered off the white crystal product and drying in oven. (Yield: 5.1g, 88%. m.p.: 134-136 ^{o}C (lit. 135-137)[19].

2.3 Preparation of [Zn(Sac-$CH_2O)_2$] (1)

An aqueous solution of Sac-CH_2OH ligand (0.500g, 2.346mmole) (15ml) with few drops of Et_3N as a base was added to a hot aqueous solution of $Zn(oAc)_2.2H_2O$ (0.257g, 1.173mmole) (20ml) was added to A white ppt. was formed. The mixture was refluxed for 3hr. The formed ppt. was filtered, washed with water and dried in oven. (61%. m.p.: 283-285 ^{o}C (decom.)

2.4 Preparation of [Zn(Sac-$CH_2O)_2$(Bipy)] (2)

A solution of Bipy (0.100g, 0.064mmole) in $CHCl_3$ (10ml) was added to a suspension of complex [Zn(Sac-$CH_2O)_2$] (0.314g, 0.064mmole) in $CHCl_3$ (10ml). A clear solution was formed. The solution was reflux for 4h at 50°C, the mixture was left to cooling at room temperature. The white ppt. then formed was filtered, washed with ethanol and dried in oven. (66%. m.p.:262-264 ^{o}C).

The following complexes [Zn(Sac-$CH_2O)_2$(Phen)] (**3)**, and [Zn(Sac-$CH_2O)_2$(en)] (**4)**, were prepared and isolated in a similar method.

2.5 Preparation of [Zn(Sac-$CH_2O)_2(Py)_2$](5)

A solution of Pyridine ligand (0.100g, 0.063 mmol) in $CHCl_3$ (10 ml) was added to a suspension of **(1)** (0.313g, 0.063 mmol) in $CHCl_3$ (15 ml) with stirring. The clear solution formed was refluxed for 4 h and then left to cool at room temperature. The white solid formed was filtered, washed with diethyl ether and dried under vacuum (Yield: 82%. m.p.: 211-212^{o}C) .

2.6 Antibacterial activity

The N-hydroxymethylsaccharin ligand and its Zn(II) complexes were tested in vitro antibacterial activity against *Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa,* as Gram-negative; *Staphylococcus aureus*, and *Bacillus subtilis*, as Gram-positive bacteria. Antibacterial activity as the zones of inhibition and inhibition percentage for all compounds are listed in Tables **4** and **5**.

3. Results and Discussion

3.1 general

All prepared complexes are stable in room temperature, are insoluble in common solvents such as MeOH, EtOH, Acetone, $CHCl_3$, but soluble in DMSO and DMF. All data of the prepared complexes presented in Tables 1-3 are in good agreement with suggested structures of complexes. The low values of molar conductance of the Zn(II) complexes in DMF indicate their non-electrolytic nature[7].

Table 1:Color, yield %, m.p. and elemental analysis for the prepared complexes

Seq.	Complexes	Color	m.p (^oC)	Yield %	Elemental analysis Calc. (Found) %		
					C	H	N
	Sac-CH_2OH	White	134-136[a]	88	45.07 (54.11)	3.31 (3.40)	6.57 (6.62)
1	[Zn(Sac-$CH_2O)_2$]	White	283-285[b]	61	39.24 (39.09)	2.47 (2.61)	5.72 (5.88)
2	[Zn(Sac-$CH_2O)_2$(Bipy)]	White	262-264	66	48.34 (48.62)	3.12 (3.32)	8.67 (6.95)
3	[Zn(Sac-$CH_2O)_2$(Phen)]	White	188-191	56	50.20 (50.34)	3.01 (2.91)	8.36 (8.43)
4	[Zn(Sac-$CH_2O)_2$(en)]	White	233-235[b]	73	39.32 (39.57)	3.67 (3.79)	10.19 (10.33)
5	[Zn(Sac-$CH_2O)_2(Py)_2$]	White	211-212	71	48.19 (48.01)	3.42 (3.72)	8.65 (8.93)

