Part (I): Synthesis, Characterization and Antibacterial Activity of Vanadium(V) Complexes with ONS Schiff bases


Samira M. Faylough

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Part (I): Synthesis, Characterization and Antibacterial Activity of Vanadium(V) Complexes with ONS Schiff bases

Part (II): Synthesis of 9-CD3-11-cis-Retinal for the Investigation of the Activation Mechanism of GPCR Rhodopsin by Solid-State $^2$H NMR Spectroscopy

By

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Bachelor of Chemistry, University of Benghazi
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A dissertation submitted to the College of Science at Florida Institute of Technology in partial fulfillment of the requirements for the degree of

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Chemistry

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Part (I): Synthesis, Characterization and Antibacterial Activity of Vanadium(V) Complexes with ONS Schiff bases


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ABSTRACT

Part (I): Synthesis, Characterization and Antibacterial Activity of Vanadium(V) Complexes with ONS Schiff bases

a dissertation by

Samira M. Faylough
Research Advisor: D. A. Knight, Ph.D.

Vanadium compounds are known to play many roles in both biology and industry, and the biochemical activity of these compounds has led to their increased importance in both biological and medical research. In the present work, some new substituents for hydrazinecarbodithioate Schiff base ligands and their dioxo vanadium(V) complexes were synthesized and characterized by $^1$H NMR, $^{13}$C NMR, DART mass, FT-IR, UV-vis, fluorescence spectrometer, and single X-ray diffraction analysis for complex $\text{K}[\text{VO}_2\{\text{(S-benzyl-3-(2-hydroxy-5-nitrophenyl) methylene dithiocarbazate)}\}]$ (212). Dioxovanadium(V) complexes $\text{K}[\text{VO}_2\{\text{(S-methyl-3-(2-hydroxy-5-nitrophenyl)methylenedithiocarbazate)}\}]$ (211) and 212 have been tested for antibacterial activity against *Burkholderia pseudomallei* and *Burkholderia mallei* strains. Ceftazidime was used as a reference in this study. Complex 212 has been shown to have higher antibacterial activity compared to complex 211. In some strains the minimum inhibitory concentration (MIC) values of complex 212 are only 2-fold higher than Ceftazidime and the antibacterial activity of this complex against *B. pseudo* is stronger than it is against *B. mallei*. Based on these results, we synthesized some hydrazine carbodithioate Schiff base ligands which have
derivatives of the benzyl group to test their antibacterial ability against *B. pseudomallei* and *B. mallei* strains. The ligands 203, 204, and 205 show a stronger intensity of fluorescence in comparison with their complexes 213, 214, , and 215, respectively. The fluorescent properties of vanadium complexes allow them to be used as cellular imaging agents in living cells.
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<td>Acac</td>
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<tr>
<td>LMCT</td>
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Finally, thanks to the Libyan government for funding me during these years.
Dedication

To my Family, Friends, and Colleagues

for loving and supporting me!
CHAPTER 1

Vanadium Complexes and their Antibacterial and Antifungal Activity

1.1 Introduction

Vanadium is one of the trace elements which has multiple roles in biology.\textsuperscript{1} Vanadium complexes have been used as antidiabetic, anticarcinogenic and antimicrobial agents\textsuperscript{2}, as well as being used to treat tuberculosis, amoebiasis, HIV, and herpes\textsuperscript{3}. The impact and effectiveness of vanadium compounds as antifungals were known more than 100 years ago.\textsuperscript{4} Because vanadate (VO\textsubscript{4}\textsuperscript{3-}) is analogous in its structural and electronic properties to the phosphate ion, vanadate compounds have been used to inhibit phosphate-metabolizing enzymes such as phosphatases and ribonucleases.\textsuperscript{5} The detection of two types of vanadium enzymes, vanadium nitrogenase and vanadium bromoperoxidase., has brought about more interest in vanadium compounds in the field of biological chemistry.\textsuperscript{6}

Vanadium complexes have shown good inhibition of microbial growth. In general, the ability of metal complexes to inhibit the growth of different types of bacteria and fungi is explained by two different theories, Tweedy’s chelation theory and Overtone’s concept.\textsuperscript{7} The chelation of a metal center with ligands enhances and increases the antimicrobial activity of the compound. The overlapping between the positive charge of the metal ion and the ligand orbitals decreases the polarity and enhances the lipophilicity of the complex. It enhances the ability of the complex to penetrate the lipid membrane and subsequently to block the binding sites of the metal
centers of microorganism enzymes that are required for microbial physiological processes as shown in Figure 1.1. Along with metal centers, the ligands of a metal complex also play an important role in their antimicrobial activity. Schiff bases are the most widely used ligands in the design of metal complexes with a variety of biological applications such as antifungal, antibacterial, anticancer, antitubercular, anticonvulsant, antiHIV, antitumor, antioxidant, antimalarial, corrosion inhibiting, and anti-inflammatory properties. Based on these properties, the development of the Schiff base ligands and their chelating complexes calls for further investigation. The coordination of metal ions with organic compounds brings about significant changes in their nature and biomimetic characteristics, therapeutic capabilities, and pharmacological properties. In this chapter, the biological evaluation of vanadium complexes as antibacterial and antifungal agents is reviewed.

1.2 Antibiotic vanadium complexes

The toxicological and pharmacological properties of organic drugs are sometimes modified during the course of the formation of their metal complexes. Nalidixic acid was the first quinolone antibiotic used in the treatment of urinary tract infection, and it showed a limited biological activity by itself; some structural modifications improved its antibiotic as well as pharmacokinetic properties. Refat et al. found that the nalidixic acid VO(II) complex showed high antimicrobial activities compared with free nalidixic acid against two types of bacteria, Escherichia coli and Staphylococcus albus, and two types of fungi, Aspergillus flavus and Asper-
In addition, ofloxacin (Ofl), a fluoroquinolone chemotherapeutic antibiotic, and its metal complexes have been used as antacids. Oxovanadium(IV) [VO(Ofl)\(_2\)(H\(_2\)O)]·5H\(_2\)O complex 2 (Figure 1.2) has been synthesized and evaluated for its antibacterial activities against Gram-positive bacteria species (Staphylococcus aureus K1, Bacillus subtilis K22, and Gram-negative species such as E. coli K32, Pseudomonas aeruginosa SW1, and Klebsiella oxytoca K42. The results showed that the vanadium complex offered higher activity against all types of bacteria compared with ofloxacin and the references (ampicillin and amoxicillin).

Ciprofloxacin (Cip) is a quinolone derivative antibacterial agent, which acts by forming ternary complexes with DNA. [VO(Cip)\(_2\)H\(_2\)O (3-9) mixed ligand complexes where X is 3:X = anthranilic acid, 4: X = glycine, 5: X = β-alanine, 6: X = L-asparagine, 7: X = DL-serine, 8: X = o-aminophenol, and 9: X = DL-valine, have been synthesized and evaluated for their antibacterial activity against E. coli, P. aeruginosa, S. aureus, B. subtilis, and S. marcescens by using the double dilution technique. All the ligands showed no antimicrobial activity, while all the vanadium complexes exhibited high antimicrobial activity. The bulkiness of the ligands, as well as the ease of coordination at the metal center seem to be responsible for the inhibition activity. Complexes 4, 5, 6, and 7 have potent activity against all organisms, while the complexes 8 and 9 exhibited high bactericidal activity towards S. merscences. Turel et al. synthesized a ciprofloxacin oxovanadium(IV) complex 10 (Figure 1.2) and studied its antibacterial activity against S. aureus, S. epidermidis, Streptococcus salivarius, Streptococcus lactis, Streptococcus foecalis, Micrococcus
Figure 1.1  A visualization of Tweedy’s chelation theory.
luteus, Bacillus cereus, Bacillus subtilis, Aeromonas, Salmonella enteritidis, E. coli, Enterobacter aerogenes, Proteus vulgaris, Klebsiella pneumoniae, P. aeruginosa, Pseudomonas fluorescens, and Shigella sonnei. Ciprofloxacin was used as a reference control. The ciprofloxacin oxovanadium(IV) complex exhibited the same antibacterial activity as the antibiotic ciprofloxacin. Sadeek et al. have tested the antibacterial activity of the moxifloxacin oxovanadium complex [VO(MOX)2H2O]·SO4.11H2O (11) (Figure 1.3) against S. aureus, B. subtilis, Brevibacterium otitidis, E. coli, P. aeruginosa and K. oxytoca, and they found that the antibacterial activity of the oxovanadium complex against Gram-positive bacteria was higher than its inhibition degree for Gram-negative bacteria. Moreover, the antibacterial activity of the complex was superior for all tested organisms compared to that of the free antibiotic ligand (moxifloxacin).

Gajera et al. have synthesized oxovanadium(IV) derivative fluoroquinolone complexes with 3-(diphenylphosphino)propionic acid 12-18 (Figure 1.3) and evaluated their antibacterial ability against E. coli (MTCC 433), P. aeruginosa (MTCC P-09), S. marcescens (MTCC 7103), B. subtilis (MTCC 7193), and S. aureus (MTCC 3160). All oxovanadium(IV) complexes exhibited higher antibacterial activity against both Gram-positive and Gram-negative bacteria than free fluoroquinolones.

