Summary of the Thesis

Synthesis and Applications of Naphthoquinone Derivatives and Their Metal Complexes

Submitted to

The Maharaja Sayajirao University of Baroda, Vadodara

For the Degree of

DOCTOR OF PHILOSOPHY
In

CHEMISTRY

By

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Thesis Outline and Key Findings

The creation of novel derivatives of the naturally occurring substance 2-hydroxy-1,4-naphthoquinone (Lawsone) and their metal complexes was the aim of the research that went into this thesis in order to learn more about their biological properties and potential applications in catalysis. Six sections make up the bulk of the thesis. **Chapter 1** covers an introduction of quinones and naphthoquinones. The role, synthesis and applications of various naphthoquinones derivatives in biological activity and materials will be elaborated. The synthesis of novel Mannich bases and their Structure Activity Relationship (SAR) are covered in **Chapter 2**. The chemistry of complexes generated from Mannich bases of lawsone is discussed in **Chapters 3** and **4**, along with their use in anticancer and antibacterial activity, as well as interactions with biomolecules including DNA and BSA. **Chapter 5** discusses the production and application of lawsone vanadium complex for catalytic oxidation. A study of the synthesis, anticancer effectiveness, and molecular docking of novel isoindolinones is presented in **Chapter 6.**

Aim and objectives

- ✓ Synthesis of mannich bases of 2-hydroxy-1,4-napthoquinone, assessment of their anticancer potential and establishment of structure activity relationship (SAR)
- ✓ Synthesis of 2-hydroxy-1,4-napthoquinone derivatives, assessment of their anticancer potential and establishment of structure activity relationship (SAR).
- ✓ Synthesis of metal complexes of 2-hydroxy-1,4-napthoquinone derivatives, Investigation of their anticancer potential.
- ✓ Investigation of catalytic application of 2-hydroxy-1,4-napthoquinone metal complexes for organic transformations.

Chapter 1:

INTRODUCTION

In medicinal chemistry research organic chemist attempts to design and synthesize a drug or pharmaceutical medicine which will help human being to get relief from various diseases. In last two decade increasing rate of various diseases led opportunity to chemists to design, synthesize and evaluate novel drug molecules. Naphthoquinones belong to one of the most important and widely distributed chemical class in quinone family. They are largely found in plants, microorganisms, and some animals. Naphthoquinones are class of organic compound derived from naphthalene and normally exists in three isomeric forms.

1] 1,2-Napthoquinone

2] 1,4-Napthoquinone

3] 2,6-Napthoquinone

Natural and synthetic naphthoquinones are known for large number of biological activities among which anticancer, antiparalytic, antibacterial, antimalarial, antiviral, anti-inflammatory, antimicrobial and antifungal. Important derivatives of naturally occurring 1,4-Napthoquinone are Lawsone, Plumbagin, Juglone and Menadione. Thymoquinone, Lapachol and Parvaquone are bioactive cytotoxic quinones **(figure 1.1)**.

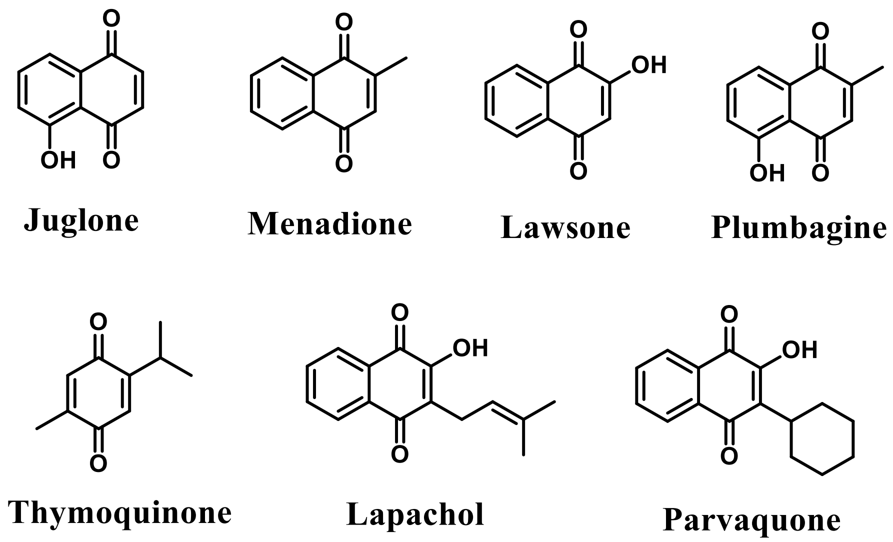

Figure 1.1 Derivatives of naturally occurring Naphthoquinones

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Position of the hydroxy group in napthaquinone compounds are crucial to their biological activity as it affects redox potential. 2-hydroxy-1,4-napthoquinone or Lawsone is one of the simplest naturally occurring napthoquinone. This red coloured pigment can be obtained from dry powdered leaves of henna.

Introducing nitrogen atom in lawsone or napthoquinone can led to increase in anticancer, antibacterial and other activities. Mannich reaction is a very useful one-pot reaction which results in *C*-aminoalkylation adjacent to a carbonyl functional group. The final product is a βamino-carbonyl compound that is also known as a **Mannich Base.** In this reaction, nucleophilic addition of an amine to a carbonyl group, followed by dehydration, forms a Schiff base that reacts in a second step with nuclophiles, such as enol or enone derivatives, to form an amino *C*-alkylated adduct. Therefore this reaction is ideal for the preparation of a library of compounds for biological screening. It is also called as Multi Component Reaction **(MCR).** In literature, there are few reports on mannich bases of lawsone and its derivatives which offer good to excellent anticancer activity **(figure 1.2)**.

