

## Design, synthesis and antibacterial activities of novel complexes of 3-Amino-2-Methyl-7-Chloro-Quinazolin 4 (3H)-One

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### Abstract

A new Ligand of 3-Amino-2-Methyl-7-Chloro-Quinazolin 4(3H)-one (L) has been synthesized in good yield by the reaction of methyl-2-amino-4-chlorobenzoate with acetic anhydride then the oxygen was replaced with nitrogen of hydrazine. When the Ligand reacts with Co (II), Zn (II) and Cu (II), new complexes are formed. The chemical structures of all prepared compounds were characterized by elemental analysis, IR, UV/Visible, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and GCMS. Moreover, molar ratio M:L was also determined. The free Ligand and their metal complexes were tested in vitro against a number of microorganisms' gram positive bacteria (*Staphylococcus aureus*, *Bacillus species* and *Enterococcus faecalis*), gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*) and fungi (*Candida albicans*) in order to assess their antimicrobial properties. All the complexes show considerable activity against all microorganisms.

**Keywords:** Anthranilic acid, 3-amino-2-methyl-7-chloro-quinazolin-4(3H)-one, Bioassay, Bacillus species, Metal complexes, Methyl-2-amino-4-chlorobenzoate, Nucleophile; Synthesis, Antimicrobial activity.

### Introduction

Quinazolinone is an important heterocyclic ring with broad spectrum of biological activities like anticonvulsant,<sup>1</sup> analgesic,<sup>2</sup> antitumor,<sup>3</sup> anti-inflammatory,<sup>4</sup> antimicrobial,<sup>5</sup> antitubercular,<sup>6</sup> antioxidant<sup>7</sup> and antiviral<sup>8</sup> activities.

Interest in coordination chemistry is increasing continuously with the preparation of organic ligands containing a variety of donor groups.<sup>9-11</sup> When the ligands have biological importance they are multiplied in many fold.<sup>12-13</sup> Quinazoline compounds are widely used in agrochemicals as plant virucides<sup>14</sup>, antifungal agents<sup>15</sup> and herbicides.<sup>16</sup> According to recent data, quinazoline nucleus has attracted the attention of medicinal chemists due to its well known anticancer activity and many substituted quinazoline derivatives have recently earned great interest in chemotherapy as antitumor drugs.<sup>17,18</sup>

Schiff bases with donors (N, O, S, etc.) have structural similarities with natural biological systems and imports in elucidating the mechanism of transamination and reamination reaction in biological system due to presence of imine (-N=CH-) group.<sup>19</sup>

Taking into consideration the use of metal complexes in the treatment of some diseases mentioned above, we have tested the antimicrobial activity of a new ligand and its metal complexes using strains of *Staphylococcus aureus*, *Bacillus species*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella Pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans* isolated from different pathological products of patients. Based on the above observations and in continuation of the drug research program, it was of interest to synthesize a novel metal complexes of Copper Cu(II), Zinc Zn(II) and Cobalt Co (II) of the title Ligand as a trial to obtain safer and potent antibacterial agents.

### Experimental

All reagents and solvents were products of sigma-Aldrich, Germany. Melting points were determined on a kofler hot stage apparatus and were uncorrected. IR spectra were recorded on a Buck scientific IR M500 instrument. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded in DMSO-*d*<sub>6</sub> at 400 MHz with HAZ VOLATILE V2. M spectrophotometer. Chemical shifts were reported in ppm relative to tetramethylsilane. Gas chromatography-Masss (GC/MS) spectra were obtained on a Finigan MAT 44S mass spectrometer operating at electron impact energy of 70eV. Elemental analysis data agreed favourably with the calculated values Analytical thin layer chromatography (TLC) was used to monitor the reactions.

### Synthesis of 2-methyl-7-chloro-benzo [1,3] -oxazin-4-one(1) and Synthesis of 3-amino-2-methyl-7-quinazolin4 (3H) one(2).

