Syntheses and Analyses of Novel Schiff Base Ligands with NN'OS Coordination Spheres

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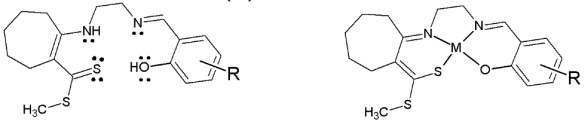
INTRODUCTION

Schiff base ligands are organic compounds characterized by at least one nitrogen double bonded to a carbon. This nitrogen and other possible atoms provide unpaired sets of electrons that allow the Schiff base to bind around a metal to form a Schiff base metal ligand complex as can be seen in Figure 1 (1). Schiff base ligands are frequently used in the fields of medicine, pharmacy, and material science (2, 3). Currently, pharmaceutical companies are developing medication using Schiff base ligand complexes in hopes of better treating diseases once deemed incurable (3).



Schiff Base Ligand Schiff Base Metal Ligand Complex

Figure 1: A characteristic trait of Schiff base ligands is a nitrogen double bonded to a carbon, as circled. When a metal binds to the unpaired electrons in the coordination sphere, the Schiff base becomes a Schiff base metal complex. Image adapted from Asadi et al. (7).

According to the Chang group, Cobalt (III) Schiff base complexes belonging to the CTC family can inhibit Herpes Simplex Virus 1 (HSV-1) and HIV-1. The exact mechanism of HSV-1 inhibition is currently unknown. However, research has shown that CTC-96 can bind to a particular protein known as maturational protease, which is composed of large concentrations of histidine. CTC-96 binds to histidine thus inactivating the protein and preventing the spread of the virus (3).

The discovery that CTC-96 binds to histidine may indicate the Schiff base metal complex's potential to treat HIV-1. Studies conducted by Louie et al. reveal that CTC compounds can bind to the zinc finger Sp1 protein, which is also composed of histidine. The Sp1 protein serves as a transcription factor that helps replicate the genome of HIV-1. By binding to this protein, CTC compounds prevent transcription of the viral genome, which ultimately prevents more HIV-1 viruses from being produced. This form of treatment is promising because it targets the genome of HIV-1. Prior to this research, HIV medication was developed to target and bind to the protein coat of the virus. However, the frequent mutations of HIV-1 cause the coat to alter in shape, thus preventing the medication from properly binding to the virus (4).

In addition to antiviral features, certain Schiff base ligand complexes also display antibacterial and tumor-suppressing features (5). A study by Saghatforoush et al. has shown that certain Cobalt (II) ligand complexes can eliminate several bacteria including *Streptococcus pyogenes* and *S. agalactiae* (6). *S. pyogenes* infection can result in several diseases such as acute pharyngitis and scarlet fever, while *S. agalactiae* infection can cause pneumonia or meningitis (8,9). The discovery of these antibacterial traits indicates that cobalt (II) ligand complexes could possibly be used in antibiotics (6).

The tumor-suppressing features of Schiff base ligand complexes were measured by Kumar et al. In this study, six novel ligand complexes were synthesized using copper (II), nickel (II), and zinc (II). Human cervical carcinoma cells were exposed to these compounds and their cytotoxicities (capability of inhibiting cell growth) were measured through a MTT Assay (10, 11). According to the results, a copper (II) complex had the highest cytotoxicity and could possibly be incorporated in medicine to suppress human cervical carcinoma growth (10).

Because of these potential biological applications of Schiff bases, the original objective of this project was to synthesize five novel asymmetric tetradentate Schiff base metal complexes with NN'OS coordination spheres. Studies have shown that such complexes could potentially serve as biological oxygen carriers, which facilitate oxygen distribution in organisms, as well as other crucial proteins, such as cytochrome c oxidase (12, 13, 14). The general procedure was provided by Asadi et al. and involved synthesizing the ligands from cycloheptanone using salicylaldehyde, 5-chlorosalicylaldehyde, 3, 5-dichlorosalicylaldehyde, 3-*tert*-butyl-2-hydroxybenaldehyde, and 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde (Figure 2) (7). The NN'OS coordination spheres of the products were then supposed to be complexed to either copper (II) or nickel (II).