a: (lit. 135-137) ; b: decompose temperature

3.2 Infrared spectra

The infrared spectral bands of the prepared ligand and its Zn(II) complexes are given in Table 2. The internally hydrogen bonded –OH band disappear in the spectra of the metal complexes, indicating the deprotonation and formation of Zn-O bond. This is further supported by the shifting of carbonyl group ν(C=O) towards higher frequency, indicating the Sac-CH_2OH bonded through the oxygen atoms of the carbonyl and hydroxyl groups [11,15-17]. The ν(C=N) vibration of the amines ligands which are shifted to a lower frequency in the complexes, indicating the coordination are happen through the nitrogen atoms of the amine ligands[18]. A new bands at 498-543 and 456-487 were assigned to (M-O) and M-N bond stretching band frequencies, respectively and served as further evidence of coordination via the nitrogen and oxygen atoms of the Sac-CH_2O and amine ligands [6,10].

Table 2: Selected IR stretching vibration bands (cm^{-1}) of prepared complexes

Seq.	Complexes	CH arom.	C-H aliph.	C=O	C=N	SO_2 asy/sy	Zn-O	Zn-N
	Sac-CH_2OH	3093	2939	1747		1340 1184		
1	[Zn(Sac-$CH_2O)_2$]	3058	2879	1686		1288 1159	523	
2	[Zn(Sac-$CH_2O)_2$(Bipy)]	3057	2897	1728	1554	1294 1160	512	456
3	[Zn(Sac-$CH_2O)_2$(Phen)]	3088	2934	1721	1562	1294 1162	498	487
4	[Zn(Sac-$CH_2O)_2$(en)]	3094	2920	1716	1523	1296 1158	512	466
5	[Zn(Sac-$CH_2O)_2(Py)_2$]	3050	2988	1712	1587	1292 1150	543	480

arom. = aromatic; aliph = aliphatic

3.3 Proton NMR spectra

The ^{1}H NMR spectra of ligand and its Zn(II) complexes recorded in DMSO-d6 are shown in Table 3. Figs. 4 and 5. The signal at 5.18 and 6.74 ppm in the ^{1}H NMR spectrum of the ligand refer to OCH_2 and OH proton, respectively. The O**H** proton disappeared in the spectra of the prepared complexes. This is a clear indication that the hydroxyl group is coordinated to the metal ion through the hydroxyl oxygen after deprotonation. All signals of the other protons are listed in the Table 3.

Table 3: ^{1}H NMR chemical shifts for some the prepared complexes in (DMSO-d^6) solvent

Compound	^{1}H -NMR Chemical shift δ (ppm)
Sac-CH_2OH	δ5.18 (s, 4H, OCH_2) ; 6.74 (bs, 1H, OH); 7.99 (td, 1H) ; 8.05 (td, 1H) ; 8.12 (dd, 1H); 8.28 (dd, 1H)
[Zn(Sac-$CH_2O)_2$]	δ5.28 (s, 4H, OCH_2) ; 6.97 (d, 1H); 7.54 (dd, 1H); 7.87 (t, 1H) ; 7.98 (d, 1H)
[Zn(Sac-$CH_2O)_2$(Bipy)]	δ 5.28 (s, 4H, OCH_2); 7.20 (d, 2H, H-bipy); 7.43-7.57 (m , 8H , H-bipy and Sac-CH_2O) ; 7.72 (d, 2H, H-bipy) ; 8.19 (d , 2H , H-bipy) ; 8.48 (d , 2H , H-bipy)
[Zn(Sac-$CH_2O)_2(Py)_2$]	δ 5.18 (s , 4H, OCH_2) ; 7.23-8.68 (m , 16H , H-py and Sac-CH_2O)

s: singlet; d: doublet; dd: doublet of doublets; td: triplet of doublets; m: multiplet; bs: broad singlet.