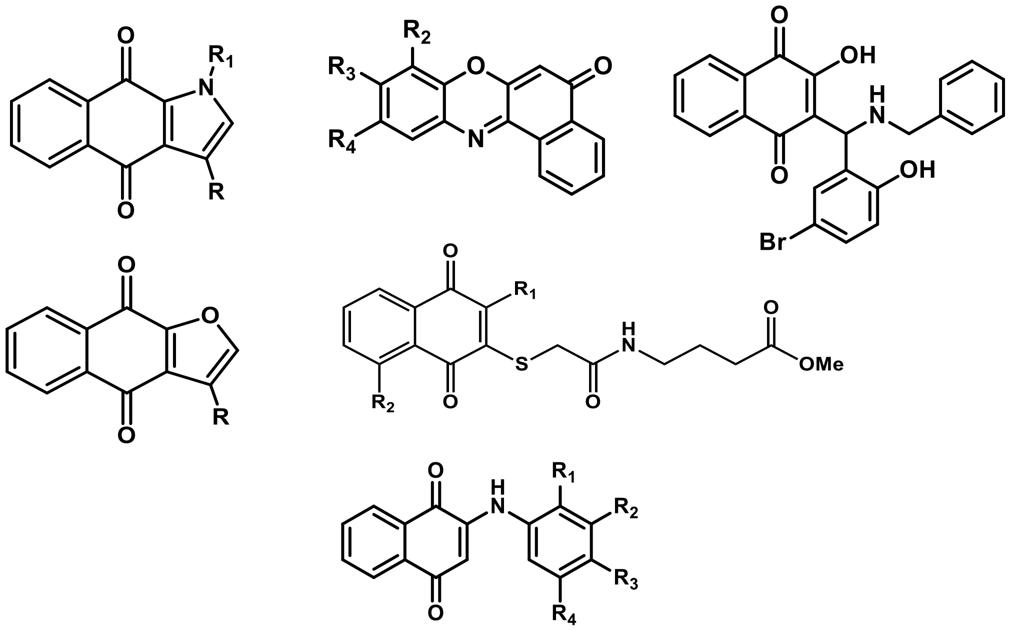


Figure 2.2 Derivatives of naturally occurring Naphthoquinones

Chapter 2:

Synthesis, Characterization and Anticancer Activity of Some 2-hydroxy-1,4-Napthoquinone (Lawsone) Based Mannich Bases

In this study, we have successfully explored a series of anticancer active 3-aminomethyl-1,4-naphthoquinone synthesized from lawsone via one pot Mannich reaction to investigate their cytotoxicity/anticancer activity effects on HepG2 cell line (Human liver carcinoma cell line). Further cell based studies have been carried out to evaluate the potent compound for its apoptosis inducing nature and heamocompatibility.

The present work is aimed to synthesize some novel Mannich bases (MB) of Lawsone with substituted aldehydes and alkyl amines using multicomponent Mannich reaction (MCR) **(figure 2.1).** As MCR is efficient and versatile tool for development of drugs.

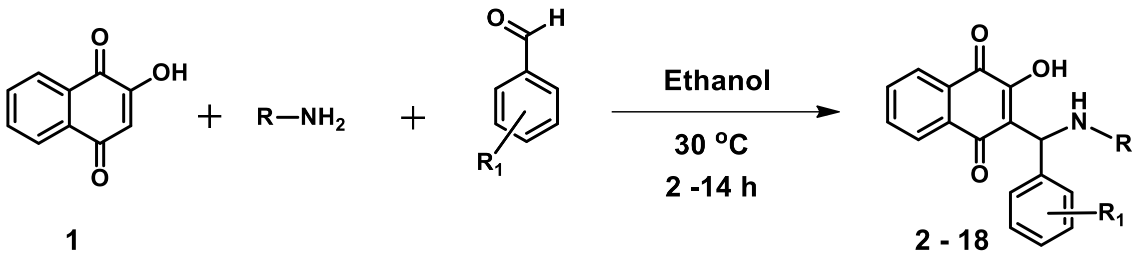


Figure 2.1: Schematic for synthesis of Mannich bases of Lawsone

These synthesized Mannich bases were evaluated for their anticancer activity against HepG2 cell lines by employing MTT assay. The results of *in vitro* cytotoxicity indicated that compounds **2, 8, 9, 15,** and **16** were most potent among the screened compounds and were comparable to standard drug doxorubicin **(figure 2.2),** Structure Activity Relationship (SAR) was also established. The most potent compounds **(2, 8, 9, 15, 16)** were subjected to DAPI, AO/EB staining and showed that cytotoxic effect through induction of apoptosis. Furthermore, they all are haemocompatible.