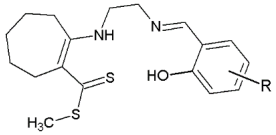
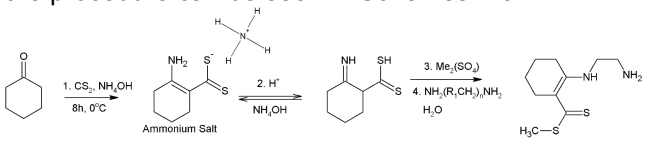


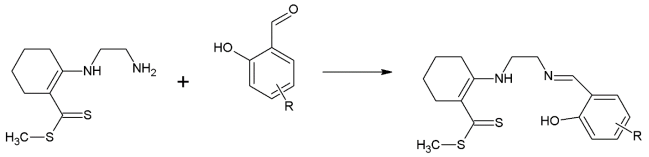
Figure 2: Ligand structure about the NN'OS coordination sphere. (R=H; 3-Cl; 3,5-Cl; 3 *tert*-butyl; and 3,5-di-*tert*-butyl) Image adapted from Asadi et al. (7).

Unfortunately, there was approximately 0% yield of the ammonium salt from reaction step 1 (Scheme 1). The original procedure provided by Asadi et al. was meant to be used for cyclopentanone rather than cycloheptanone (7). Therefore, it was concluded that the steric hindrance caused by the addition of two additional carbons to the ring resulted in the ammonium salt to be too unstable. As an alternative,

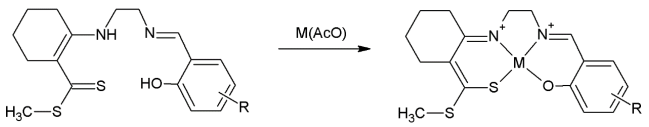
cyclohexanone was used as the starting reagent, thus the new novel Schiff base ligands would have a six carbon ring instead of seven. The general outline of the procedure can be seen in Schemes 1-3.



Scheme 1: Several reactants will be used to convert cyclohexanone into an amine intermediate (far right compound). Image adapted from Asadi et al. (7).

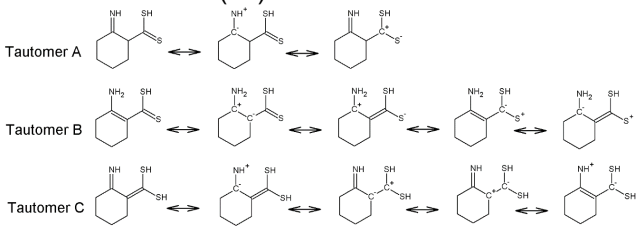


Scheme 2: Synthesis of the Schiff base ligand. (R= H; 3-Cl; 3,5-Cl; 3 *tert*-butyl, 3,5-di-*tert*-butyl) Image adapted from Asadi et al. (7).



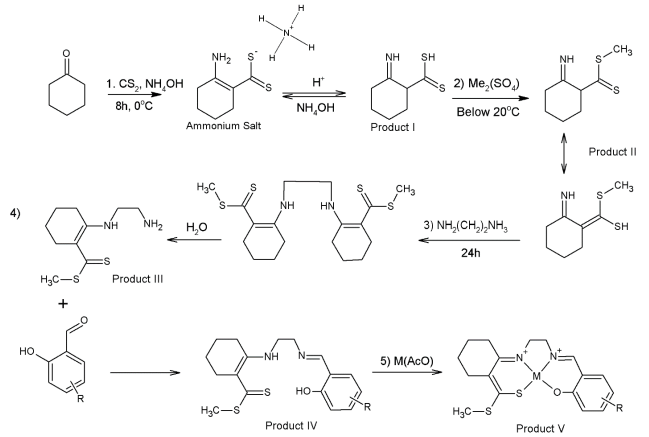
Scheme 3: Coordination of the Schiff base ligand to a metal through the use of the appropriate metal acetate. (M=Co^{2+} or Ni^{2+}) Image adapted from Asadi et al. (7).

Throughout the project, the possibility of tautomers was considered while analyzing ^{13}C and ^{1}H NMR spectra of each reaction product. Tautomers are isomers that have different rearrangements of bonds and atoms (15). There are three tautomers for the product of reaction step 1, as can be seen in Scheme 4 (16). Theoretically, since tautomer A, which is shown as the product of step 2 in Scheme 1, has the least number of resonance structures, it would be the least stable and the least prevalent. Because both tautomers B and C have the same number of resonance structures, they would be equally common (17). Based on prior studies, however, tautomer B is the most common (16).