The fluoroquinolones have shown significant antibacterial activity against Gram-negative bacteria such as E. coli, but their antibacterial activity against Gram-positive
Figure 1.2  Structures of complexes 1, 2, and 10.
bacteria such as *S. aureus* was low. This activity depends on both bicyclic heteroaromatic pharmacophores and their substituents. VO(pr-norf)$_2$(H$_2$O) (19) (Figure 1.4), where pr-norf is *N*-propyl-norfloxacin, has shown identical antibacterial activity to that of the free ligand against *E. coli*, *P. Aeruginosa*, and *S. aureus* while UO$_2$(pr-norf)$_2$ has shown higher antibacterial activity against *S. aureus* than the free ligand. Sulfasalazine (H3Suz) is a derivative of mesalazine, a drug which is used to treat inflammatory bowel disease. An oxovanadium sulfasalazine (H3Suz) complex [VO(SuzH)(H$_2$O)$_2$]·6H$_2$O (20) (Figure 1.4) was synthesized and tested for its antibacterial activities against *E. coli* and *B. subtilis* as well as its antifungal activities against *tricoderma* and *penicillium*. Oxovanadium sulfasalazine exhibited no active antibacterial or antifungal properties against any of the bacteria and fungi which were included in this study.11

The biocidal activities of the complexes VO(H$_2$O)$_3$Y$^{2-}$ (21), VO(H$_2$O)Y$_2$$^{2-}$ (22), VY$_3$ (23) (Figure 1.5), where Y is a cephalosporin antibiotic (cephradine), towards different kinds of bacteria including *E. coli*, *B. subtilis*, *Shigella flexneri*, *S. aureus*, *P. aeruginosa*, and *salmonella typhimurium* and fungal species including *Trichophyton longifusus*, *Candida albicans*, *A. flavus*, *Microsporum canis*, *Fusarium solani*, and *Candida glaberata* were investigated. All the compounds exhibited great activity against *E. coli* and *B. subtilis* while their activity against *S. aureus* species was moderate. On the other hand, all complexes exhibited high antifungal activity toward *T. longifusus*, *C. albicans*, *F. solani* and *C. glaberata*, and moderate activity against *M. canis*.
Figure 1.3  Structures of complexes 11-18.
Figure 1.4 Structures of complexes 19 and 20.
The cefuroxime oxovanadium(IV) complex, \([\text{VO}(\text{C}_{15}\text{H}_{15}\text{N}_{4}\text{O}_{8}\text{S})]\cdot\text{HSO}_{4}\) (24) (Figure 1.5) was synthesized, and its antibacterial activity against \(K.\ pneumoniae, B.\ subtilis, P.\ aeruginosa, E.\ coli\) and \(S.\ aureus\) was investigated. The complex showed a higher MIC value compared to cefuroxime against all of the bacterial species used in this study.\(^{21}\)

Enrofloxacin vanadium(V) complexes with different organic ligands such as aniline, pyridine, \(o\)-tolidine and triethylamine and dimethylformamide \(^{25-29}\) (Figure 1.5) were synthesized and tested for their antibacterial activity against \(E.\ coli, P.\ aeruginosa, K.\ pneumonia, S.\ aureus, S.\ epidermidis,\) and \(B.\ pumilus\). All complexes showed higher antibacterial activity compared with enrofloxacin.\(^{22}\)

1.3 Macrocyclic vanadium complexes

Macrocyclic metal complexes have been found to have antibacterial, antifungal, and anticarcinogenic properties, and they have numerous applications in the areas of coordination, bioinorganics, and catalysis.\(^{23, 24}\) Pawar \textit{et al.} screened the oxovanadium(V) complexes 30 and 31 (Figure 1.6) and their macrocyclic Schiff base ligands (HDAA) and (EDAA), respectively, at four different concentrations for their antibacterial activity against \(E.\ coli(\cdot), S.\ aureus(\cdot), M.\ luteus(\cdot),\) and \(B.\ licheniformis(\cdot)\). The antibiotic standard used in this study was ofloxacin. The vanadium complexes and their ligands showed potent antibacterial activities in all types of tested bacterial species. The IC\(_{50}\) values of the complexes 30 and 31 were in the range of 0.20 mg/mL to 0.35 mg/mL.\(^{23}\) Also, oxovanadium macrocyclic Schiff
Figure 1.5  Structures of complexes 21 and 29.

Complex | Ligand
--- | ---
25 | Aniline
26 | Pyridine
27 | Tolidine
28 | Triethylamine
29 | Dimethylformamide
base TSCB and HDCB complexes 32 and 33, respectively (Figure 1.6), were synthesized and evaluated for their antibacterial activities against *S. aureus, B. licheniformis, M. luteus,* and *E. coli.* Ofloxacin was used as the standard control. The vanadium complexes of TSCB and HDCB (32, 33) exhibited higher bactericidal effects compared with their corresponding ligands.\(^{25}\) Pawar *et al.* synthesized oxovanadium complexes (34, 35) (Figure 1.6) with macrocyclic Schiff base ligands TSCA and SCHA, respectively, and tested their antibacterial ability against strains of bacteria including *S. aureus, B. licheniformis, E. coli,* and *M. luteus* (ATCC). The bioactivity of these complexes was compared with that of the standard antibiotic ofloxacin.\(^{26}\) All the tested compounds showed strong to moderate antimicrobial activity against all the types of bacteria tested. Oxovanadium complexes have exhibited higher antibacterial activities than their corresponding ligands.\(^{26}\) Leelavathy *et al.* have synthesized unsymmetrical macrocyclic binuclear oxidovanadium(IV) complexes 36-40 (Figure 1.7) and investigated their antifungal activity against fungi such as *Aspergillus ochraceous, Paecilomyces variotii* and *Botrytis cinerea* and antibacterial activity against *Serratia sp., M. luteus* and *P. vulgaris.* Complexes 39 and 41 showed higher antifungal activity than complexes (36-38). Also, they noticed that the *P. vulgaris* exhibited more resistance to all complexes than *Serratia sp.*\(^{27}\) In addition, the tetraaza macrocyclic vanadium complex [VOZ]-SO\(_4\), (41) (Figure 1.7) where Z is afforded by condensation of 4,4'-diaminodiphenylmethane, formaldehyde and *p*-anisidine, has been tested for antifungal activity against *A. niger, A. fluvus, Trichoderma harizanum, Trichoderma*
Figure 1.6 Structures of complexes 30 and 35.
viridae, and Rhizoctonia solani. Complex 41 showed potent antifungal activity against most of the fungal strains. Raman et al. have tested vanadium complex 42 (Figure 1.7) for antibacterial activity against the bacteria S. typhi, S. aureus, K. pneumoniae, B. Subtilis, S. flexneri, and P. aeruginosa. Vanadium complex 42 exhibited higher antimicrobial activity than the corresponding a tetraaza macrocyclic Schiff base ligand which was synthesized from 1,2-(diimino-4΄-antipyrinyl)-1,2-diphenylethane and o-phenylenediamine.

1.4 Five membered heterocyclic Schiff base vanadium complexes

Five membered heterocycles including nitrogen and sulphur atoms such as triazole, 1, 3, 4-oxadiazole, and thiadiazole derivatives have exhibited chemotherapeutic and pharmacotherapeutic properties. Square-pyramidal oxovanadium(IV) complexes 43-47 (Figure 1.8) of bidentate triazole Schiff bases have been synthesized and studied for their antibacterial and antifungal activities against E. coli, Shigella flexneri, P. aeruginosa, S. typhi, S. aureus, B. subtilis, T. longifusus, C. albicans, A. flavus, M. canis, F. solani, and C. glabrata. Imipenem was used as the standard drug for antibacterial activity, and miconazole and amphotericin B were used as antifungal activity standards. Oxovanadium(IV) complexes showed moderate antibacterial activity against bacteria strains while all compounds showed potent antifungal activity against most of the fungal strains. Also, vanadium(IV) triazole Schiff bases complexes 48-51 (Figure 1.8) have been synthesized and evaluated for their antibacterial activity against Escherichia coli, Shigella flexenari,
Figure 1.7  Structures of complexes 36-42.
*Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis* bacterial strains and studied for their antifungal activity against *Trichophyton longifuscus*, *Candida albican*, *Aspergillus flavus*, *Microscopum canis*, *Fusarium solani* and *Candida glaberata.\(^{31}\) Imipenem, miconazole, and amphotericin B were used as the standard drugs. Due to the presence of a larger number of nitrogen atoms in the oxovanadium(IV) Schiff base complexes 50 and 51, they showed the most active antibacterial activity compared with oxovanadium complexes 48 and 49 and all free Schiff base ligands. In addition, all ligands and complexes exhibited moderate antifungal activity.\(^{31}\)

The antibacterial and antifungal activity of triazole oxovanadium complexes have been discussed and reported by Chohan et al.\(^{32}\) In this study, oxovanadium(IV) triazole-derived Schiff base ligands 52-56 (Figure 1.9) have been synthesized and exhibited significantly enhanced biological activity against *E. coli*, *S. flexneri*, *P. aeruginosa*, *Salmonella typhi*, *S. aureus* and *B. subtilis*, *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani*, and *C. glabrata*.\(^{32}\) In another study, oxovanadium(IV) thienyl derived triazole Schiff base complexes 57-61 (Figure 1.9) have been synthesized and studied for their antibacterial activity against *E. coli*, *S. flexneri*, *P. aeruginosa*, *S. enterica*, *Serover typhi*, *S. aureus*, and *B. subtilis* and antifungal activity against *T. longifuscus*, *C. albican*, *A. flavus*, *M. canis*, *F. solani*, and *C. glabrata*. Imipenem, miconazole, and amphotericin B were used as controls in this study. The antibacterial activity of complexes 60 and 61 is higher than that of complexes (57-59) due to the chloro and nitro substituents, which form the hydrogen
bonding with the bacterial proteins and thus increase the bioactivity of these complexes. Also, all the oxovanadium(IV) complexes exhibited different antifungal abilities depending on ligands and types of fungi.33