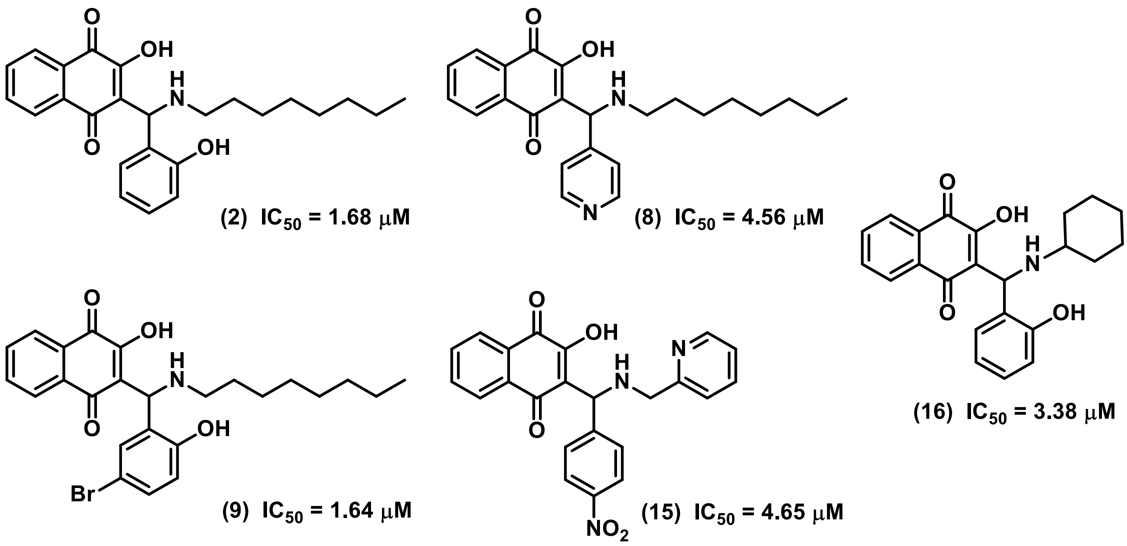


Figure 2.2 Structures of most potent MB

Chapter 3:

Synthesis, Characterization, DNA/BSA Binding and Cytotoxicity Studies of Cu(II) and V(IV) Complexes Derived from Mannich Bases of Lawsone

This chapter illustrates synthesis of a series of anticancer active Cu(II) complexes and a vanadium complex of 3-aminomethyl-1,4-naphthoquinone derived from Lawsone Mannich bases. The geometry optimization and FMO analysis of the complexes were analyzed by means of DFT calculations and compared with Single Crystal X-ray data. In order to get an insight into the charge distribution over the complexes MEP surface diagram was also scrutinized. The interactions of complexes towards biomolecules (CT-DNA and BSA) was investigated in detail with the aid of UV-vis and florescence techniques to demonstrate their biological potential. Further, in *vitro* anticancer activity of selected complexes was successfully evaluated against HepG2 (Human liver carcinoma cell lines) using MTT assay along with apoptosis assessment (AO/EB staining). In addition *in vivo* studies were also performed.

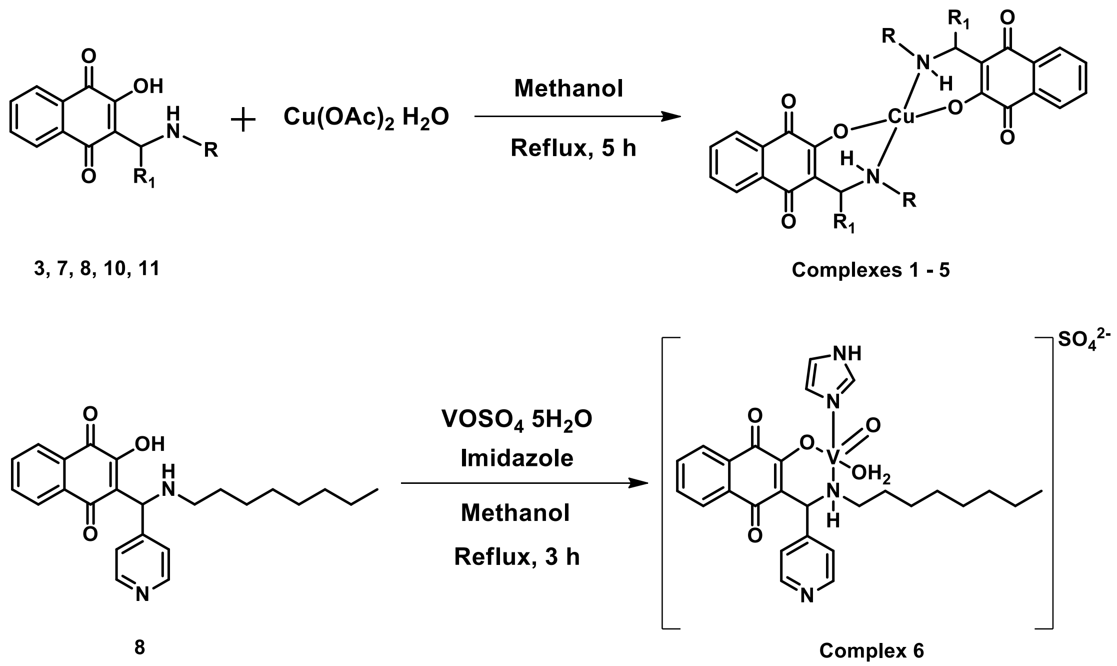
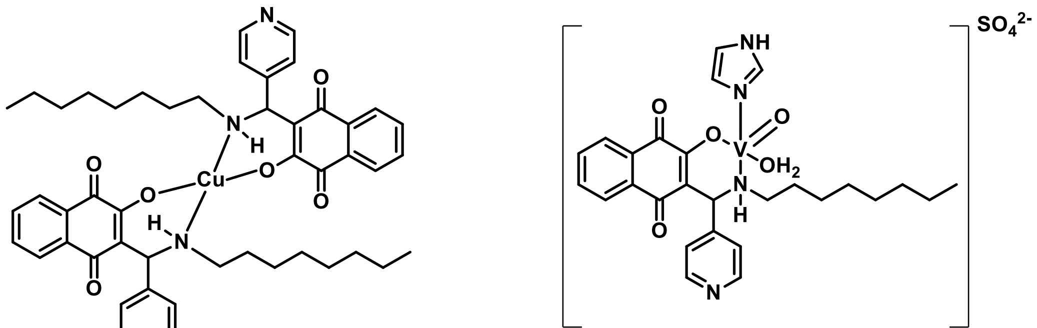