Scheme 4: Possible tautomers of the reaction 1 product and their first order resonance structures. Adapted from Bordás et al. (16).

Experimental



Scheme 5: Full scheme of Schiff base metal ligand complex synthesis. (R= H; 3-Cl; 3,5-Cl; 3 *tert*-butyl, 3,5-di-*tert*-butyl and M=Co^{2+} or Ni^{2+}). Adapted from Asadi et al. (7).

Step 1: CS_2 Addition. Three different procedures were used to bind CS_2 to cyclohexanone (Scheme 5). The first procedure used was the most similar to that of the Asadi groups (18). 5.21mL of cyclohexanone (0.05mol) was added to 50mL of NH_4OH. 3.93mL of CS_2 (0.065mol) was then added to the mixture, and the solution was mixed with a magnetic stir bar for 8 hours at 0°C. At the end of the 8 hour period, the solution was filtered and the ammonium salt was washed with ethyl ether prior to being placed in a vacuum dessicator overnight. Product I was synthesized when the dried salt was dissolved in acetic acid, which was heated to 60-70°C, according to a 1g:10mL ratio. H_2O was then added dropwise until the solution became yellow, and Product I was filtered and recrystallized with methanol (18). A small sample of the product was dissolved in dimethyl sulfoxide (DMSO) and analyzed with ^{13}C and ^{1}H NMR. Batch 1 and Batch 2 were synthesized in this manner.

The second procedure involved mixing 5.21mL cyclohexanone (0.05mol) with 5.56mL of THF. This mixture was added to a mixture of 16.67mL NH_4OH and 6.56 mL of CS_2 (0.1085mol). The solution was then mixed for 8 hours below 18°C (19). The ammonium salt was filtered and acetic acid was heated as previously described and added to the salt until it dissolved completely. Therefore, the 1g:10mL ratio was disregarded. Product I was analyzed with ^{13}C and ^{1}H NMR as previously described. Batch 4 was synthesized in this manner.

The third procedure was the most effective. 30.82mL of cyclohexanone (0.297mol) was mixed

with 27.50mL of CS_2 (0.457mol) and 100 mL of NH_4OH. The solution was mixed for 6 hours at 0°C and then filtered and dried. Protonation was conducted and Product I was analyzed (20). Batch 3 was synthesized in this manner.

Step 2: Methylation. To methylate, 0.1731g NaOH was mixed with 15mL of H_2O. 0.75 g of Product I (0.004mol) was partially dissolved in this mixture and 0.4104mL $(Me)_2SO_4$ (0.005mol) was slowly added dropwise. The solution was stirred for approximately 4 hours under 20°C and then filtered. Product II was dried in a vacuum dessicator overnight and dissolved in DMSO prior to ^{13}C and ^{1}H NMR analysis (16). Batches 1-3 were methylated in this manner. The amounts used were according to Batch 1 yields, but the same molar ratios were maintained for all batches.

Step 3: Amination. Amination occurred when 0.437g Product II (0.002mol) was added to a mixture of 4.08 mL of methanol and 0.78mL of ethylenediamine (0.0116mol). The solution was stirred at room temperature for 48 hours and filtered. H_2O was added to the filtrate until it turned yellow. This filtrate was then filtered for Product III and analyzed using ^{13}C and ^{1}H NMR in the same manner previously described (16).

Results

Table 1: The yields of product at each stage of the synthesis.

	Stage	Theoretical Yield (g)	Yield (g)	% Yield
Cycloheptanone	Ammonium Salt	5.457	0.021	0.385
Batch 1	Ammonium Salt	9.518	3.321	34.89
	Product I	3.024	Missing	Missing
	Product II	0.509	0.293	57.47
Batch 2	Ammonium Salt	9.518	3.791	39.83
	Product I	3.452	1.00	28.97
	Product II	0.757	0.555	73.35
Batch 3	Ammonium Salt	56.611	28.063	49.57
Cut 2A	Product I	2.240	0.826	36.88
	Product II	0.525	0.437	83.24
	Product III	0.537	0.050	9.311
Cut 2B	Product I	2.112	0.958	45.35
	Product II	0.823	0.571	69.38
	Product III	0.615	0.219	35.61
Cut 3	Product I	4.797	1.764	36.77
	Product II	1.907	1.619	84.90
Batch 4	Ammonium Salt	9.518	3.496	36.73

Use provided contact information for additional information regarding NMR spectroscopy.