Vanadyl(IV) triazole complexes 62-65 (Figure 1.9) have been tested for their antibacterial and antifungal activities against some types of bacteria such as E. coli, S. flexneri, P. aeruginosa, S. typhi, S. aureus, and B. subtilis and fungal strains such as T. longifusus, C. albicans, A. flavus, M. canis, f. solani, and C. glabrata. All complexes showed higher antibacterial and antifungal activities compared with free corresponding ligands and were less active than the reference drugs (imipenum, miconazole, and amphotericin B).34

Oxovanadium(IV) triazole Schiff base complexes 66-71 (Figure 1.10) have been synthesized and tested for their antibacterial and antifungal activities against E. coli, S. flexneri, P. aeruginosa, S. typhi, S. aureus, and B. subtilis bacterial strains and fungi such as T. longifusus, C. albican, A. flavus, M. canis, F. solani, and C. glabrata. All oxovanadium(IV) complexes showed significant biological activity against all organisms compared with the corresponding ligands.35 Sumrna et al. have tested the antibacterial and antifungal activities of six oxovanadium(IV) triazole complexes 72-76 (Figure 1.10) against bacteria strains, for example, E. coli, S. flexneri, P. aeruginosa, S. typhi, S. aureus, and B. subtilis and fungal species such as T. longifusus, C. albicans, A. flavus, M. canis, F. solani, and C. glabrata. All compounds showed antibacterial and antifungal activities with different degrees of inhibition.36
Figure 1.8  Structures of complexes 43-51.
Figure 1.9  Structures of complexes 52-65.
Oxovanadium(IV) triazole complexes 77-81 (Figure 1.10) have been synthesized and estimated for their antibacterial and antifungal activities against *E. coli*, *S. flexenari*, *P. aeruginosa*, *S. typhi*, *S. aureus*, and *B. subtilis* bacterial strains and fungal strains such as *T. longifucus*, *C. albican*, *A. flavus*, *M. canis*, *F. solani*, and *C. glaberata*. All complexes displayed differing levels of inhibitory effect against different types of bacteria and fungus, and complexes 80 and 81, which have chloro and nitro substituents, showed the greatest activity. Oxovanadium(IV) complexes 82-86 (Figure 1.11) of 4-amino-5-(substituted-phenoxyacetic acid)-1,2,4-triazole-3-thiol with benzil have been synthesized and screened for their antibacterial activity against *E. coli*, *S. typhi*, *S. aureus* and *B. subtilis*, and in vitro antifungal activity against *A. niger*, *Colletotrichum falcatum*, and *Colletotrichum pallescensce*. In this paper, it was reported that triazole vanadium complexes damage the fungal cell wall and stop the synthesis of proteins that are important to fungal growth. In addition, complex 82, which has a 2-Cl-substituent, exhibited higher activity than other complexes against all fungi but less than the standard drug (fluconazole).

The different antifungal results are caused by the difference in the permeability of the cells and the activity of the ribosomes. In addition, vanadium complexes 82 and 83 showed considerable antibacterial activities compared with complexes 84, 85, and 86 and their relevant ligands against Gram-positive bacteria while all compounds exhibited no activity against Gram-negative bacteria due to the structure of the Gram-negative bacterial cell wall. Shashidhara *et al* have tested oxovanadium(IV) complexes 87-90 (Figure 1.11) as antibacterial and antifungal agents against *E. coli*,
Figure 1.10  Structures of complexes 66-81.
B. cirrolagellosus, A. riiger, and C. albicans. The results were compared with cotrimoxazole and fluconazole, which are used as standards in this study.\textsuperscript{9} The antibacterial and antifungal activities of all ligands showed them to be weakly active against all the organisms compared with their vanadium(IV) complexes. Moreover, vanadium(IV) complex 87 exhibited high activity against a bacterium, R. cirrolagellosus, but it showed itself to be weakly active against fungi.\textsuperscript{9}

Six oxovanadium complexes containing 2, 5-substituted 1, 3, 4-oxa/thiadiazole derivatives as ligands 91-96 (Figure 1.12) were synthesized and tested for their ability to inhibit growth of some bacterial species include S. aureus, E. coli, P. mirabilis, and P. aeruginosa. It was seen that the oxovanadium(IV) complex of PIOA showed high antibacterial activity due to its ionic nature, but the highest activity was shown by PITA and its vanadium complex against P. aeruginosa. ATYP, PITA, and their vanadium complexes showed the best MIC values against P. mirabilis.\textsuperscript{39}

Heterocyclic thiadiazole oxovanadium(IV) complexes 97-100 (Figure 1.12) have been synthesized and tested for antifungal activity against fungi A. niger, C. falcatum, and C. pallescence and antibacterial activity against E coli, Salmonella typhi, S.aureus, and B. subtilis bacterial strains. Complex 98 showed the highest antifungal activity among all compounds. Also, all complexes exhibited higher antimicrobial activities against Gram-positive bacteria than free ligands, but the structure of the Gram-negative bacterial cell wall resisted the activity of all
Figure 1.11  Structures of compounds 82-90.
compounds. Schiff bases of 4-aminoantipyrine and its complexes exhibited high levels of biological activity as antitumor agents, fungicides, bactericides, anti-inflammatoryities, and antivirals. Schiff base V(IV) complexes 101-103 (Figure 1.13) have been synthesized and tested for their antibacterial activity against S. aureus (Oxford 6538), E. coli (ATCC 10536), and C. albicans (ATCC 10231) bacterial strains. All Schiff base ligands showed some inhibition ability to the growth of bacterial strains; however, this antibacterial activity increases with the formation of vanadium complexes. In addition, the anionic sulfate group on the vanadium complex coordination sites causes a rise in the antimicrobial activity of these complexes, but they show poor activity compared to tetracycline, which was used as the control drug in this study.

Isatin (1H-indole-2,3-dione) and its derivatives have shown a lot of biological activity. Isatinic quinolyl hydrazone VO(II) complex 104 (Figure 1.13) has been synthesized and tested for its antibacterial activity against S. aureus, S. pyogenes, P. Phaseolicola, and P. fluorescens. The isatinic quinolyl hydrazone VO(II) complex exhibited high antibacterial activity against the studied organisms relative to the references (chloramphenicol and cephalothin). Pragathi et al. tested the octahedral oxovanadium complex [VO(BNCM)SO_4(H_2O)]^{2+} where BNCM is 1H-benzimidazole-2-methanamine, N-[4-chlorophenyl)methylene], as an antibacterial agent against B. subtilis, S. aureus, E. coli, and P. aeruginosa. Streptomycin was used as the standard in the assay. The results show that the oxovanadium complex exhibited high antibacterial activity against all bacterial species as compared with BNCM. The
Figure 1.12 Structures of compounds 91-100.
Figure 1.13  Structures of compounds 101-104.
The lipophilic nature of the vanadium ion in the complex and the formation of a hydrogen bond through the azomethine nitrogen with the active centers of the cell constituents might lead to the resulting interferences with the normal cell process, resulting in the bacterial death. Furthermore, the nuclease activity of the vanadium complex studied showed that the interaction of \([\text{VO(BNCM)}\text{SO}_4(\text{H}_2\text{O})]^2+\) with pUC19 DNA cleaved the DNA in the presence of hydrogen peroxide.\(^{43}\) In addition, oxido-vanadium(IV) complexes \([\text{VO(A)}(\text{H}_2\text{O})], [\text{VO(B)}(\text{H}_2\text{O})] \) and \([\text{VO(C)}(\text{H}_2\text{O})]\), where A is 2,6-diaminopyridine, named 1,1′-(pyridine-2,6-diyl)bis(3-(benzoyl)thiourea), B is 1,1′-(pyridine-2,6-diyl)bis(3-(2-chlorobenzoyl) thiourea) and C is 1,1′-(pyridine-2,6-diyl)bis(3-(4-chlorophenyl)thiourea), and the dioxo-vanadium(V) complex \([\text{H}_2\text{Met}]\text{[VO}_2(\text{dipic-OH})]\text{2H}_2\text{O}\), where dipic is chelidamic acid, H\(_2\)dipic-OH and H\(_2\)Met is metformin, have been synthesized and evaluated for their antibacterial activities in vitro against \(E. \text{coli}, P. \text{aeruginosa, S. aureus, and E. faecalis}\). Compared to standard drugs, vanadium complexes showed good antibacterial activities against all of the bacterial strains, and had higher activity than the free ligands.\(^{44}\)

The Schiff base ligands of Oxovanadium(IV) complexes 105-108 (Figure 1.14) exhibited moderate antibacterial activity against \(E. \text{coli, P. aeruginosa and S. aureus}\) and high antifungal activity against \(A. \text{flavus and A. niger}\). However, they showed lesser activity against \(S. \text{pyogenes and Cladosporium}\).\(^{45}\) The triazole Schiff base oxovanadium(IV) complexes 110-114 (Figure 1.14) exhibited high antibacterial activity against Gram-negative bacteria strains such as \(E. \text{coli, S. flexenari, P. aeruginosa, and S. typhi}\) and two Gram-positive bacteria, \(S. \text{aureus and B. subtilis}\).
Figure 1.14 Structures of compounds 105-114.