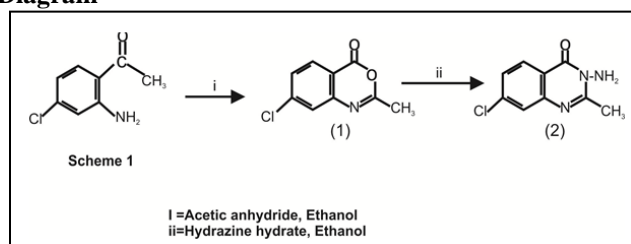
A mixture of 1.97g (0.005mol) Methyl-2-amino-4-chlorobenzoate or 4-chloroanthranilate with 10mL, 1.02g (0.01mol) acetic anhydride in 30ml ethanol medium was heated under reflux with stirring using magnetic stirrer until the reaction mixture showed no trace of starting material by TLC data (about 2 h). Ethanol was removed in vacuum and the solid product was recrystallized from proper solvent, Yielding 0.81g (92%) mp: 87 -89°C.

A mixture of equimolar amounts (1.59, 0.005mol) of 2-methyl-2-chloro-4H-benzo[1,3]-oxazin-4-one and hydrazine hydrate (0.93g, 0.001mol) in 30ml boiling ethanol was heated under reflux with stirring using magnetic stirrer until the reaction mixture showed no trace of starting material by TLC data (about 3 h). The white precipitate formed was then filtered, washed three times with 20ml of distilled water [20ml x 3]. The white crystals were dried and recrystallized from dimethylformamide (DMF) to give pure

3-amino-2-methylquinazolin-4 (3H) -one Yielding 1.48g (92%) mp: 114 - 116°C.

**Design, synthesis and bioassay of novel metal complexes**  
0.01 mol) in 30 mL boiling ethanol was stirred using a magnetic stirrer until the reaction mixture showed no trace of starting material when TLC was developed (about 3 h). The reaction mixture was concentrated in vacuum under reduced pressure using rotary evaporator. The solid product (white crystals) was dried and recrystallized from proper solvent, Yielding 1.48g (92%); mp, 114 – 116°C.

#### Diagram



#### Synthesis of complexes

Reactions of the ligand with metal ions in 1: 2 molar ratio in ethanol medium yield the complexes. Ligand is bidentate in character and coordinate through oxygen and nitrogen donor atoms. All the complexes are fairly stable and can be stored for long periods at room temperature.

#### Metal complexes synthesis

A hot ethanolic solution (20ml) of corresponding metal salt (0.005mol) was mixed with hot ethanolic solution of the Ligand (0.01mole). The mixture was refluxed for 5 hours on a water bath on cooling the contents, a coloured complex was separated out in each case. The product was filtered, washed with 50% ethanol and dried in vacuum over  $\ell_4O_{10}$ . Purity of the obtained complexes was checked by TLC.<sup>20</sup>

#### Complexes formation in solution study

Complexes of Ligand with metal ions were studied in solution using DMF as solvent in order to determine (M: L) ratio in the complex following the molar ratio method<sup>21</sup>. A series of solutions were prepared having a constant concentration ( $10^3M$ ) of metal ion and Ligand (L). The (M/L) ratio was determined from the relationship between the absorbance and the mole ratio of (M/L). The results are listed in Table 1.

#### Antibacterial activity testing

Agar wall diffusion method was utilized for the antibacterial activities<sup>22</sup>. Seven species: *Staphylococcus aureus* (ATCC 10145), *Bacillus species* (NCTC 8236), *Enterococcus faecalis*, (NCTC 6571), *Esherishia coli* (ATCC 25922), *Klebsiella pneumonia* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 10145) and *Candida albicans* (ATCC 24433) Stock cultures were used. The test organisms were supplied by the pharmaceutical

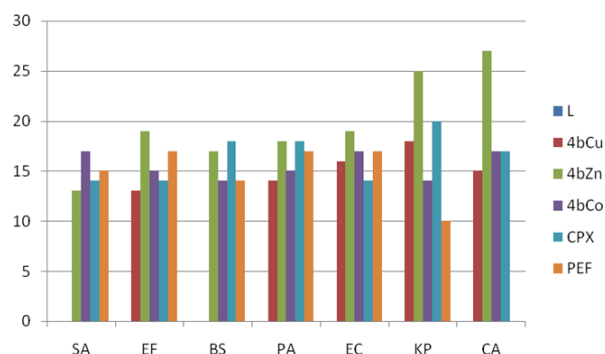
Microbiology Department of the University of Benin. The test organisms were cultured overnight in nutrient broth, diluted to the turbidity of McFarland standard. Broth culture (0.5mL) was seeded on nutrient agar and is allowed to dry. Then various concentrations of the compound (20 – 640mg/mL) were introduced. The culture plates were then incubated at 37°C for 24hours. The results were assessed by measuring the zone of growth inhibition by the test compound<sup>23</sup>. Activity and inactivity were observed in accordance with the standard and accepted method. Fig. 2