The original objective of this project was to synthesize a Schiff base ligand from cycloheptanone based on a procedure by the Asadi group (7). To synthesize the ammonium salt, the first procedure from the Experimental Section was used. As can be seen in Table 1, the yield for ammonium salt using cycloheptanone was only 0.385%; therefore, there was not enough product synthesized for subsequent steps to be conducted. To confirm that the ammonium salt was synthesized, ^{13}C and ^{1}H NMR was run on the product. The salt would not dissolve in $CDCl_3$, which was the solvent used at the time, and clean spectra were not produced. However, ^{13}C NMR did provide strong evidence that the desired product was synthesized (Figure 1). Peaks representing all eight of the unique carbons can be seen on the spectrum with three additional peaks representing impurities. After running ^{13}C and ^{1}H NMR on the two reagents cycloheptanone and CS_2, it was concluded that the three impurity peaks were remnants of cycloheptanone. The ^{1}H NMR spectrum was inconclusive and can be seen in the Supplementary Section along with the reagent spectra.

Three more attempts were made to synthesize ammonium salt from cycloheptanone. The first two attempts incorporated slight alterations to the temperature of the starting materials and the use of HCl to synthesize Product I (7). The third attempt involved the use of the second procedure in the Experimental Section. However, the yields for these additional attempts were approximately 0%. As a result, cyclohexanone was used as the starting reagent instead. The ammonium salt, which was an orange solid, of Batch 1 and 2 was synthesized using the first procedure and resulted in 34.89% and 39.83% yield respectively (Table 1). During the eight hour mixing period for Batch 1, the ammonium salt became so thick that the magnetic stir bar could no longer mix the reagents. To circumvent this issue, Batch 2 was mixed with a larger stir bar. This change resulted in the 4.94% yield difference between the two batches. Because ammonium salt does not dissolve in DMSO, ^{13}C and ^{1}H NMR was not used at this stage.

The yield for Batch 1 Product I prior to recrystallization was not measured because the original intention was to only conduct measurements for Product I recrystallized. Because the yield was only 16.30%, however, approximately 0.20g of Product I crude from Batch 2 was recrystallized. The crude and recrystallized products were both analyzed through ^{13}C and ^{1}H NMR, and were identical. Because NMR signals were low for the spectra of both crude and recrystallized products, results were deemed inconclusive and the same experiment was conducted for Product I of Batch 3 Cut 2A. In comparison to its crude product, the recrystallized spectra was less intense, which means that product was actually lost during recrystallization. Therefore, recrystallization was deemed detrimental and unnecessary.

Out of all three ammonium salt procedures, the third procedure was most successful, as can be seen in the 48.57% yield of Batch 3 (Table 1). Because Batch 3 was so large, it was divided into 4 cuts prior to proceeding. Cut 1 was lost because protonation of the salt was attempted using a method published by Padma et al., which was meant to be used for ammonium salt made from cyclopentanone. The salt was supposed to be dissolved in H_2O and protonated with HCl (20). Unfortunately, the salt did not dissolve in H_2O. Cuts 2A-B and 3 were used to proceed to the next step, while measurements for Cut 4 were not made and Batch 4, which was synthesized using the second procedure, was not used due to time constraints.

Although three different methods were used for ammonium salt synthesis, Product I, which resembled bright orange crystals, was still successfully synthesized. This can be seen in the NMR spectra of Batch 3 Cut 3 (Figures 2 and 3). In the ^{13}C NMR spectrum, all seven unique carbons have been identified. However, there are three peaks that are unaccounted for, one of which is the peak at 119.2854ppm. Based on a ^{13}C spectrum generated by ACDLabs' NMR predictor, this peak belongs to Tautomer B.