<table>
<thead>
<tr>
<th>Complex</th>
<th>R</th>
<th>R'</th>
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<tbody>
<tr>
<td>105</td>
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</tr>
<tr>
<td>106</td>
<td>CH₃</td>
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</tr>
<tr>
<td>107</td>
<td>C₂H₅</td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>C₃H₇</td>
<td></td>
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<tr>
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<td>Cl</td>
</tr>
<tr>
<td>114</td>
<td>H</td>
<td>NO₂</td>
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Also, they showed high antifungal activity against *T. longifucus, C. albicans, A. flavus, Microscopum canis, Fusarium solani*, and *Candida glaberata*. Complexes 113 and 114 showed the highest antibacterial and antifungal activity due to the existence of chloro and nitro substituents, and complex 114 showed the most activity with an MIC value of 0.02 µg mL\(^{-1}\) against *E. coli*.\(^{37}\)

1.5. Thiosemicarbazones and dithiocarbazate vanadium complexes

Oxovanadium(IV) complexes \([\text{VOD}_2]\) and \([\text{VO(D)}_2]\text{SO}_4\) (115-124) Figure 1.15, where DH=thiosemicarbazones, have been screened for their antibacterial and antifungal activities. Both the thiosemicarbazone ligands and their oxovanadium(IV) complexes showed less antifungal activity against *Helminthosporium oxyzae* than *A. niger*. Furthermore, the antibacterial activity was evaluated for ligands and complexes against the bacteria *E. coli, P. pyocyancus, and S. citrus*. Ligand TCTH and its oxovanadium(IV) complexes showed the highest antibacterial activity against *E. citrus*.\(^{46}\) In another study, vanadium complexes 125-127 (Figure 1.15) of thiosemicarbazone, semicarbazone, and thiocarbohydrazone ligands were synthesized and evaluated for their antibacterial and antifungal activities against *Rhizobium bacteria* and *Fusarium oxysporium* fungus. Complex 125 showed a higher antibacterial activity than its free ligand while ligands of complexes 126 and 127 showed a higher effect as an antibacterial than their metal complexes. Moreover, all metal complexes showed lower antifungal effect than their free ligands.\(^{47}\) It has been noted that the nature of the metal ion has a significant impact on the
antimicrobial ability of different metal complexes with the same ligands against *S. aureus* bacteria with the following order: nickel(II) > vanadyl(II) > cobalt (II) > copper(II) ≈ palladium(II) >> iron(III).\(^{47}\) Pahontu *et al.* reported that the thiosemicarbazone dioxo vanadium complex, \([\text{VO}_2(\text{M})\cdot 2\text{H}_2\text{O}],\) where \(\text{HM}\) is 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone 4-ethyl-thiosemicarbazone \(^{128}\) (Figure 1.15), showed higher antibacterial activity against *E. coli*, *S. abony*, *S. aureus*, and *B. cereus* and higher antifungal activity against *C. albicans strains* than the free ligand.\(^{48}\) Aswar *et al.* synthesized manganese(II), iron(II) cobalt(II), nickel(II), copper(II), zinc(II), cadmium(II) and oxovanadium(IV) complexes with a Schiff base (methyl-3-(2-hydroxy-5-chlorophenyl)methylene thiocarbazate), and the antibacterial activities of the complexes and the free ligand have been tested against *E. coli*, *S. aureus*, *P. Mirabilis*, and *S. typhi*. Both the ligand and complexes exhibited antibacterial activity towards all bacterial strains. The oxovanadium(II) complex \(^{129}\) (Figure 1.15) and Co(II) complexes offered very good activity against all bacterial strains while the Mn(II), Fe(II), and Ni(II) complexes exhibited moderate activity against all bacteria strains except *E. coli*.\(^{49}\)

The \([\text{V(C}_1\text{H}_{20}\text{O}_4\text{N}_2\text{S}_2])\cdot \text{Cl}]\) complex with the ligand *S*-benzyl-\(\beta\)-N-(2-hydroxy-5-bromophenyl) methylenedithiocarbazate was synthesized and evaluated for antimicrobial activities against six different bacterial species: *S. aureus*, *B. subtilis*, *K. pneumoniae*, *Salmonella typhi*, *P. aeruginosa*, and *S. flexneri*. This work showed that the vanadium(III) complex possesses higher antimicrobial activity than that of
the free ligand of the complex and the control. The superior activity of these metal chelates is in accordance with Overtone’s concept and the chelation theory.\(^{50}\)

Oxovanadium(IV) \([\text{VOE}(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}\), where HE is hydrazinecarbodithioic acid, 2-[1-(3,6-dihydro-4-hydroxy-2,6-dioxo-2H-1,3-thiazin-5-yl)ethylidene]methyl ester were synthesized and tested for antibacterial and antifungal activity against \(S. \ aureus, S. \ pyogenes, P. \ phaseolicola, \) and \(P. \ fluorescens\), and fungus strains such as \(F. \ oxysporum\) and \(A. \ fumigatus\). The oxovanadium complex exhibited moderate activity against all bacteria strains and low activity against the fungi.\(^{51}\)

Cis-dioxovanadium(V) complexes \([\text{VO}_2(\text{aptsc})], [\text{VO}_2(\text{apmtsc})]\) and \([\text{VO}_2(\text{apptsc})]\) (130-132) (Figure 1.15), where Haptsc=2-acetylpyridinethiosemicarbazone; Hapmtsc = 2-acetylpyridine-N(4)-methyl-thiosemicarbazone and Happtsc = 2-acetylpyridine-N(4)-phenyl-thiosemicarbazone, respectively, have been synthesized and estimated against the strains of the \(M. \ tuberculosis \ H_37Rv \ ATCC \ 27294\) for their anti-mycobacterium tuberculosis activities. All complexes showed higher anti-mycobacterium tuberculosis activity compared to the free thiosemicarbazone ligands.\(^{52}\) El-Asmy et al. found that \([\text{VO}_2(\text{H}_3\text{BTS})(\text{SO}_4)] \cdot 2\text{H}_2\text{O}\), where \(\text{H}_3\text{BTS}\) is 1-(3,4-dihydroxybenzylidene)thiosemicarbazide, showed almost the same values of inhibition to Gram-positive and Gram-negative bacteria and demonstrated that direct contact between the vanadium complex and DNA is necessary to degrade the DNA.\(^{53}\)

1.6. Mixed vanadium-metal complexes

Mixed metal complexes \([\text{(T-4H)}(\text{CHC}_6\text{H}_4\text{OH})_2\text{VOMoX}_4] \cdot \text{mH}_2\text{O}\), where HT is
Figure 1.15 Structures of compounds 115-132.
diethylene triamine (dien) or triethylenetetraamine (trien), X=O or S, and m=6 or 10, have been synthesized. The antibacterial activity of [(trien-4H) (CHC₆H₄OH)₂ VO(MoS₄)]·10H₂O has been tested against S. aureus and Acinetobacter. The complex was found to have higher antibacterial activity against Acinetobacter than against S. aureus.⁵⁴ Narayanan et al. have synthesized a hetero binuclear cryptate, [GdVOL(DMF)] (133) (Figure 1.16) where DMF is a ligand which is synthesized by the condensation of tris-(2-aminoethyl)amine and 2,6-diformyl-4-methylphenol. This complex was tested against bacteria S. aureus, B. cereus, E. coli, and Vibrio cholera, and a fungal culture A. niger for its antimicrobial activity. Complex [GdVOL] exhibited a moderate inhibition toward all of the bacteria but not against the A. niger fungal pathogen.⁵⁵

1.7. Vanadium oxygen donor ligand complexes Vanadium(III)

complexes V(LCOO), where L represents oxygen donor ligands 134-141 (Figure 1.17), have been synthesized. The antibacterial and antifungal activity of complexes which have ligands 135, 137, 138, 140 and 141 were evaluated against E.coli, S. aureus, B. subtilis, P. aeruginosa, S. flexneri, and S. typhi and fungi such as T. longifusus, M. canis, C. albicans, F. solani, A. flavus, and C. glaberata by the agar well diffusion method. Complexes which have ligands 135, 137, 138, 140 and 141 exhibited strong activity against E. coli and B. subtilis and moderate activity against S. aureus species, while antifungal activity of the complexes which have ligands 135, 138, 140 and 141 showed strong activity against C. glaberata and moderate activity against T. longifusus, M. canis, C. albicans, and F. solani.⁵⁶
Figure 1.16 Structure of compound 133.
Muhammad et al. have synthesized oxovanadium(IV) carboxylates VO(H₂O)(G)₂, where G is a non-steroidal anti-inflammatory drug, 142-145 (Figure 1.17) and evaluated their antibacterial activity against E. coli, B. subtilis, S. aureus, P. aeruginosa, and S. typhi by utilizing the agar well-diffusion methods; imipenum was used as a reference. Moreover, all the oxovanadium(IV) carboxylates were tested against fungi such as T.longifusus, C. albicans, A. flavus, M. canis, F. solani, and C. glaberata, using the tube diffusion test, with miconazole and amphotericin B used as references. The results showed that most of the oxovanadium(IV) complexes exhibited biological activity against different types of bacteria and fungi, and their activity depended on the nature of the ligand. 57 Schiff base vanadium complexes (146-151) (Figure 1.18) were synthesized and tested for their antibacterial activity against B. subtilis, S. aureus E. coli, S. flexenari, P. aeruginosa, and Salmonella typh, and antifungal activity against T. longifusus, C. albicans, A. flavus, M. canis, and F. solani. All vanadium complexes exhibited higher antibacterial and antifungal activity than the corresponding ligand but lower activity than the standard drug controls (imipinem, miconazole and amphotericin.B). 58 Additionally, vanadium complexes VCl₂ₙ (bazc)₂(HJ¹⁻²)ₙ (152-155) (Figure 1.18), where HJ¹= C₆H₅O CH₂C(O)NHO⁻, HJ²= C₆H₅CH=CHC (O) NHO⁻, and n = 1 and 2 have been synthesized and tested for their antibacterial activity against E. coli, S. aureus, P. mirabilis, P. aeruginosa, S. epidermidis, and S. flexneri. All complexes have been shown to be more active than free ligands. Furthermore, complexes 153 and 155 have
Figure 1.17  Structures of compounds 134-145.
Figure 1.18  Structures of complexes 146-155.
exhibited potent activity against \textit{S. epidermidis} and \textit{S. flexneri} compared to the commercial antibiotic streptomycin, which was used as a standard in this study, and more efficient against \textit{P. mirabilis} compared to complexes 152 and 154.\textsuperscript{59}