#### Results and Discussion

The reaction of the 4-chloroantranilate or methyl-2-amino-4-chlorobenzoate with acetic anhydride yield the cyclic compound 2-methyl-7-chloro-4H-benzo[1,3]-oxzin-4-one (1) as shown in the mechanism. The reaction of this compound with hydrazine yields the new Ligand 3-amino-2-methyl-7-chloro-quinazolin 4(3H)-one (2) as shown in the mechanism.

**Antimicrobial activity of ligand and tested complexes against tested standard organization control drugs**  
Ciprofloxacin (CPX) For Bacteria  
Ketonaxol (PEF) For Fungus

Ligand (L), Complex 1 (4bCu), Complex 2 (4bZn), Complex 3 (4bCo)



**Figure 1:** The effect of Ligand toward studied bacteria. SA = *Staphylococcus aureus*, BS = *Bacillus species*, EF = *Enterococcus faecalis*, EC = *Escherichia coli*, KP = *Klebsiella pneumonia*, PA = *pseudomonas aeruginosa* and CA = *Candida albicans* (4c = L)

Significantly different from Ligand at  $P < 0.05$ , values are in mm

**Table 1:** Physical characteristics of complexes

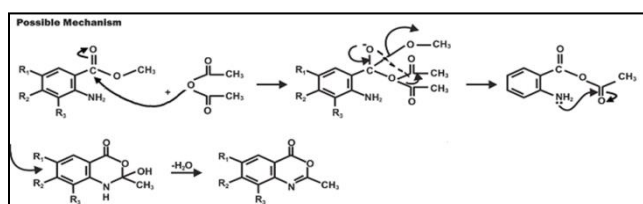
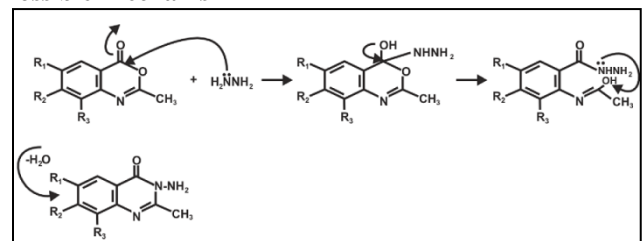
No	Complexes	Colour	M.P °C	Yields (%)	M: L
C <sub>1</sub>	CoL <sub>2</sub> U <sub>2</sub>	Green	248-250	82	1: 2
C <sub>2</sub>	CuL <sub>2</sub> U <sub>2</sub>	Brown	250-252	88	1: 2
C <sub>3</sub>	ZnL <sub>2</sub> U <sub>2</sub>	White	244-246	84	1: 2

**Table 2:** Elemental chemical analysis data of metal complexes

Elemental Analysis							
No	Complexes	Theoretical Calculated Values			Actual Calculated Values		
		C%	H%	M%	C%	H%	M%
C <sub>1</sub>	CoL <sub>2</sub> U <sub>2</sub>	46.63	3.83	12.21	46.53	3.81	12.27
C <sub>2</sub>	CuL <sub>2</sub> U <sub>2</sub>	45.63	4.83	12.21	45.33	4.81	12.30
C <sub>3</sub>	ZnL <sub>2</sub> U <sub>2</sub>	47.92	3.45	13.14	47.82	3.40	13.00

**Table 3:** Infrared absorption frequencies (cm<sup>-1</sup>) of ligand and its complexes

No	Complexes	V(NH)	V(C=N)	V(C=O)	M-O	M-N
L		3340	1569	1690	-	-
C <sub>1</sub>	CoL <sub>2</sub> U <sub>2</sub>	3270	1501	1630	448	442
C <sub>2</sub>	CuL <sub>2</sub> U <sub>2</sub>	3210	1503	1635	450	440
C <sub>3</sub>	ZnL <sub>2</sub> U <sub>2</sub>	3215	1499	1640	452	450