The spectral data were in good agreement with the suggested structures of prepared complexes, and the saccharin derivative ligand in complex **1** was coordinated through the oxygen atoms of carbonyl and hyrdoxymethyl groups as bidentate ligand to give tetrahedral geometry around the Zn(II) ion, whereas in complexes **2-5** the Sac-CH_2OH bonded via the oxygen atom of hyrdoxymethyl group as monodentate ligand

and the amine ligand bonded through the nitrogen atoms to give tetrahedral geometry around the Zn(II) ion. '

3.4 Antibacterial activity

From the results obtained, the antibacterial activity of Zn(II) complexes is found to be higher than that of a free N-hydroxymethyl-saccharin ligand, against the same microorganism under identical experimental conditions. This is similar to earlier observations [4,5,21]

Resulting data of these investigations revealed that the inhibitory effect is susceptible to the concentration of the compound used for inhibition so that the activity is greatly enhanced at the higher concentration of compound. In case of antibacterial activity, the [Zn(Sac-$CH_2O)_2$(Bipy)] complex was has highest antibacterial activity against bacteria species when compared with other Zn(II) complexes. And the [Zn(Sac-$CH_2O)_2$(Bipy)] complex was found highly effective against B. subtilis, and P. aeruginosa, in the following order B. subtilis > P. aeruginosa > E. coil > S. aureus, and highest percentage inhibition in same complex (see Fig. 1), whereas the [Zn(Sac-$CH_2O)_2(Py)_2$] complex was has lowest antibacterial activity.

"This increased activity of the Zn(II) complexes upon chelation is attributed to chelation theory, according to which, the chelation reduces the polarity of the metal atom mainly because of the positive charge of the metal partially shared with donor atoms present on the ligand and there is electron delocalisation over the whole chelate ring. This, in turn, increases the lipophilic character of the metal chelate and favors its permeation through the lipid layers of the bacterial membranes[9,21]. Generally, it is suggested that the chelated complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms. Other factors such as solubility, conductivity and dipole moment, affected by the presence of metal ions, may also increase the biological activity of the metal complexes compared to the ligand". [21].

'The compounds exhibited a broad spectrum of activity against the tested microbes. Some of the compounds however exhibited increased antimicrobial activity with increased number of chelate rings. Hence, chelation may serve as a useful tool in the design of potential antimicrobial agents".

Table 4: Antimicrobial activity of the prepared complexes

Compound	Conc.	Diameter of inhibition zone of growth (mm)				
		S.aureus	*E. coli*	*P.aeruginosa*	*B. subtilis*	*S. typhi*
Sac-CH_2OH	50	5	5	4	2	3
	100	7	6	7	4	5
[Zn(Sac-$CH_2O)_2$]	50	11	12	13	7	12
	100	13	9	21	9	14
[Zn(Sac-$CH_2O)_2$(Bipy)]	50	9	17	18	22	15
	100	14	21	22	26	19
[Zn(Sac-$CH_2O)_2$(Phen)]	50	8	16	15	8	9
	100	11	10	17	9	13
[Zn(Sac-$CH_2O)_2$(en)]	50	10	12	16	8	9
	100	14	9	19	14	10
[Zn(Sac-$CH_2O)_2$(Py)]	50	7	9	13	10	8
	100	9	11	16	12	12
Ciprofloxacin [a]	100	32	35	32	30	34

Antimicrobial activity: >15 significant; 10-14mm, moderate activity; <10 weak activity ; [a] standard

Table 5: Antimicrobial percentage of the N-hydroxymethylsaccharin ligand and their Zn(II) complexes for maximum concentration (100 µg mL^{-1})

Compound	% inhibition				
	S. typhi	*B. subtilis*	*P.aeruginosa*	*E. coli*	*S.aureus*
Sac-CH_2OH	19	15	22	25	28
[Zn(Sac-$CH_2O)_2$]	33	61	44	45	31
[Zn(Sac-$CH_2O)_2$(Bipy)]	37	59	49	51	35
[Zn(Sac-$CH_2O)_2$(Phen)]	43	56	39	48	33
[Zn(Sac-$CH_2O)_2$(en)]	45	51	51	39	35
[Zn(Sac-$CH_2O)_2(Py)_2$]	51	54	29	44	46
Ciprofloxacin	100	100	100	100	100