Ashraf et al. found that octahedral vanadium complexes which have various nonsteroidal anti-inflammatory drugs as ligands (Figure 1.19) have shown high antibacterial activity against \textit{E. coli}, \textit{B. subtilis}, \textit{S. flexenari}, \textit{S. aureus}, \textit{P. aeruginosa} and \textit{S. typhicompare} compared to a standard drug (imipenem) and high antifungal activity against \textit{T. longifusus}, \textit{C. albicans}, \textit{F. solani} compared with a commercially available drug (miconazole). On the other hand, no vanadium complexes showed antifungal activity against \textit{A. flavus}.\textsuperscript{2} Peroxovanadate polymeric complexes \textit{Na}_3[V_2O_2(O_2)₄(carboxylate)]⁻PA, \textit{Na}_2[VO(O_2)₂(carboxylate)]⁻PMA, where PA is poly(acrylate) and PMA is poly(methacrylate), have been synthesized and tested for their antibacterial activity against \textit{S. aureus} and \textit{E. coli}. The pV (dimeric structures in PAV) showed the highest antibacterial activity against all organisms in this study compared to PMAV and PAV. Figure 1.20 shows the scanning electron micrographs of sodium poly(acrylate), sodium poly(methacrylate), PAV and PMAV and their complexes.\textsuperscript{60} Vanadium(IV) complexes of hydroxamic acids [\textit{VCl}_2.n(acac)₂(HT⁻²₃)ₙ], where \textit{HT}₁ is phenoxyacetohydrox- amate ion, \textit{HT}² is cinnamylhydroxamate ion, and \textit{n}=1 and 2, exhibited high antibacterial activity against \textit{E.coli} and \textit{S. aureus} with an inhibition of bacterial growth at 31.25 μg mL\(^{-1}\) and 62.5 μg mL\(^{-1}\) respectively. While earlier studies showed promising results, comparison is not feasible because of differences in assayed strains and methods;
Figure 1.19  Structures of anti-inflammatory drugs and their vanadium complexes.
Figure 1.20  Scanning electron micrographs of (a) sodium poly(acrylate), (b) sodium poly(methacrylate), (c) PAV and (d) PMAV.
however, bioactivity of these complexes is seen as likely. Cevik et al. reported that 
\([\text{V}_2\text{O}_2(\text{SO}_4)_2(\text{H}_2\text{O})_6]\) showed similar weak antibacterial and antifungal activity 
compared to that of commercial \(\text{VOSO}_4\cdot\text{xH}_2\text{O}\) compounds against \(\text{B. cereus, N. asleroides,}
and \(\text{C. albicans, and good activity against C. freundii.}\)

1.8. Vanadium-N/O, N/S, and N/N complexes.

Vanadium(III) complex \([\text{V}(\text{NTA})(\text{H}_2\text{O})_2]\cdot\text{H}_2\text{O}\) (156) (Figure 1.21), where NTA 
is 2,2',2"-nitrilotriacetic acid, was synthesized and tested for its antimicrobial 
activities against \(\text{B.subtilis, S. aureus, E. coli, and P. aeruginosa}\) and antifungal 
activity against \(\text{A. niger, A. flavus, S. cerevisiae, and C. albicans.}\) Complex 156 
showed a higher range of inhibition, with diameters from 20.00 to 14.33 mm. The 
bacteria \(\text{A. flavus}\) was found to be more sensitive to the antibiotic activity of complex 
156 whereas \(\text{P. aeruginosa}\) was less sensitive to it. Al-Amiery et al. have estimated 
the antibacterial and antifungal activities of oxovanadium complex 157 (Figure 1.21) 
against \(\text{S. aureus, B. cereus, E. coli, P. aeruginosa, K. pneumoniae, and P. vulgaris}\) 
and fungi such as \(\text{A. niger, and C. albicans.}\) They noted that the oxovanadium 
complex showed higher antibacterial and antifungal activity compared to its free 
ligand at low concentrations. Oxovanadium(IV) complex (158) (Figure 1.21) with 
the Schiff base ligand OVPTH, which was formed from \(p\)-toluichydrazide and 2-
hydroxy-3-methoxy benzaldehyde, was synthesized and its antibacterial activity 
against \(\text{S. typhi, E. faecails, and E. coli}\) were evaluated. The vanadium complex 158 
exhibited higher antibacterial activity than the free ligand OVPTH.
Dhahagani et al. found that the antimicrobial activities of VO(Z\textsubscript{1,2})\textsubscript{2} complexes 159 and 160 (Figure 1.21), where HL\textsuperscript{1} is 2-[(4 morpholinophenyl imino) methyl] 4-chloro-phenol and HZ\textsuperscript{2} is 2-[(4 morpholinophenyl imino) methyl] 4-bromophenol), against the bacteria *C. vialacium*, *S. Flexinari*, *S. aureus*, *P. aeruginosa*, *S. byogenes*, and fungi such as *P. chrysogenum*, *A. niger*, and *C. albicans* were moderate due to the formation of a hydrogen bond between the complexes and the cell constituents.\textsuperscript{66}

Oxovanadium(IV) Schiff base complexes (161, 162) (Figure 1.21) have been synthesized and tested for their antibacterial activity against *E. coli*, *S. aureus*, and *S. fecalis*. Depending on the type of bacteria, the oxovanadium complexes exhibited high antibacterial activity compared with the corresponding Schiff bases; however, with changes in concentration in some cases they showed comparable activity.\textsuperscript{67} Rudbari et al. have tested the antibacterial activity of vanadium complex VQ\textsubscript{2} (163) Figure 1.21, where HQ is 2-(allyliminomethyl)phenol, against *s. aureus* and *E. coli* by using the disk-diffusion method. The vanadium complex showed no activity compared with the ligand, which exhibited a good antibacterial activity.\textsuperscript{68}

Liu et al. have synthesized a vanadium complex [VOI]·H\textsubscript{2}O (164) (Figure 1.22), where HI is \textit{N,N’-bis(3-ethoxy-2-hydroxybenzylidene)ethylenediamine}, and evaluated its antibacterial activity against *B. subtilis*, *E. coli*, and *S. aureus*. The antibacterial activity of vanadium complex was high compared with that of the free ligand but weaker than that of penicillin and kanamycin which were used as reference drugs in this study.\textsuperscript{69} An oxovanadium complex [VOE\textsubscript{2}] (165) (Figure 1.22), where
Figure 1.21 Structures of complexes 156-163.
HE is \( N-(4\)-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one)pyridoxaldimine \) has been evaluated for its antibacterial activity against bacterial strains such as \( E. \) coli, \( S. \) aureus, \( K. \) pneumonia, and \( P. \) aeruginosa. The oxovanadium complex 165 exhibited the same or lower antibacterial activity compared with its free ligand against \( S. \) aureus, \( K. \) pneumoniae and \( E. \) coli and higher antibacterial activity than the free ligand against \( P. \) aeruginosa.\(^{70}\)

Garg et al. have evaluated the antibacterial and antifungal activities of oxovanadium complexes (166-173) (Figure 1.22) at different concentrations against \( E. \) coli and \( P. \) cepacia and fungi such as \( Rhizoctonia \) bataticola and \( A. \) alternata. They noted that all complexes showed good antibacterial and antifungal activities compared to their corresponding ligands.\(^{71}\) Schiff base ligands OHAPPTH , OVPPTH, and their vanadium metal complexes 174, 175 (Figure 1.23), respectively, have been synthesized and tested for their antibacterial activity against two bacteria, \( E. \) coli and \( B. \) subtiles. Streptomycin was used as a reference drug. As would be expected from the base chelation theory, the oxovanadium complexes showed more biological activity than their free ligands. The antibacterial activity of the two vanadium complexes exhibited strong to moderate antibacterial activity compared to the control, streptomycin. Also, complex 174 showed higher activity against \( E. \) coli and \( B. \) subtiles than complex 175.\(^{72}\) Vanadium(III) complex 176 (Figure 1.23) was synthesized and tested for antibacterial activity against several bacteria and fungi at 200 \( \mu \)g mL\(^{-1}\). The vanadium complex 176 was found to be a better antibacterial agent in \( S. \) aureus and \( S. \) typhi as compared to the other tested bacteria. The antibacterial
Figure 1.22  Structures of complexes 164-173.
activities of different metals with the same ONS ligand can be expressed in the order \( \text{Mo} > \text{V} > \text{Ti} \).\(^{73}\)

A square pyramidal vanadium complex \([\text{VO} \{\text{N},\text{N}^-\text{-bis}(4\text{-methyl- benzylidene})\text{benzene-1,2-diamine}\}]_2\text{·}(\text{ClO}_4)_3\) has been synthesized and tested against the bacteria \( \text{B. cereus, E. coli, P. aeuruginosa, and S. aureus} \) and fungi \( \text{A. flavus, A. niger, C. albicans, and Penicillinium} \) by the disc diffusion method; ciprofloxacin and amphotericin-B were used as the standards. The antibacterial and antifungal activity of the vanadium complex were compared with some metal complexes such as \( \text{Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Zr(IV), and Ru(III)} \) with the same Schiff base ligand. The antimicrobial activity of the Schiff base and the vanadium complex exhibited a weak-to-good activity depending on the kind of bacteria and fungi, while the \( \text{Cu(II)} \) complex showed greater activity among the other metal complexes for all of the tested antimicrobial species.\(^{74}\)