The remaining two peaks left unaccounted for were within the 20.0-40.0ppm region. These two peaks are the result of alternating chair conformations of the cyclohexane ring. To prevent as much steric repulsion as possible, carbons within the cyclohexane ring enter a formation resembling a chair. There are two different chair conformations that can form. Often times a ring-flip, which is the interconversion between these conformations, can occur (Scheme 6). During a ring flip, two carbons move, while the other four remain in place. As a result, the two moving carbons enter different environments in the ring, causing the NMR to detect them as unique carbons (21).

Scheme 6: Alternating chair conformations of a cyclohexane ring. Adapted from McMurry (21).

The ^{1}H NMR spectrum did not provide as much information as that of the ^{13}C NMR (Figure 3). Although all unique hydrogens except for hydrogen 2 were assigned to a peak, they were not done so with absolute certainty because the peaks overlapped each other. The cause of this overlapping can be attributed to the presence of additional tautomers. At 3.5ppm, there was a peak that could not have been attributed to tautomers. Compared to all other peaks, this was the second most intense, meaning that the unique hydrogen it represents was the second highest in concentration. To determine the source of this peak, the NMR solvent, DMSO, was spiked with isopropanol and analyzed using ^{1}H NMR. The unidentified peak appeared at 3.5ppm in this sample as well, which meant that DMSO was contaminated. Spectra for all batches of Product I were identical.

Following confirmation that Product I was synthesized, all batches except for Batch 4 underwent methylation to form Product II, which resembled brown pellets. According to literature, Product II was supposed to be recrystallized with 1:1 methanol:water (16). The product was not able to dissolve in this solvent. After conducting a solvent test using methanol, ethanol, and ethyl acetate and realizing most batches had less than 1g of Product II, it was deemed unnecessary to recrystallize.

Based on the ^{13}C NMR spectra for Product II of each batch, there appears to be no evidence that Product II was successfully synthesized. However, the ^{1}H NMR spectra of each batch does indicate one additional peak at within the 2.3-2.5ppm range that represents the hydrogens on the additional methyl group (Figure 4). Though this peak is very distinct in the spectrum of Batch I, it is relatively subtle in the spectra of other batches, thus suggesting that only a small portion of each batch was methylated.

Upon confirmation that Product II was synthesized, the amination procedure was conducted to synthesize Product III. Because of time constraints, only Batch 3 Cut 2A and 2B underwent this procedure. Based on the NMR spectra, Product III was not synthesized. ^{13}C spectra for both cuts did not have the two additional peaks that should represent the two new carbons on Product III. In addition, there were no additional peaks on the ^{1}H NMR spectrum either. Therefore, the Schiff base ligand complex was not able to be synthesized by the end of the research period.

Because there has been a lack of literature regarding the synthesis of a Schiff base ligand similar to that of Asadi et al. using cyclohexanone, such a task may not be possible. Although there has been published literature reporting the syntheses of compounds similar to it, none have even reported successfully synthesizing Product II (19, 22-23). According to Takeshima et al., Product I is extremely unstable and could result in alternative compounds, one of which can be seen in Figure 5 (19). This possibility may explain the inconclusive ^{1}H NMR spectra for all the products. In addition to possible tautomers, other structures may also be present in the sample.

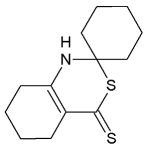


Figure 5: Possible alternative structure to Product I according to Takeshima et al. (19).

Despite the unstable nature of Product I, ^{13}C NMR spectra have confirmed that a majority of each Product I sample is composed of the intended compound. The alternative compound in Figure 5 was likely only synthesized, if at all, in very small concentrations since the ^{13}C NMR spectra do not reveal the additional six peaks needed within the alkane region. Spectra for Product II are not as promising. Because the additional peak in all ^{1}H NMR spectra of Product II is relatively small, they cannot be considered completely conclusive of a successful synthesis. However, the presence of this peak in all ^{1}H NMR spectra of Product II does provide substantive evidence that methylation did occur. Based on NMR spectra for Product III, amination did not occur.

CONCLUSION

According to the spectra for ammonium salt from cycloheptanone, the ammonium salt was synthesized. However, the consistent lack of yield produced prevented further progression into the procedure. As a result, the objective of the project was changed from using cycloheptanone to synthesize a Schiff base metal ligand complex with an NN'OS coordination sphere similar to that of the Asadi group, to using cyclohexanone instead. Interest in synthesizing this ligand can be attributed to potential biological applications (12-14).