Figure 3.1 Schematic for synthesis of copper complexes 1-5 and vanadium complex 6

The objective of present work is to synthesize Cu(II) and V(IV) complexes of some moderately active Mannich bases (MB) of Lawsone **(figure 3.1)** and characterization using various techniques such as FT-IR, UV Visible, EPR, TGA, ESI-MS, Cyclic Voltammetry and

Single crystal XRD. Further, to determine potential changes in anticancer activity on HepG2 cell line as compared to their precursor ligands along with interaction with CT-DNA and BSA.

Copper and vanadium complexes were synthesized from bioactive Lawsone based ligands and tested for anticancer activity. Single crystal X-ray diffraction analysis revealed that complex containing copper atom (complex **1),** is in slightly distorted square planar geometry. The geometrical parameters of complex **1** obtained by XRD correlated with theoretical DFT studies. Some important parameters such as bond length, bond angle, HUMO and LUMO energy gap (ΔE) were also determined using theoretical DFT study. The CT-DNA and BSA binding of complexes were studied by employing UV-visible and fluorescence spectroscopy. The spectroscopic data have confirmed that all complexes efficiently bind with CT-DNA via intercalative or groove binding mode. Copper (II) complex **4** exhibits the highest binding affinity with binding constant K_b = 3.57 x 10^6 M^{-1} for CT-DNA. The results of BSA binding study revealed that quenching of proteins occurs in a static mode. It is noteworthy that complex **5** exhibited higher protein binding affinity than other complexes (K_a = 15.790 x 10^7 M^{-1}), whereas vanadium complex **6** also has higher protein binding affinity compared to copper complexes **1-3** (K_a = 1.37 x 10^6 M^{-1}). A theoretical investigation using DFT allows rationalization of experimental findings.



Complex 4, IC_{50}= 3.32 μM

Complex 6, IC_{50}= 2.71μM

Figure 3.2 Structure of most potent Cu complex 4 and V complex 6

A significant cytotoxic potential of complexes **3-6** was recorded against HepG2 cell line (by MTT assay) wherein; the findings are in agreement with experimental results and comparable to known anticancer drug cisplatin. A prominent green fluorescence following AO/EB staining in treated HepG2 cells implies to trigger of an apoptotic cascade that also corroborates with the observed cytotoxicity. The biological studies imply that these new compounds could serve as a non-platinum anticancer compound with remarkable cytotoxic effectiveness.

The study proved that complexation of moderately active MB with Copper and Vanadium metal enhances anticancer activity on HepG2 cell line along with effective binding with CT-DNA and BSA.

Chapter 4:

Synthesis, Characterization and applications of some metal Complexes Derived from 2-(((2-(dimethylamino)ethyl)amino)(4-nitrophenyl)methyl)-3-hydroxynaphthalene-1,4-dione

This chapter discusses another set of three metal complexes of Vanadium, Ruthenium and Nickel derived from novel MB. The interaction with CT-DNA and BSA were investigated using UV-vis and florescence techniques to determine their biological activity potential. Further, in *vitro* cytotoxicity activity of all complexes was successfully evaluated against HepG2 (Human liver carcinoma cell lines).

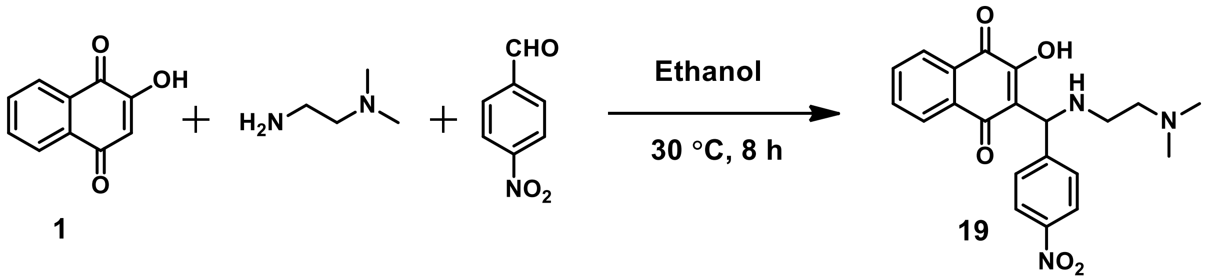


Figure 4.1 Synthesis of MB 19

The objective of present work is to synthesize various metal complexes (M = V, Ru, Ni) derived from tridentate MB ligand **(figure 4.1 & 4.2)** and characterization using various sophisticated techniques such as FT-IR, UV Visible, EPR, TGA, ESI-MS, powder XRD and 51V NMR. Furthermore, geometry optimization by DFT calculations and their biomolecular interaction with CT-DNA and BSA and cytotoxicity activity against HepG2 cell line using resazurin dye.