**Possible Mechanism**

Where, R<sub>1</sub> = H, R<sub>2</sub> = Cl, R<sub>3</sub> = H

**Elemental analysis**

The C, H and M contents (both theoretically calculated values and actual values) are in accordance with the formula ML<sub>2</sub>Cl<sub>2</sub> indicating that the ligand is neutral. This can be explained by the absence of any deprotonating agent during the synthesis. The complexes are generally soluble in common organic solvents Table 2.

**Infrared spectra**

The study and comparison of the IR spectra of the ligand and its complexes imply that the ligand is bidentate, with the carbon oxygen and nitrogen coordination sites. The presence of ring vibrations and C-H absorption makes the spectra fairly complicated for complete assignment of individual bonds in Table 3. In the IR spectra of the complexes, a considerable negative shift in V(C=O) is observed, indicating a decrease in the stretching force constant of the C=O bond as a consequence of co-ordination through the carbonyl-oxygen atom of the free base. Another important band, which occurs at 1503cm<sup>-1</sup> is attributed to V(C=N) azomethine mode<sup>24-25</sup> and remain unaffected after complexation. The bond due to NH<sup>+</sup> stretching in free ligand occur in the 3340cm<sup>-1</sup> region, in the spectra of all the

complexes. This band is shifted to lower frequency and appears in the 3200 – 3270cm<sup>-1</sup> region indicating the involvement of the N-atom in coordination.

**UV-vis Spectra**

Ligand synthesized showed three absorption bands in DMF. The first band observed at 235nm represents the ( $\bar{\Lambda}-\bar{\Lambda}^*$ ) transition while the second and the third bands (which have higher intensity than the first band due to conjugated system) appear at 360 and 370nm and represent the ( $n-\bar{\Lambda}$ ) transition. Generally, the bands of the newly synthesized complexes are shifted to shorter or longer wavelengths than that of ligands, but the high intensity of these bands is indicative of complex formation. The origin of the band observed at about 700nm in the electron spectra of complexes has been identified as d-d transition. In these spectra the bands observed at 300 – 400nm could be assigned to nitrogen-metal charge transfer absorption. The electronic absorption bands for the ligand and complexes are classified into two distinct groups, those belonging to liquid transitions appeared in the uv region while d-d transitions appeared in the visible region. These transitions are assigned in relevance to the structures of complexes<sup>20</sup>.

**Antibacterial analysis evaluation**

The antibacterial analysis results show that the synthesized compounds exhibit antibacterial activities, and it is important to note that the metal chelates exhibit more pronounced inhibitory effects than the parent ligands. The increased activity of the metal chelates can be explained on the basis of chelation theory. It is known that chelation makes the complexes more powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand. It is established that, in a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligands, and there may be  $\bar{\Lambda}$ -electron delocalization over the whole chelate<sup>26</sup>. This increases the lipophilic character of the metal chelate and favours its permeation through the lipid layer of the bacterial membranes. The

increased lipophilic character of these complexes seems to be responsible for their enhanced antibacterial activity. It may be suggested that these complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms. It has also been proposed that the ultimate action of the toxicant is the denaturation of one or more proteins of the cell, which as a result impairs normal cellular processes. There are other factors which also increase the activity, including solubility, conductivity and bond length between the metal and the ligand.

Activity of the ligand and metal complexes was tested against some human pathogenic microbes including Gram positive (*Staphylococcus aureus*, *Bacillus species* and *Enterococcus aureus*), Gram negative (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas ariginosa*) and fungi (*Candida albicans*) by the Agar well diffusion method in Figure 2. From the result obtained from the method, it was found highly active even at low concentrations.

### Statistical analysis

All data were expressed as the mean  $\pm$  SEM; the student's t-test was applied to determine the significance of the difference between the ligand and the test compounds.