1.9. Vanadium ONO/NNO complexes

A vanadium(III) Schiff base complex \([\text{VYCl}] \) \(^{177}\) (Figure 1.24), where \( \text{HY} \) is \( 1,6\text{-bis}(2\text{-}(5\text{-bromo-2-hydroxybenzylideneamino)}\text{-4-chlorophenoxo})\text{hexane}) \), has been synthesized and studied for antibacterial activity against \( \text{P. aeuriginosa, S. aureus, L. pneumophila subsp pneumophilia, B. subtilis, M. luteus, E. hirae, and E. coli} \). The vanadium complex showed weak to moderate activities against \( \text{L. pneumophila subsp pneumophilia, B. subtilis, M. luteus, E. hirae, and E. coli} \), and was inactive for \( \text{P. aeuriginosa} \) and \( \text{S. aureus} \).\(^{10}\) Also, Cornejo et al. reported that the
Vanadium(III) Schiff base complex 178 (Figure 1.24) showed considerable antibacterial activity against *P. aeruginosa, S. aureus, L. pneumophila, B. subtilis, M. luteus, E. hirae*, and *E. coli* when its activity was compared with reference drugs such as amikacin and tetracycline.\textsuperscript{75}

Ebrahimipour *et al.* have synthesized oxido-vanadium(V) complexes including [VO(I)(MeOH)(OMe)], [VO(I)(OEt)], and [VO(I)(OPr)], 179-181 (Figure 1.24), where HI is a tridentate Schiff base ligand *(E)-N’-((2-hydroxynaphthalen-1-yl)methylene)benzohydrazide*. Their antimicrobial activities against *E. coli* (*ATCC 2922*), *S. aureus* (*ATCC 29213*), and *S aureus* (MRSAa, *ATCC 33591*) were studied. The oxido-vanadium(V) complexes exhibited a higher antimicrobial activity than the free ligand against *S. aureus* (*ATCC 33591* and 29213) and *E. coli* (*ATCC 25922*). The complexes [VO(I)(OEt)], [VO(I)(OPr)], and especially [VO(I)(MeOH)(OMe)] showed clear anti-MRSA activity despite the fact that methicillin-resistant *S. aureus* (MRSA) resists other antibiotic drugs.\textsuperscript{76}

Hydrazones possess a wide range of biological activities, such as antibacterial, antifungal, and antitumor. Octahedral vanadium(V) complexes with two hydrazone ligands, [VOD\textsuperscript{a} D]-CH\textsubscript{3}OH, [VOD\textsuperscript{b}D], where D\textsuperscript{a} (182, Figure 1.25) is *(E)-N’-(2-hydroxy-5-methoxybenzylidene)-2-hydroxybenzohydrazide*, D\textsuperscript{b} (183, Figure 1.25) is *(E)-N’-(3,5-dichloro-2-hydroxybenzylidene)-4-methoxybenzohydrazide* and D is benzohydroxamic acid, were synthesized and their antibacterial activities were studied against two Gram (+) bacterial strains (*B. subtilis* and *S. aureus*) and two Gram (−) bacterial strains (*E. coli* and *P. fluorescence*); penicillin G was used as the
Figure 1.23  Structures of complexes 174-176.
In addition, vanadium complexes were tested for their antifungal activities against *C. albicans* and *A. niger*, and ketoconazole was used as a control. Vanadium complexes exhibited medium to strong activity against *B. subtilis*, *S. aureus*, and *E. coli* and inactivity against *P. fluorescence*, while they did not show any activity against fungal strains.77

An oxovanadium complex [VO(H₂J)(SO₄)(H₂O)], where H₂J is 4-hydroxy-5-[N-(2-hydroxyphenyl) ethanimidoyl] 2H-1,3-thiazine-2,6(3H)-dione, showed high antibacterial activity against Gram-negative bacteria *Proteus vulgaris* and no activity against Gram-positive bacteria *S. aureus*. In addition, it exhibited good antifungal activity against *Candida albicans*.78

Oxovanadium (IV) complexes 184-187 (Figure 1.25) have been estimated for their antibacterial and antifungal activities against *B. subtilis*, *S. aureus*, *S. faecalis*, *E. Coli*, *P. aeruginosa*, and *E. aeruginosa* and fungi such as *C. albicans* and *A. niger*. All complexes showed higher antibacterial activity against Gram-positive bacteria than against Gram-negative bacteria while they showed no activity against *A. niger*.79

Derivatives of hydroxamic acids have been shown to be highly antibiotic, antifungal agents and are used as food additives and tumor inhibitors, among other applications. Derivatives of hydroxamic acid vanadium complexes [VCl₂-n(acac)₂ (HW¹⁻²)n], where HW¹ is a phenoxyacetohydroxamate ion and HW² is a cinnamyl-hydroxamate ion and n=1 and 2, have been synthesized and tested for their antibacterial activities against *S. aureus* and *E. coli* by using the minimum inhibitory
Figure 1.24  Structures of complexes 177-181.
concentration method. All the derivatives of hydroxamic acid vanadium complexes showed high antibacterial activity compared with the hydroxamate ligands.\textsuperscript{61}

Oxovanadium(V) complexes, $[\text{VOP}^{1-3}\text{(OEt)(EtOH)}]$ (188–190) Figure 1.25, where $\text{H}_2\text{P}^1$ = salicylhydrazone of diacetyl monoxime; $\text{H}_2\text{P}^2$= 4-methoxy salicylhydrazone of diacetyl monoxime and $\text{H}_2\text{L}^3$= 4-hydroxy salicylhydrazone of diacetyl monoxime, have been synthesized, and the antibacterial activity of the complexes has been inspected against \textit{Escherichia coli}, \textit{Bacillus}, \textit{Proteus} and \textit{Klebsiella}. Complex 188 exhibited a high antibacterial activity compared with complexes 189 and 190.\textsuperscript{80} In addition, Sharma et al found that the minimum inhibitory concentration of the free ligands performed poorly (62.5–250 $\mu$g mL$^{-1}$), which was similar to the results of Dash et al. (MIC $\sim$15.62–500 $\mu$g mL$^{-1}$).\textsuperscript{81}

Liu et al. synthesized and characterized vanadium Schiff base complexes $[\text{VOE}^\text{a}(\text{OMe})]\cdot0.5\text{MeOH}\cdot0.6\text{H}_2\text{O}$ and $[\text{VOE}^\text{b}(\text{OMe})(\text{MeOH})]$ where $\text{E}^\text{a}$ is $[\text{N}^\prime-(2$-hydroxynaphthyl) ethylidene]-4-nitrobenzohydrazide and $\text{E}^\text{b}$ is $\text{N}^\prime(3,5$-dichloro-2-hydroxybenzylidene)-3-nitrobenzohydrazide. The antibacterial activities of aroylhydrazone ligands and their vanadium complexes was evaluated against strains \textit{B. subtilis}, \textit{E. coli}, and \textit{S. aureus}. The vanadium complex $[\text{VOE}^\text{b}(\text{OMe})(\text{MeOH})]$ exhibited medium antibacterial activities againist \textit{B. subtilis} and \textit{S. aureus} with MIC values of 1.56 and 3.13 $\mu$g mL$^{-1}$ compared with HE$^\text{b}$.\textsuperscript{82}

A square pyramidal oxovanadium complex $[\text{VO(U)}]\cdot\text{H}_2\text{O}$ (191) (Figure 1.25), where $\text{U}$ is a tetradeinate Schiff base ligand derived from 2-hydroxy-5-chloroacetophenone and carbohydrazide, has been synthesized. The Schiff base and
Figure 1.25  Structures of compounds 182-194.
its metal complex have been screened for their antibacterial activity against *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus*, and *B. subtilis* by the cup plate method. The results showed that the vanadium complex is more potent in its antibacterial activity than the free ligand. Another Schiff base ligand *N*,*N*-1,2-propylene-bis(3-methyl-salicylideneimine was coordinated with a vanadium(V) ion to afford a complex [VOU(NCS)] by using a microwave-assisted synthesis method. The Schiff base ligand and vanadium complex have been studied for antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. The vanadium complex has been seen to have clearly enhanced antibacterial activity against Gram-positive bacteria but is inactive against Gram-negative bacteria.

Cis diox-ido-vanadium(V) complexes, [NH(CH₂CH₃)₃][VO₂(B¹)] and [NH(CH₂CH₃)₃][VO₂(B²)] 192-193 (Figure 1.25), where HB¹ is 2-amino-3-(E)-(2-hydroxy-benzylidene) amino) maleonitrile and HB² is 2-amino-3-((E)-(2-hydroxy-3-methoxy benzyl idene) amino) maleonitrile, were synthesized and tested for their antibacterial activity against *P. aeruginosa* ATCC27853, *S. aureus* PTCC 1112, *M. luteus* PTCC 1110, *B. cereus* PTCC 1015, *Escherichia. coli* PTCC 1330, *Listeria monocytogenes*, *K. pneumoniae*, *E. faecalis*, and a fungal species *C. albicans* PTCC 5027. Both complexes showed antibacterial activity against all Gram-positive bacteria and against *Candida albicans* while ligands L¹ and L² showed no antibacterial activity.

An octahedral dinuclear oxovanadium(V) complex, [VO₂W]₂ (194) Figure 1.25, where HW is the 2-[(2-ethylaminoethylimino)methyl]-6- methylphenol, has been
synthesized and screened for its antibacterial activity against *S. aureus*, *E. coli*, and *C. albicans*, and tetracycline was used as the control. The complex 194 showed greater antimicrobial activities against *S. aureus*, *E. coli*, and *C. albicans*, when compared to the free Schiff base.