The CT-DNA and BSA binding of complexes were studied by employing UV-visible and fluorescence spectroscopy. The spectroscopic data have confirmed that complexes **7 & 8** efficiently bind with CT-DNA via intercalative or groove binding mode. The vanadium complex **7** exhibits the highest binding affinity with binding constant K_b = 3.56 x 10^5 M^{-1} for CT-DNA. The results of BSA binding study revealed that complex **7** exhibited higher protein binding affinity (K_a = 3.42 x 10^8 M^{-1}) and quenching of proteins occurs in a static mode. Whereas ruthenium complex **8** also has slightly lower protein binding affinity (K_a = 8.21 x 10^7 M^{-1}).

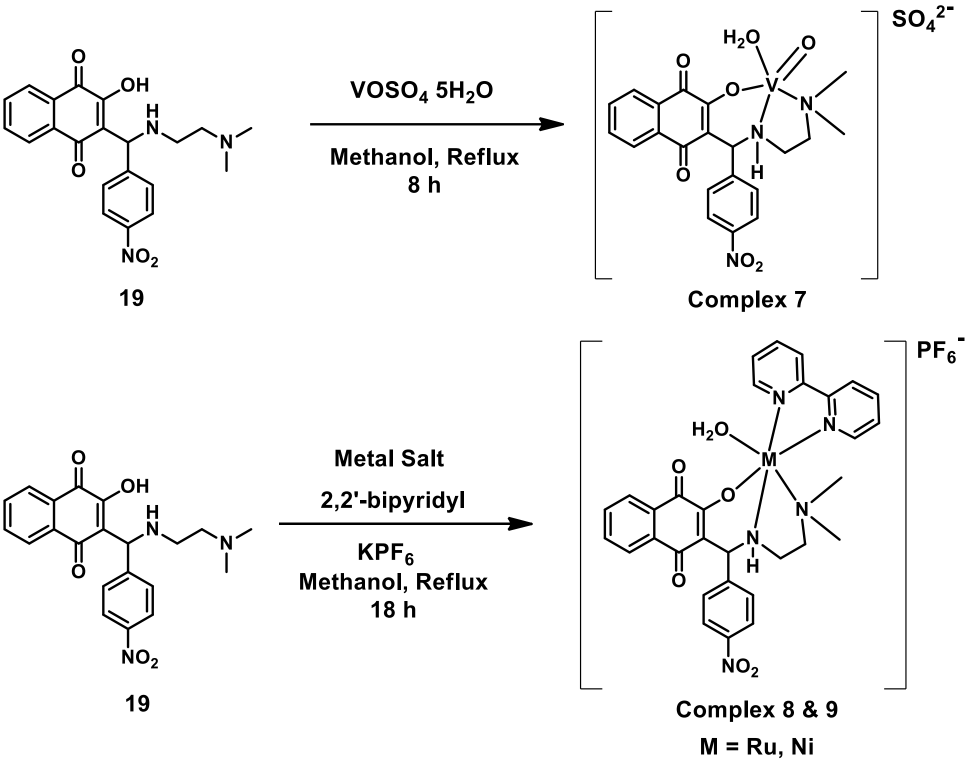


Figure 4.2 Schematic for synthesis of complexes 7-9

The cytotoxic potential of synthesized complexes **7-9** was recorded against HepG2 cell line (by resazurin dye).The cytotoxic activity implies that complex **8** (IC_{50} = 50.05 μM) is most potent among complexes **(figure 4.3)**.

In conlcusion, using the oxygen and two nitrogen atoms (ONN) donor Mannich base ligand, novel V(IV), Ru(II), and Ni(II) metal complexes were successfully synthesized. Different methods, such as molar conductivity, spectroscopic, and analytical approaches, were used to characterize the ligand and complexes (1H NMR, 13C NMR, Uv-Visible, FT-IR, ESI-MS and ESR). Bond length, bond angle, HUMO and LUMO energy gaps (E), among other crucial geometry optimization parameters, were also established. It is feasible to conclude that the successful synthesis of metal complexes under particular circumstances from these various spectroscopic analyses. UV-visible and fluorescence spectroscopy were used to confirm the intercalative and groove binding interactions of complexes with biomolecules such as CT-DNA and BSA. All available spectroscopic techniques demonstrated unequivocally that V(IV) and Ru(II) complexes exhibit the strongest binding with both DNA and BSA, but Ni(II) complex showed only modest contact. DFT research backed up the experimental results. The cytotoxicity potential of ligand and complexes was assessed using a resazurin dye-based assay on HepG2 cells, and the results showed that the ruthenium complex had significantly higher activity than ligand and other complexes, and that it was comparable to standard medication. Antibacterial test results also revealed that the Ruthenium and Vanadium complexes showed enhanced activity. The inactivity of nickel complex indicates the importance of role of metal.