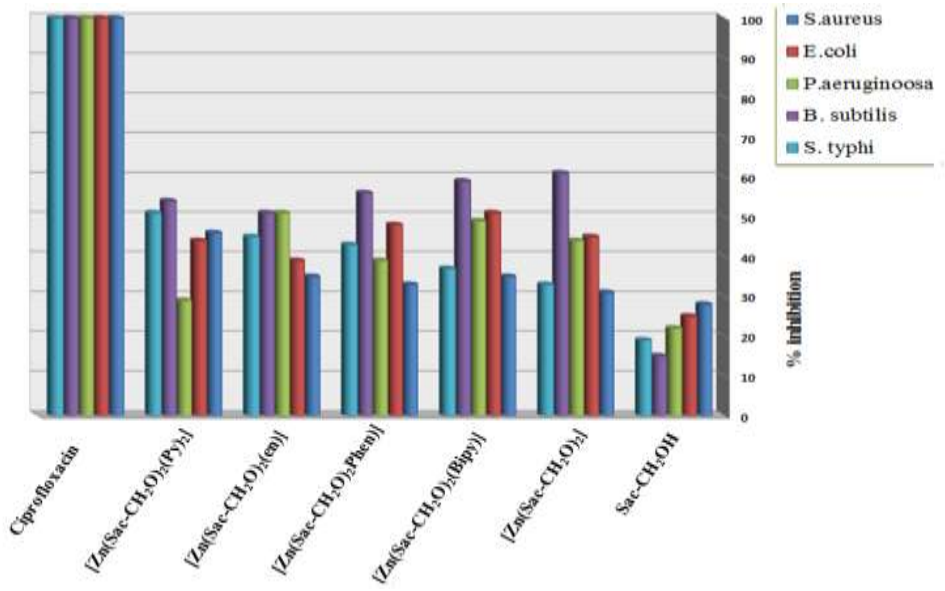


Figure 1 : Percentage inhibition of Sac-CH_2OH ligand and their Zn(II) complexes against bacteria species

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References

1- Agrawal, O.P., 1985.Synthetic Organic Chemistry; Goel Publishing House: Merrut, India.

2- Ahmad, M. Zia-ur-Rehman, Siddiqui, H. L., Fasih-Ullah, and Parvez , M. M., 2011 Microwave assisted synthesis and structure–activity relationship of 4-hydroxy-*N*′-[1-phenylethylidene]-2*H*/2-methyl-1,2-benzothiazine-3-carbohydrazide 1,1-dioxides as anti-microbial agents. European J. Med. Chem., 46, 2368-2377

3- Al-Janabi, A. S. M., Irzoqi,A. A., and Ahmed,S. A. O., 2016. Synthesis and Characterization of Mixed Ligands Cadmium (II) Complexes with N-Hydroxymethylsaccharinate and diphosphines. Tikrit J. of Pure Science, 21 (3):54 -60

4- Al-Jibori, S.A., Al-Janabi, A. S., Basak-Modi, S., Mohamed, S. S., and Schmidt. H., 2015. Mixed ligand palladium(II) complexes of N-hydroxymethylsaccharin (Sac-CH2OH): synthesis, characterization and biological studies. Transition Met. Chem., 40(8):917-921.

5- Badwaik, V.B. and Aswar, A.S. 2007. Synthesis, characterization, and biological studies of some Schiff base complexes. Russ. J. Coord. Chem., 33:755-760.

6- Ferraro, J.R., 1971. Low Frequency Vibrations of Inorganic and Coordination Compounds". Plenum Press, New York.

7- Geary, W.J., 1971. The use of conductivity measurements in organic solvents for the characterization of coordination compounds. Coord. Chem. Rev., (7) :81-122.