1.10. Conclusion

Much attention has been paid of late to the chemistry of vanadium due to its importance in processes involving both biology and industry. The recent discoveries of biochemical activity of vanadium compounds have led to increased interest from both the biological and the medical areas. We review here the antibacterial and antifungal properties of vanadium complexes with diverse kinds of ligands at three different oxidation states, 2+, 3+, 4+, and 5+. 
CHAPTER 2

Synthesis and Spectroscopic Characterization of Schiff Base Ligands

2.1 Introduction

The condensation of a primary amine with aldehyde or ketone is used to generate the azomethine group of a Schiff base, and the number of the coordinating atoms extant in the Schiff base ligand determines the coordination number of the metal complex.\textsuperscript{86} Metal complexes that have NOS tridentate Schiff base ligands are stable and have shown interesting physico-chemical properties.\textsuperscript{87} Dithiocarbazate is considered an important mixed hard-soft nitrogen-sulfur ligand, and small modifications in its derivative structures have been shown to have different bioactivity applications.\textsuperscript{88, 89} For more than 30 years, the derivatives of dithiocarbazic esters and their complexes have been studied both in regard to their structures and biological activities.\textsuperscript{90} S-alkyl/aryl dithiocarbazates and their metal complexes have shown high antibacterial, antifungal, anticancer, and insecticidal activities. In this study, we investigate the synthesis and characterization of some new hydrazinecarbodithioate substituent Schiff base ligands. The synthesis strategy of these compounds is outlined in Scheme (2.1). All compounds were characterized by \textsuperscript{1}H NMR, \textsuperscript{13}C NMR, DART mass spectroscopy, FT-IR, and UV-vis spectrometry.
Scheme 2.1 Synthesis of Schiff base ligands.

Figure 2.1 Thione and thiol tautomers.
2.2. Synthesis and spectroscopic characterization of dithiocarbazates

In the first step, the carbon disulfide in absolute EtOH was added dropwise to the solution of hydrazine monohydrate and KOH in 70% EtOH. The yield of a yellow oil (potassium dithiocarbazate) produced from this step depends on the time during which the addition of CS\(_2\) takes place; for the best yield, the process should take more than one hour. Then the potassium dithiocarbazate was dissolved in 40% EtOH, and methyl iodide or benzyl bromide derivatives in absolute EtOH were added to achieve derivative dithiocarbazate compounds 195, 196, 198, 199, and 200. The first step requires a low temperature (0° C) and absolute EtOH to dissolve the methyl iodide or benzyl chloride derivatives listed above before adding them to the potassium dithiocarbazate. THF was used instead of absolute EtOH to dissolve the 4-nitrobenzyl bromide to form compounds 197. The reason behind the change in solvent is that 4-nitrobenzyl bromide can dissolve only in hot absolute EtOH and would recrystallize before reacting with the potassium dithiocarbazate at 0° C. \(^1\text{H}\) NMR, \(^{13}\text{C}\) NMR, and DART mass were used to characterize compounds 195-200. These compounds and their Schiff base ligands have the thioamide function \(-\text{NHC}(\text{S})\text{SR}\) and undergo transformation to thione and thiol tautomers as shown in figure 2.1.\(^91\)

The \(^1\text{H}\) NMR and \(^{13}\text{C}\) NMR spectra of compounds 195-200 were recorded in DMSO-\(d^6\) which helps to visualize the amine protons, as, for example, at 5.07 ppm for compound 195. The proton peak of NH thiol form exhibited at 10.80 ppm while
the SH thione form exhibited a single peak at 2.45 ppm. The (-CH$_3$) protons displayed a single peak at 2.38 ppm in $^1$H NMR and at 16.38 ppm in the $^{13}$C NMR spectra. In addition, the $^{13}$C NMR spectra showed a thiol peak (C=S) at 201.42 ppm and a thione peak (C-SH) at 157.59 ppm. The positive DART mass: m/z= [M+H]$^+$ found 123.0246 which corresponds to the structure of compound 195.

In the $^1$H NMR spectra of 4-benzyl hydrazinecarbodithioate 196, the absence of –SH proton resonance and the appearance of the single NH proton peak at 10.87 ppm indicate the presence of compound 196 in thiol form. The singlet peak at 4.39 ppm is assigned to –SCH$_2$ protons while the broad signal peak at 5.12 ppm is assigned to NH$_2$ protons. Also, the phenyl ring protons appear at 6.91-7.61 ppm. The positive DART mass: m/z= [M+H]$^+$ found 199.0557 which corresponds to the structure of compound 196. The $^1$H NMR and $^{13}$C NMR of compounds (196-200) are present in the thiol form, as shown in the experimental section.

2.3. Synthesis and spectroscopic characterization of Schiff base ligands

The Schiff base ligands were procured in good yields by the condensation of equimolar amounts of 2-hydroxy-5-nitrobenzaldehyde or 4-(N,N-diethylamino)-2-hydroxybenzaldehyde with hydrazine carbodithioate substituents in regular EtOH with added drops of acetic acid. All spectra of these Schiff base ligands are summarized in the experimental section. The $^1$H NMR and $^{13}$C NMR spectra of compounds (201-209) were recorded in DMSO-d$_6$. The absence of a SH single peak in the $^1$H NMR and a ν(SH) band in the IR spectra indicate that the Schiff base ligands are present in the thione form in both the solid-state and in solution. This is supported
by the presence of ν(C=S) strong bands at points located between 1023 and 1102 cm⁻¹ and of ν(NH) bands between 3085 and 3516 cm⁻¹ in compounds 201-209. The ν(OH) phenolic stretch in free ligands shows as a broad band at several points in the range between 3015 and 3641 cm⁻¹, and in some cases the stretch bands of ν(OH) and ν(NH) overlap. The azomethine hydrogen in ¹HNMR for free ligands appear in the range between 8.28 and 8.72, and their IR spectra display bands in the 1620-1632 cm⁻¹ range. In addition, the base peak in DART mass spectra for all compounds is consistent with the proposed molecular formula as shown in the experimental section.

2.4. Experimental

2.4.1. General data

All ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer and referenced to internal tetramethylsilane (TMS). IR spectra were recorded on a Nicolet Magna FT-IR spectrometer, UV-Vis spectra were recorded on a Hewlett Packard 8453 diode array UV-Vis spectrophotometer, and fluorescence spectra were recorded on a Horiba Spex Fluoro Max-3 spectrophotometer. Mass spectra (MS) were recorded on a JEOL AccuTOF JMS-T100LC mass spectrometer in DART.

All chemicals and solvents were used as received without further purification: hydrazine hydrate, absolute ethanol, and THF (Sigma-Aldrich); methyl iodide, carbon disulphide, 4-vinylbenzyl chloride, 2-hydroxy-5-nitrobenzaldehyde, anhydrous Na₂SO₄, and potassium hydroxide (Fisher); benzyl chloride, 4-nitrobenzyl
chloride, 4-bromobenzyl bromide, methyl 4-(chloromethyl)benzoate, and 4-(diethylamino) salicylaldehyde (ACROS Organics); 4-fluorobenzyl chloride (Oakwood Products); 4-(bromomethyl)benzoic acid (Alfa Aesar); ethanol, dichloromethane (VWR), and DMSO, (Cambridge Isotope Laboratories).

*S*-methyl hydrazinecarbodithioate (195).\(^{93,94}\) A 250-mL round-bottom flask equipped with a magnetic stir bar was charged with 70% ethanol (35 mL), KOH (5.611 g, 0.1000 mol), 55% hydrazine hydrate (5.611 g, 0.1000 mol). The mixture was stirred for 15 mins in an ice bath. Then carbondisulfide (4.86 mL, 0.100 mol) was added dropwise with constant stirring using an addition funnel over a period of one hour. Two layers had formed, a light yellow upper layer and a clear oil dark yellow bottom layer. The bottom oil layer was separated by removing it with a pipette, added to 40% ethanol (30 mL) in 250-mL round bottom flask, and then cooled to 0 °C using an ice bath. Methyl iodide (6.22 mL, 0.100 mol) was added dropwise via an equalized addition funnel over a period of 1.5 hours. After all the methyl iodide was added the white powder formed which were separated by filtration, washed three times with cold distilled water, and dried under oil-pump vacuum to give 195 (9.76 g, 80%). Positive DART mass (m/z): [M+H]\(^+\) calc. for C\(_2\)H\(_7\)N\(_2\)S\(_2\) 123.0045, and found 123.0246.

*S*-benzyl hydrazinecarbodithioate (196).\(^{93,94}\) A 250 mL round-bottom flask equipped with a magnetic stir bar was charged with 70% ethanol (35 mL), KOH (5.611 g, 0.1000 mol), 55% hydrazine hydrate (4.86 mL, 0.156 mol). The mixture was stirred for 15 mins in an ice bath. Then carbondisulfide (4.86 mL, 0.100 mol)
was added dropwise with constant stirring using an addition funnel over a period of one hour. Two layers had formed, a light yellow upper layer and a clear oil dark yellow bottom layer. The bottom oil layer was separated by removing it with a pipette, added to 40% ethanol (30 mL) in 250 mL round bottom flask, and then cooled to 0 °C using an ice bath. Benzyl chloride (11.5 mL, 0.100 mol) was added dropwise via an equalized addition funnel over a period of 1.5 hours. After all the benzyl chloride was added the white powder formed which were separated by filtration, washed three times with cold distilled water, and dried under oil-pump vacuum to give 169 (16.43 g, 83%). Positive DART mass (m/z): [M+H]^+ calc. for C_{8}H_{11}N_{2}S_{2} 199.0358, and Found 199.0557.