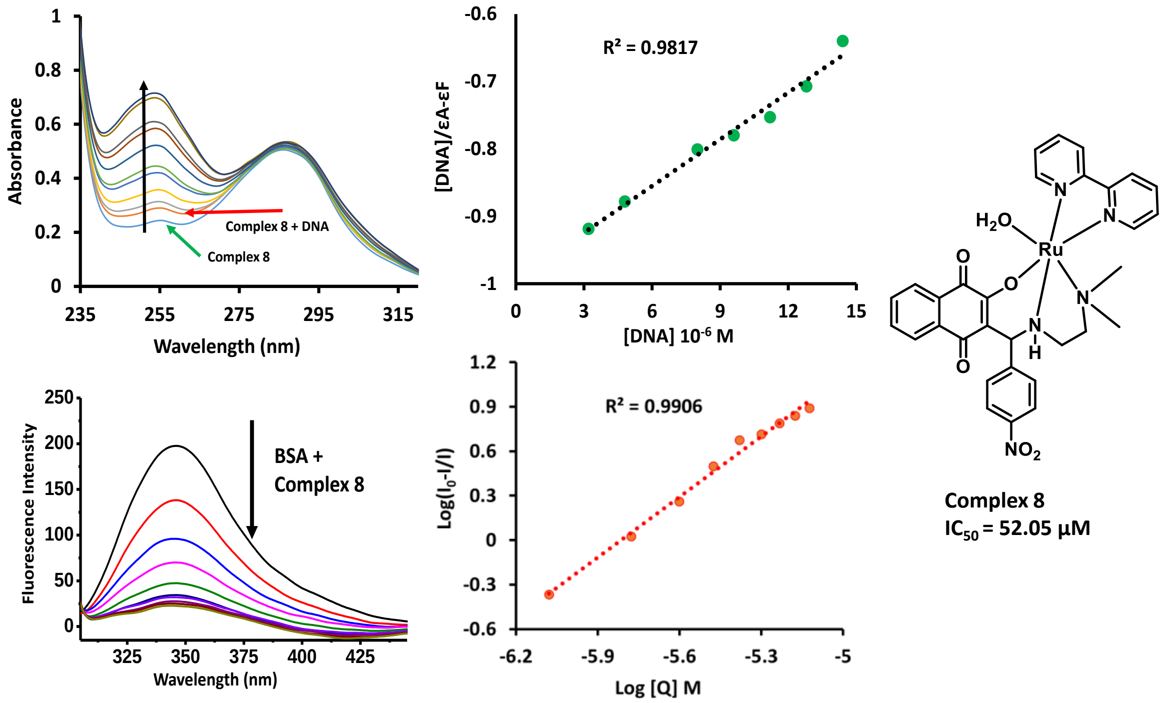


Figure 4.3 Structure of potent complex 8, plots of CT-DNA and BSA binding

Chapter 5:

Synthesis of Lawsone Vanadium complex for catalytic oxidation of diphenylmethane

This chapter discusses synthesis, characterization and applications of novel Vanadium complex of Lawsone **(figure 5.1).** Synthesized V(IV) complex characterized using various spectroscopic techniques such as UV-visible, FT-IR, HRMS, TGA and ESR. Further application of synthesized vanadium complex in selective oxidation of methylene (CH_2) to carbonyl (C=O).

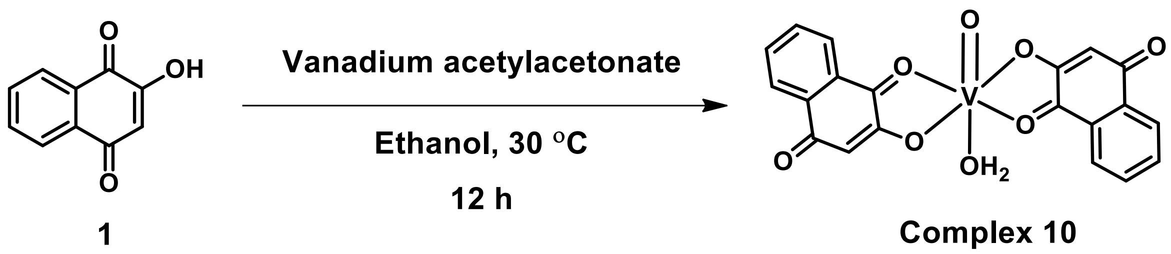


Figure 5.1 Schematic for synthesis of vanadium complex 10

Further, in this chapter theoretical calculation of complex **10** performed to determine optimize geometry using DFT calculations. The reaction conditions such as amount of catalyst, amount of oxidant, time, and temperature optimized for oxidation of diphenyl methane. The schematic and optimized condition for oxidation of arylalkane to arylketone is shown in **Figure 5.2**. The oxidized product will be isolated and percent conversion and yield will be determined. The structure of isolated products characterised using ^{1}H NMR.

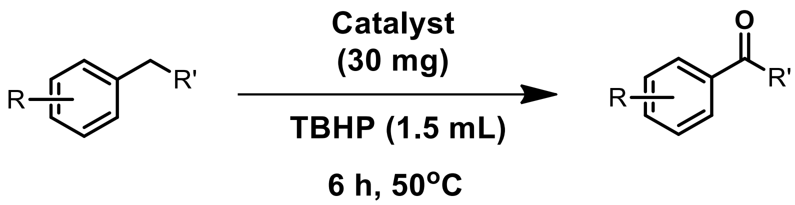


Figure 5.2 Catalytic oxidation of aryl alkanes with complex 10

In conclusion to this chapter, the vanadium (IV) complex was synthesized using naturally occurring lawsone as a ligand. The complex was characterized using a variety of spectroscopic methods, including powder XRD, FTIR, UV, and mass spectrometry. DFT computations verified the complex formation and established the optimized geometry. The complex was used as a catalyst for the high conversion and 92% isolated yield of benzophenone at 50°C within 6 hours without the formation of intermediate. Additionally, it preferentially transforms substituted arylalkanes into the appropriate ketones. This study can be extended to various substituted arylalkanes and in organic synthesis, and it may offer some guidelines for future use.