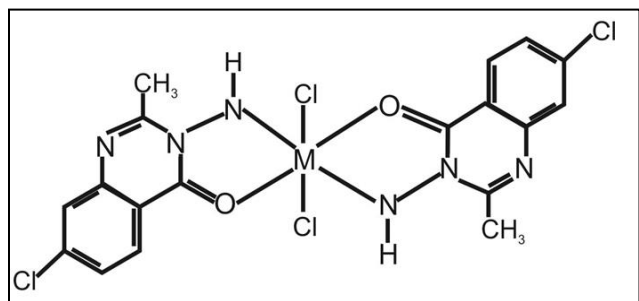


Fig. 2: Proposed structure of the complexes

### Conclusion

The Ligand has a lower activity than all the synthesized complexes against the test microorganisms. From the results, we may conclude that the ligand act as bidentate uni-negative ligand, coordination through one of nitrogen and oxygen. From this study, all the complexes are found to be mononuclear, based on the IR spectroscopy data. Based on the physicochemical and the spectra studies, the tentative structures proposed for the complexes is shown in Figure 2.

### Abbreviations

TLC-Thin Layer Chromatography, SEM-Standard Error Mean, IR-Infrared Spectra, UV/Visible-UV-Visible Spectra, <sup>1</sup>H NMR-Proton Nuclear Magnetic Resonance, <sup>13</sup>C NMR-Carbon thirteen Nuclear Magnetic Resonance, GCMS Gas Chromatography Mass Spectroscopy, L-Ligand, 4bCu-Copper complex 1, 4bZn-Zinc complex 2, 4bCu-Colbert complex 3, CPX-Ciprofloxacin, PEF-Ketonaxol.

### Conflict of interest

The author declares no conflict of interest.

### Funding

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### Author declaration

The author hereby declares that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by me.

### Ethics approval and consent to participate

Ethic approval, consent to participate and the procedure used were approved by the Ethic approval committee of Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria.

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### Declaration statement

The author declares there is no conflict of interest.

### References

- Govindaraj Saravanan, Veerachamy Alagarsamy, Chinnasamy Rajaram Prakash, Design, synthesis and anticonvulsant activities of novel 1-(substituted / unsubstituted benzylidene)-4-(4-(6, 8-dibromo-2-(methyl / phenyl)-4-oxoquinazolin-3(4H)-yl)phenyl)semicarbazide derivatives, *Bioorganic & Medicinal Chemistry Letters* 2012;22:3072-8.
- Alagarsamy V, Rajasolomon V, Meena R and Ramseshu KV. Synthesis, analgesic, anti-inflammatory and anti-bacterial activities of some novel 2-butyl 3-substituted quinazolin-4(3H)-ones. *Biol Pharm Bull* 2005;28(6):1091-4.
- Cao S L, Feng Y P, Jiang Y Y, Synthesis and in vivo anti-tumour activity of 4(3H)-quinazolinone derivatives with dithiocarbamate side chains. *Bio Org Med Chem* 2005;15:1915-7.
- Alagarsamy V, "Synthesis, Analgesic and Antiinflammatory Activities of Some Novel 2, 3- Disubstituted Quinazolinone-4(3H)-one," *Biol Pharm Bull* 2003;26(4):557-9.
- Kant P. Synthesis and anti-microbial activities of some new 2-substituted 3(1''-aryl-4''-nitrophenyl imidazol-5''-yl) amino quinazolin-4-ones. *Indian J Heterocycl Chem* 2006;15:221-4.
- Nandy P, Vishalakshi MT and Bhat AR. Synthesis and antitubercular activity of mannich bases of 2-methyl 3H-quinazolin-4-ones. *Indian J. Heterocycl. Chem* 2006;15:293-4.
- Rajasekaran S, GopalKrishna R, Sanjay Pai PN and Gurpreet Singh S. Synthesis, Antibacterial and invitro Antioxidant Activity 2,3-Substituted Quinazolin-4(3H)-ones. *J Chem Pharm Res* 2010;2(1):482-8.
- Kumar KS, Ganguly S, Veerasamy R, Clercq ED, "Synthesis, antiviral activity and cytotoxicity evaluation of Schiff bases of some 2-phenyl quinazolinone-4(3)H-ones." *Eur J Med Chem* 2010;45:5474-9.
- Hancock RD, Martell AE. Ligand design for selective complexation of metal ions in aqueous solution. *Chem Rev* 1989;9:1875-914.
- Bhyrappa P, Young JK, Moore JS, Suslick KS. DendrimerMetalloporphyrins: Synthesis and Catalysis. *J Am Chem Soc* 1996;118:5708-11.