Based on NMR spectra for each product, only Product I and II were synthesized. Spectra for Product III showed no difference from those of Product II, thus suggesting that amination did not occur. Because of time constraints, this issue was not circumvented and the Schiff base was not synthesized. Based on the paucity of literature found regarding the successful syntheses of Product II and III, the objective of this project is likely not possible. According to Takeshime et al., Product I is extremely unstable and can enter alternative reactions to produce different compounds (19). Though the ^{13}C NMR spectra of all batches confirm that Product I was made, its unstable nature likely contributed to the difficulties in synthesizing Product II and III.

Had there been more time, two changes may have been made to the syntheses of Product II and III. As previously mentioned, the ^{1}H NMR peak representing the successful synthesis of Product II was most distinct only in the spectrum of Batch 1, which suggests that a majority of this sample was methylated. The only difference between the methylation procedure for Batch 1 and those of the other batches was that Batch 1 Product I was recrystallized. Based on the spectra of Product I recrystallized and its untouched counterpart, it was originally deemed that recrystallization was detrimental and unnecessary because the procedure resulted in a smaller yield that was as pure, if not less, as Product I prior to recrystallization. Since a higher portion of recrystallized Product I seemed to be methylated, the benefits of the procedure should have been reconsidered. Unfortunately, Batch 1 Product II was not used for amination to determine if it could have made a difference in the synthesis of Product III.

In regards to the synthesis of Product III, more than 4.08mL of methanol should have been used as the solvent. While conducting the procedure, Product II of both Cut 2A and Cut 2B did not completely dissolve into solution. To compensate for this, the mixing period was extended from 24 hours, as suggested by the literature, to 48 hours (16). However, the products still did not dissolve, and neither Cut 2A nor Cut 2B resembled the pale yellow solution described in the literature. By increasing the amount of solvent, Product II of both cuts may better react with ethylenediamine.

Because the steric hindrance produced from the cyclohexane ring makes Product I unstable, the chances of Product III being synthesized is still slim (19). If this project were to be conducted again, cyclopentanone would be used since it would be following the procedure of the Asadi group more closely (7). However, the amine bridge of Product III would be altered. Rather than using ethylenediamine, which was done so according to literature, other amine bridges would be used, such as propylenediamine and 1-methylethylenediamine.

REFERENCES

1. GUweb.Gonzaga.edu. http://guweb.gonzaga.edu/faculty/cronk/biochem/S-index.cfm?definition=Schiff_base (Accessed March 9, 2015).
2. Hadjeb, R; Barkat, D. Determination of acid dissociation constants of some substituted salicylideneanilines by spectroscopy. Application of the Hammett relation. *Arabian J. Chem.* **2014**, pp 1-6.
3. Chang, E; Simmers, C; Knight, D. A. Cobalt Complexes as Antiviral and Antibacterial Agents. *Pharmaceuticals* **2010**, *3*, pp 1711-1728.
4. Louie, A. Y; Meade, T. J. A cobalt complex that selectively disrupts the structure and function of zinc fingers. *Proc. Natl. Acad. Sci.* **1998**, *95*, pp 6663-6668.
5. Iqbal, J; Tirmizi, S. A; Wattoo, F. H; Imran, M; Wattoo, M. H. S; Sharfuddin, S; Latif, S. Biological Properties of Chloro-salicylidene Aniline and Its Complexes with Co (II) and Cu (II). *Turk J. Biol.* **2006**, pp 1-4.
6. Saghatforoush, L. A; Chalabian, F; Aminkhani, A; Karimnezhad, G; Ershad, S. Synthesis, spectroscopic characterization and antibacterial activity of new cobalt (II) complexes of unsymmetrical tetradentate (OSN_2) Schiff base ligands. *Eur. J. Med. Chem.* **2009**, *44*, pp 4490-4495.
7. Asadi, M; Mahammadi, K; Esmaielzadeh, S; Eternadi, B; Fun, K. F. Some new Schiff base ligands giving a NNPS coordination sphere and their nickel (II) complexes: Synthesis, characterization and complex formation. *Polyhedron* **2009**, *28*, pp 1400-1418.
8. Textbookofbacteriology.net. http://textbookofbacteriology.net/streptococcus.html (Accessed March 10, 2015).