Chapter 6:

Novel substituted isoindolinones derived from Lawsone: Synthesis, Characterization, DFT calculations and biological studies

In this study, we report the synthesis of substituted isoindolinone derivatives via three-component Mannich-cascade cyclization reaction of lawsone, 2-formyl benzoic acid, and primary amines in presence of acetic acid as a catalyst at room temperature with moderate to high yields. In addition, DFT calculations of synthesized compounds were performed to determine molecular structure, dipole moment, Molecular Electrostatic Potential (MEP) maps and global reactivity parameters. Further, in *vitro* anticancer activity of selected compounds was evaluated against HepG2 (Human liver carcinoma cell lines) employing MTT assay. The compound with high activity was further evaluated for in *vivo* studies.

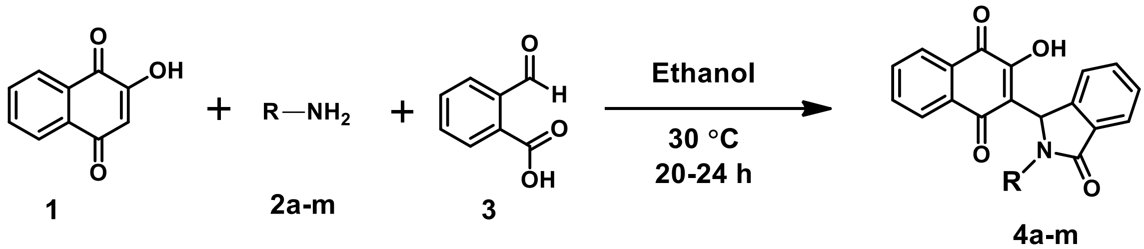
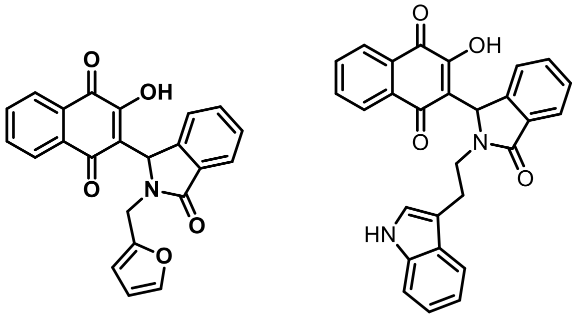


Figure 6.1 Schematic for synthesis of substituted isoindolinones

The aim of present work is to develop an environmentally benign and efficient Mannich type multicomponent reaction approach for the synthesis of a series of novel substituted isoindolinones derived from Lawsone. This protocol features direct construction of C-N and C-C bonds via metal-free method at room temperature using an environment-friendly solvent **(figure 6.1).** The cytotoxic activity of selected compounds was evaluated using MTT assay against HepG2 cell lines (Human liver cancer cell line). The data revealed that screened compounds displayed excellent to moderate activity which is comparable with standard anticancer drugs **(figure 6.2).**



4i, IC_{50} = 5.29 μM **4j, IC_{50} = 39.35** μM

Figure 6.2 Structures of potent isoindolinones

To summarize, we have developed an efficient method towards the synthesis of biologically active substituted isoindolinone via multi-component Mannich reaction using naturally occurring Lawsone. This approach offers the advantage such as the use of environmentally benign solvent, acetic acid as catalyst, column chromatography free workup procedure. The HOMO-LUMO energy gap (ΔE) and MEP surface diagram were also determined using a theoretical DFT study. The compound **4b** with 4-methoxybenzene substitution showed the lowest (-1.859 eV) while compound **4l** with n-butyl substitution showed the highest (-2.833 eV) HOMO-LUMO energy gap. The electrostatic potential data derived from MEP surface diagram revealed that carbonyl oxygen of isoindolinone and naphthoquinone has a higher electron density and it plays an important role in the biological activity through hydrogen bonding with the target and was supported by description of global reactivity parameters. Furthermore, ADME parameters revealed that compounds has good bioavailability. The IC_{50} value for cytotoxic activity of compound **4i** was found to be 5.29 μM which is comparable with standard drug. Further, in vivo studies corroborate with in-vitro data. The cancer-induced Balb mice demonstrated noteworthy decrement in levels of biomarkers MMP-2 and MMP-9 indicated significant tumor regression when compound **4i** was injected in animals. It was also evident by the histoarchitecture of the liver. Thus isoindolinone derived from lawsone can be effective as an anticancer drug.