8- Hanson, P.R., Probst, D.A., Robinson, R.E. and Yau, M. 1999. Cyclic sulfonamides via the ring-closing metathesis reaction. Tetrahedron Lett., 40,4761 -4764.

9- Jadhav, S.M., Shelke,V.A., Shankarwar, S.G., Munde A.S. and Chandrashekar, T.K. 2014. Synthesis, spectral, thermal, potentiometric and antimicrobial studies of transition metal complexes of tridentate ligand. J. Saudi Chem. Soc., 18:27-34.

10- Jorgensen, C. 2004. Optical Spectra and Chemical Bonding in Inorganic Compounds". Springer, Germany.

11- Keskioglu, E., Gunduzalp, A.B., Cete, S., Hamurcu, F. and Erk, B., 2008. Cr(III), Fe(III) and Co(III) complexes of tetradentate (ONNO) Schiff base ligands: Synthesis, characterization, properties and biological activity. Spectrochim. Acta A., 70(3):634-640.

12- Marllon N., Oliveira, M. and Mattos C. S., 2013. Ritter reaction of N-(hydroxymethyl)saccharin with nitriles: synthesis of new N-(amidomethyl)saccharins. 15th Brazilian Meeting on Organic Synthesis – 15th BMOS – November 10-13, 2013 - Campos do Jordão, Brazil.

13- Melissa, D. A., Paolo, G., Simone C., Daniela S., Rosalba F., and Adriano M., 2017. Open saccharin-based secondary sulfonamides as potent and selective inhibitors of cancer-related carbonic anhydrase IX and XII isoforms. J. of Enz. Inh. & Med. Chem., 32(1): 51-59

14- Moree, W. J. Van der Marel, G. A., and Liskamp, R.M., 1991. Peptides containing a sulfinamide or a sulfonamide moiety: New transition-state analogues, Tetrahedron Lett., 32:409-412.

15- Murukan, B. and Mohanan, K. J., 2008. Synthesis, characterization and antibacterial properties of some trivalent metal complexes with [(2-hydroxy-1-naphthaldehyde)-3-isatin]-bishydrazone. Enzyme Inhib. Med. Chem., 22:65-68.

16- Murukan,B., Kumari, B. S. and Mohanan, K. J., 2007, Synthesis, spectral, electrochemical and antibacterial studies of copper(II) complexes with isatin derived bishydrazone and different co-ligands, Coord. Chem., 60:1607-1610.

17- Nakamoto, K., 1986, Infrared and raman spectra of inorganic and coordination compounds". Wiley Inter-science, New York.

18- Raju, K.C. and Radhakrishnan, P. K. 2003. Complexes of copper(II) with 2,3-dimethyl-4-formyl(benzhydrazide)-1-phenyl-3-pyrazolin-5-one. Synth. React. Inorg. Met. Org. Chem., 33:1307-1318.

19- Rough, W. R., Gwaltney, S. L., Cheng, J., Scheidt, K.A., McKerrow, J.H., and Hansell,E. 1988. Vinyl Sulfonate Esters and Vinyl Sulfonamides: Potent, Irreversible Inhibitors of Cysteine Proteases. J. Am. Chem. Soc., 120:10994-10995(Communication).

20- Shin,S., Cho, E. and Won, C., 2000. Enhancing Effects of Fatty Acids on Piroxicam Permeation Through Rat Skins, Drug Dev. Ind. Pharm., 26(5):563-566

21- Thangadurai, D.T. and Natarajan, K. 2001. Synthesis and characterization of new Rh(III) complexes containing tetradentate Schiff bases. Synth. React. Inorg. Met. Org. Chem., 31:549-553.

22- Yeung, K.S., Meanwell, N.A., Li, Q., and Gao, Y., 1998. A facile construction of 4-hydroxymethylbenzisothiazolone-1,1-dioxide, Tetrahedron Lett. 39:1483-1486.