9. Medscape.com. http://emedicine.medscape.com/article/229091-overview (Accessed March 10, 2015).
10. Kumar, K. S; Chityala, V. K; Subhashini, N. J. P; Prashanthi, Y, Shivaraj. Synthesis, Characterization, and Biological and Cytotoxic Studies of Copper (II), Nickel (II), and Zinc (II) Binary Complexes of 3-Amino-5-methyl Isoxazole Schiff Base. *Hindawi Publishing Corporation* **2012**, *2013*, pp 1-7.
11. Moleculardevices.com. http://www.moleculardevices.com/applications/areas-research/cytotoxicity (Accessed March 10, 2015).
12. Menati, S; Azadbakht, A; Taeb, A; Kakanejadifard, A; Khavasi, H. R. Synthesis, characterization and electrochemical study of synthesis of a new Schiff base (H_2cddi^{t}butsalen) ligand and their two asymmetric Schiff base complexes of Ni (II) and Cu (II) with NN'OS coordination spheres. *Spectrochim. Acta. Part A* **2012**, *97*, pp 1033-1040.
13. Pereira, E; Gomes, L; de Castro, B. Synthesis, spectroscopic and electrochemical study of nickel-(II) and –(I) complexes with Schiff-base ligands giving a NN'OS co-ordination sphere. *J. Chem. Soc.* **1998**, pp 629-636.
14. Jameson, G; Ibers, J. Biological and Synthetic Dioxygen Carriers. In *Bioinorganic Chemistry*; Bertini, I; Bertini, G; Harry, B; Stephen, J; Valentine, J., Ed.; University Science Books: Mill Valley, California, 1994; pp 167-252.
15. McMurry, J. *Organic Chemistry*, 6th ed.; Thomson Brooks/Cole: Belmont, 2005; pp 821.
16. Bordás, B; Sohár, P; Matolcsy, G; Berencsi, P. Synthesis and Antifungal Properties of Dithiocarboxylic Acid Derivatives. II.[1] Novel Preparation of 2-Alkylamino-1-cyclopentene-1-dethiocarboxylic Acids and Some of Their Derivatives. *J. Org. Chem.* **1972**, *37*, pp 1727-1730.
17. Academics.keene.edu. http://academics.keene.edu/rblatchly/OrgoCommon/hand/bond/ResonanceRules.html (Accessed August 25, 2015).
18. Takeshima, T; Yokoyama, M; Imamoto, T; Akano, M; Asaba, H. The Reaction of Active Methylene Compounds with Carbon Disulfide in the Presence of Ammonia. III. The Reaction of Cyclopentanone and Cycloheptanone. *J. Org. Chem.* **1968**, *34*, pp 730-732.
19. Takeshima, T; Yano, A; Fukada, N; Hirose, Y; Muraoka, M. Synthesis and Reactions of 2-Iminocyclohexanedithiocarboxylic Acid. *J. Chem. Research (S)*, **1979**, pp 140-141.
20. Padma, M; Madhu, C; Sarada, R; Venkateswara Rao, B. Synthesis of 2-aminocyclopent-1-ene-1-carbodithioic acid (ACA) Capped Silver nanoparticles, Characterisation and Fluorescence application. *Int. Journal of Engineering Research and Applications* **2015**, *5*, pp 100-108.
21. McMurry, J. *Organic Chemistry*, 6th ed.; Thomson Brooks/Cole: Belmont, 2005; pp 115-119.
22. Takeshima, T; Fukada, N; Miyauchi, T. Preparation of β-Alkyliminio-dithiocarboxylic Acids. *J. Chem. Soc., Perkin Trans. 1* **1974**, pp 914-916.
23. Takeshima, T; Fukada, N; Ishii, T. Oxidation and Bimolecular Condensation Reactions of 2-Alkylimino-cyclopentanedithiocarboxylic Acids, 2-Oxocyclpentanedithiocarboxylic Acids, and 3-Methyl-5-oxo-1-phenyl-Δ^2-pyrazoline-4-dithiocarboxylic Acid. *J. Chem. Soc., Perkin Trans. 1* **1976**, pp 1706-1708.