References :

1. Jordao, A. K.; Pinto, A. C.; Vargas, M. D.; da Silva, F. C.; Ferreira, V. F.; *RSC. Adv.*, **2015, 5**, 67909-67943
2. Bhasin, D.; Chettier, S. N.; Etter, J. P.; Mok, M.; Li, P. K.; *Bioorg. Med Chem.*, **2013, 21,** 4662-4669
3. Mahal, K.; Ahmad, A,; Schmitt, F.; Lockhauserbaumer, J.; Starz, K.; Pradhan, R.; Padhye, S.; Sarkar, F. H.; Koko, W. S.; Schobert, R.; Ersfeld, K.; biersack, B.; *Eur. J. Med. Chem.* **2017, 126,** 421-431
4. Sreelatha, T.; Kandhasammy, S.; Dinesh, R.; Sruthy, S.; Mukesh, D.; Karunagaran, D.; Balaji, R.; Mathivana, N.; Perumal, P. T.; *Bioorg. Med. Chem.* **2014, 24,** 3647-365
5. Neves, A. P.; da Silva, G. B.; Vargas, M. D.; Pinheiro, C. B.; Visentin, L. C.; Filho, D. B. M.; Araujo, A. J.; Costa-Lotufo, L. V.; Pessao, C.; de Moraes, M. U.; *Dalton trans.*, **2010, 39,** 10203-10216
6. Baiju, T. M.; Almeidu, R. G.; Sivananda, S.T.; de Simone. C. A.; Brito. L. M.; Cavalcanti, B. C.; Pessaro, C.; Namboothiri, I. N. N.; da Silva, E. N.; *Eur. J. Med. Chem.* **2018, 151,** 686-704
7. Royo. S. O.; Torrejon, C.; Caudrado, I.; Molina, R. H.; Hortelano, S.; Braun, A. E.; Heras, B. D.; *Bioorg. Med. Chem.*, **2013, 21,** 2471-2477
8. Oliveira. K. M.; Liany, L. D.; Correa, R. S,; Deflon, V. M.; Cominetti. M. R.; Batista, A. A. *J. Inorg. Biochem.* **2017, 176,** 66-76.
9. Patil. S. R.; Choudhary. A. S.; Sekar. N. *New. J. chem.* **2016, 40,** 6803-6811.
10. Tian. Y.; Liu. Q.; Liu. Y.; Zhao. R.; Li. G.; Xu. F. *Tetrahedron Lett.* **2018, 59,** 1454-1457.
11. Sashidhara. K. V.; Singh. L. R.; Palnati. G. R.; Avula. S. R.; Kant. R. *Synlett.* **2016, 27,** 2384-2390.
12. Chang, Y.P.; Furness. L.; Levason. W.; Reid. G.; Zhang. W.; *J. Fluor. Chem.* **2016, 191,** 149–160.
13. Wellington, K. W. *RSC Adv.* **2015, 5**, 20309–20338.
14. Jelly, R., Lewis, S. W., Lennard, C., Lim, K. F. & Almog, J. *Chem. Commun.* **2008,** 3513–3515
15. Patel, M. *et al.* Coumarin-carbazole based functionalized pyrazolines: Synthesis, characterization, anticancer investigation and molecular docking. *RSC Adv.* **2021, 11**, 27627–27644.

Publications

1. **Nariya, P.;** Kumar, S.; Seshadri, S.; Thakore, S. Novel substituted isoindolinones derived from Lawsone: Synthesis, Characterization, DFT and biological studies, Journal of heterocyclic chemistry, ***Journal of Molecular Structure,* 2022,** 1267, 133601.

2. **Nariya, P.**; Shukla, F.; Vyas, H.; Devkar, R.; Thakore, S. Synthesis, characterization, DNA/BSA binding and cytotoxicity studies of Mononuclear Cu(II) and V(IV) complexes of Mannich bases derived from Lawsone**, *Journal of Molecular Structure,* 2022,** 1248, 131508.

3. **Nariya, P.**; Shukla, F.; Vyas, H.; Devkar, R.; Thakore, S. Synthesis and characterization of Mannich bases of lawsone and their anticancer activity, ***Synthetic Communications.* 2020,** 11, 1724.

4. **Nariya, P.;** Srivastava, R.; Thakore, S. Synthesis, Characterization, DFT calculation and Biological activities of metal complexes derived from tridentate (ONN donor) Mannich base, ***Inorganic Chemistry Communications (under review)***

5. **Nariya, P.;** Prajapati, S.; Thakore, S. Synthesis and characterization of Lawsone-Vanadium (IV) complex for catalytic oxidation of diphenylmethane to benzophenone, ***Inorganica Chimica Ata (under review)***

Conferences and Paper Presentations

1. International Conference on Organic, Medicinal and Pharmaceutical Chemistry 2022, ICOPMP-2022, 24th-27th February 2022, organized by Navrachana University, Vasna-Bhayli, Vadodara, Gujarat **(Oral presentation)**

2. Two Days Virtual International Conference on Emerging Trends in Medicinal Chemistry 2021, ETMC – 2021, 18th -19th March 2021 organized by Department of Chemistry, Sardar Vallabhbhai National Institute Technology (SVNIT), Surat, Gujarat **(Poster presentation)**

3. National seminar on Advances in Chemistry of Bioactive Molecules, ACBAM-2020, 17th-18th January 2020 organized by Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat **(Poster presentation)**

4. National Symposium on Advances in Chemical Research, ACR-2019, 24^{th} February 2019 organized by Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat **(Poster presentation)**

5. National Seminar on Applied Polymer Science and Technology, NSAPST-2019, 24^{th}-25^{th} January 2019 organized by Department of Chemistry, Sardar Patel University, Vallabh Vidhyanagar, Gujarat **(Poster presentation)**

6. Frontiers in Heterogeneous Catalysis HETCAT-2018, 8^{th}-9^{th} December 2018 organized by Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat **(Poster presentation)**