11. Castillo-Blum SE, Barba-Behrens N. Coordination chemistry of some biologically active ligands. *Coord Chem Rev* 2000;196:3-30.
12. Mohan G, Rajesh N Synthesis and anti-inflammatory activity of N-pyridinobenzamide-2-carboxylic acid and its metal chelates. *Indian J Pharm* 1992;24:207-11.
13. Kong D, Reibenspies J, Mao J, Clearfield A. Novel 30-membered octaazamacrocyclic ligand: synthesis, characterization, thermodynamic stabilities and DNA cleavage activity of homodinuclear copper and nickel complexes. *Inorg Chim Acta* 2003;342:158-70.
14. Hung RQ, Li HY, Ma JA, Qiu DW (1996). Synthesis of O-(4-Quinazolinyloxy) Ethers and Their Antiviral Activity. *Chem J Chin Univ* 1996;17:571-5.
15. Dandia A, Singh R, Sarawagi P. Green chemical multicomponent one-pot synthesis of fluorinated 2,3-disubstituted quinazolin-4(3H)-ones under solvent-free conditions and their antifungal activity. *J. Fluorine Chem.* 125, 1835-1840 (2004).
16. Khan IA, Hassan G, Khan MA. Efficacy of Post-emergence Herbicides for controlling Weeds in Canola. *Asian J Plant Sci* 2003;2:294-6.
17. Jin Y, Li HY, Lin LP, Tan JZ, Ding J, Luo XM et al, Synthesis and antitumor evaluation of novel 5-substituted-4-hydroxy-8-nitroquinazolines as EGFR signaling-targeted inhibitors. *Bioorg Med Chem* 2005;13:5613-22.
18. Wissner A, Berger DM, Boschelli DH, Floyd MB Jr, Greenberger LM, Gruber BC et al., 4-Anilino-6,7-dialkoxyquinoline-3-carbonitrile Inhibitors of Epidermal Growth Factor Receptor Kinase and Their Bioisosteric Relationship to the 4-Anilino-6,7-dialkoxy-quinazoline Inhibitors. *J Med Chem* 2000;43:3244-56.
19. E. Keskioglu, A. Balaban Gunduzalp, and S. F. Cete, *Spectrochem. Acta A*, 2008;70:634-40.
20. Osarodion Orarumwense., Lucky Okunrobo, (2015). Design synthesis and bioassay of novel metal complex of 3-amino 6,8-dibromo-2-methyl quinazolin-4(3H)-One. *Pharm Chem J* 48:(11):718-21.
21. Nada A.M.A, Alkady M.Y, and Fakry H.M. *Bioresources*. 2007;3(1):46-59.
22. Okeke M. I, Iroegbu C.U, Eze E.N, Okoli A.S, Esimone C.O. Evaluation of extracts of the root of *Landolphia owerriense* for antibacterial activity. *J Ethnopharmacol* 2001;78:119-27.
23. Mackie R, McCartney. *Practical Medicinal Microbiology* 3<sup>rd</sup> edition, Vol.2 Churchill Livingstone (Publishers), London and New York. 1984;121(141):100-106.
24. Radhakrishnan P, Lingegomda V, Molagavalli V., Zoures Wang Zar F.A. Positive PPD test in health-care workers (HCW). A study of the compliance of antibiotic prophylaxis. *J Gen Intern Med* 20002;17(15):209.
25. Agarwal R.K, Prakash J. Reactions of Imine-oxime Ligands. *Polyhedron* 10, 2567.(1991).
26. Om Prakash, Soumitra, K.S, Chandra, M.T. "Reactions of Cp<sub>2</sub>MC<sub>2</sub> (M=Ti or Zr) with Imine – Oxime Ligands. Formation of Metallacycles" *Molecules* 2005;10:653-8.

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