4-nitrobenzyl hydrazinecarbodithioate (197). A 250 mL round-bottom flask equipped with a magnetic stir bar was charged with 70% ethanol (35 mL), KOH (5.611 g, 0.1000 mol), 55% hydrazine hydrate (4.86 mL, 0.156 mol). The mixture was stirred for 15 mins in an ice bath. Then carbondisulfide (4.86 mL, 0.100 mol) was added dropwise with constant stirring using an addition funnel over a period of one hour. Two layers had formed, a light yellow upper layer and a clear oil dark yellow bottom layer. The bottom oil layer was separated by removing it with a pipette, added to 40% ethanol (30 mL) in 250 mL round bottom flask, and then cooled to 0 °C using an ice bath. 4-nitrobenzyl bromide (21.6 g, 0.100 mol) in THF 150 mL was added dropwise via an equalized addition funnel over a period of 1.5 hours. After all the 4-nitrobenzyl bromide was added the white powder formed which were separated by filtration, washed three times with cold distilled water, and
dried under oil-pump vacuum to give 179 (14.52 g, 60%). Negative DART mass (\(m/z\)) for \(\text{C}_8\text{H}_8\text{N}_3\text{O}_2\text{S}_2\): \([\text{M-H}]^–\) Calc. 242.0063, and Found 242.0214.

**4-bromobenzyl dithiocarbazate (198).** A 250 mL round-bottom flask equipped with a magnetic stir bar was charged with 70% ethanol (35 mL), KOH (5.611 g, 0.1000 mol), 55% hydrazine hydrate (4.86 mL, 0.156 mol). The mixture was stirred for 15 mins in an ice bath. Then carbon disulfide (6 mL, 0.1 mol) was added dropwise with constant stirring using an addition funnel over a period of one hour. Two layers had formed, a light yellow upper layer and a clear oil dark yellow bottom layer. The bottom oil layer was separated by removing it with a pipette, added to 40% ethanol (30 mL) in 250-mL round bottom flask, and then cooled to 0 °C using an ice bath. 4-bromobenzyl bromide (25.0 g, 0.100 mol) in ethanol (100 mL) was added dropwise via an equalized addition funnel over a period of 1.5 hours. After all the 4-bromobenzyl bromide was added the white powder formed which were separated by filtration, washed three times with cold distilled water, and dried under oil-pump vacuum to give 198 (20.8 g, 75%). Positive DART mass (\(m/z\)): \([\text{M+H}]^+\) calc. for \(\text{C}_9\text{H}_{10}\text{BrN}_2\text{S}_2\) 276.9469, and found 276.9900.

**4-vinylbenzyl dithiocarbazate (199).** A 250 mL round-bottom flask equipped with a magnetic stir bar was charged with 70% ethanol (35 mL), KOH (5.611 g, 0.1000 mol), 55% hydrazine hydrate (4.86 mL, 0.156 mol). The mixture was stirred for 15 mins in an ice bath. Then carbon disulfide (4.86 mL, 0.100 mol) was added dropwise with constant stirring using an addition funnel over a period of one hour. Two layers had formed, a light yellow upper layer and a clear oil dark yellow bottom...
layer. The bottom oil layer was separated by removing it with a pipette, added to 40% ethanol (30 mL) in 250 mL round bottom flask, and then cooled to 0 °C using an ice bath. 4-vinylbenzyl chloride (14.1 mL, 0.100 mol) in ethanol (50 mL) was added dropwise via an equalized addition funnel over a period of 1.5 hours. After all the 4-vinylbenzyl chloride was added the white powder formed which were separated by filtration, washed three times with cold distilled water, and dried under oil-pump vacuum to give 199 (19.48 g, 87%). Positive DART mass (m/z): [M+H]+ was calculated for C_{10}H_{13}N_{2}S_{2} 225.0515, and Found 225.1609

**4-benzoic acid methyl ester dithiocarbazate (200).** A 250 mL round-bottom flask equipped with a magnetic stir bar was charged with 70% ethanol (35 mL), KOH (5.611 g, 0.1000 mol), 55% hydrazine hydrate (4.86 mL, 0.156 mol). The mixture was stirred for 15 mins in an ice bath. Then carbondisulfide (4.86 mL, 0.100 mol) was added dropwise with constant stirring using an addition funnel over a period of one hour. Two layers had formed, a light yellow upper layer and a clear oil dark yellow bottom layer. The bottom oil layer was separated by removing it with a pipette, added to 40% ethanol (30 mL) in 250 mL round bottom flask, and then cooled to 0 °C using an ice bath. Methyl 4-(bromomethyl) benzoate (22.9 g, 0.100 mol) in ethanol (100 mL) was added dropwise via an equalized addition funnel over a period of 1.5 hours. After all the methyl 4-(bromomethyl) benzoate was added the white powder formed which were separated by filtration, washed three times with cold distilled water, and dried under oil-pump vacuum to give 200 (18.52 g, 75%).
Positive DART mass: m/z=[M+H]^+ was calculated for C_{10}H_{13}N_{2}O_{2}S_{2} 257.0413 and Found 257.0503.

**S-methyl 3-(2-hydroxy-5-nitrophenyl)methylene dithiocarbazate (201).** A warm solution of S-methyl hydrazinecarbodithioate 195 (1.22 g, 0.0100 mol) in ethanol (100 mL) was added to a 250 mL round bottom flask contains a boiling solution of 2-hydroxy-5-nitrobenzaldehyde (1.67 g, 0.0100 mol) in ethanol (30 mL). Then a few drops of acetic acid were added, and the solution was refluxed in an oil bath for 4 hours. A yellow solid powder formed which was separated by filtration, washed with ethanol, and dried under oil-pump vacuum to give 201 (2.17 g, 80%).

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.46 (s, 1H), 11.86 (s, 1H), 8.57 (d, $J = 2.8$ Hz, 1H), 8.55 (s, 1H), 8.20 (dd, $J = 9.1$, 2.8 Hz, 1H), 7.11 (d, $J = 9.1$ Hz, 1H), 2.56 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 198.51, 162.49, 140.92, 140.01, 127.15, 121.70, 120.32, 117.00, 16.79. The FT-IR spectrum of compound 201 showed bands at 3419, 1632, 1571, 1513, 1345, 1187, 1102 and 969 cm$^{-1}$. Negative DART mass (m/z): [M-H]$^-$ was calculated for C_{10}H_{13}N_{2}O_{2}S_{2} 272.0158, and Found 271.9956.

**S-benzyl-3-(2-hydroxy-5-nitrophenyl)methylene dithiocarbazate (202).** A warm solution of S-benzyl hydrazinecarbodithioate 196 (1.23 g, 6.20 mmol) in ethanol (75 mL) was added to a boiling solution of 2-hydroxy-5-nitrobenzaldehyde (0.990 g, 5.92 mmol) in ethanol (30 mL). Several drops of acetic acid were added to the flask, and the solution was refluxed for 4 hours. A yellow solid powder formed which was separated by filtration, washed with ethanol, and dried under oil-pump vacuum to give 202 (1.2 g, 55.7%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.50 (s, 1H),
11.82 (s, 1H), 8.55 (s, 1H), 8.50 (d, J = 2.9 Hz, 1H), 8.18 (dd, J = 9.1, 2.9 Hz, 1H), 7.44 (d, J = 7.1 Hz, 3H), 7.34 (t, J = 7.3 Hz, 3H), 7.28 (t, J = 7.3 Hz, 1H), 7.09 (d, J = 8.8 Hz, 0H), 4.52 (s, 2H).  
$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 196.61, 162.52, 141.30, 139.98, 136.81, 129.21, 128.47, 127.24, 121.68, 120.20, 116.99, 37.46.  
The FT-IR spectrum of compound 202 showed bands at 3085, 2947, 2846, 1620, 1595, 1571, 1512, and 1046 cm$^{-1}$. Positive DART mass: m/z= [M+H]$^+$ calcd. 348.0471 and found 348.0402.

$S$-4-nitrobenzyl-3-(2-hydroxy-5-nitrophenyl)methylene dithiocarbazate (203). A warm solution of 4-nitrobenzyldithiocarbazate 197 (2.31 g, 9.50 mmol) in ethanol (75 mL) was added to a boiling solution of 2-hydroxy-5-nitrobenzaldehyde (1.59 g, 9.50 mmol) in ethanol (30 mL). Several drops of acetic acid were added to the flask, and the solution was refluxed for 4 hours. A yellow solid powder formed which was separated by filtration, washed with ethanol, and dried under oil-pump vacuum to give 203. (2.54 g, 68%). $^1$HNMR (400 MHz, DMSO-d$_6$) δ 13.58 (s, 1H), 11.85 (s, 1H), 8.56 (s, 1H), 8.53 (d, J = 2.8 Hz, 1H), 8.19 (m, 3H), 7.72 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 9.1 Hz, 1H), 4.70 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-d$_6$) δ 195.80, 162.51, 146.41, 145.73, 141.47, 139.92, 130.26, 127.28, 123.39, 121.52, 120.08, 116.94, 36.12. The FT-IR spectrum of compound 203 showed bands at 3397, 3304, 3066, 2561, 1604, 1572, 1511, 1330, 1093 and 939 cm$^{-1}$. Positive DART mass: m/z= [M+H]$^+$ calcd. 393.0322 and found 391.0201.

$S$-4-bromobenzyl-3-(2-hydroxy-5-nitrophenyl)methylene dithiocarbazate (204). A warm solution of 4-bromo benzyl dithiocarbazate 198 (3.6 g, 0.013 mol) in ethanol (100 mL) was added to a boiling solution of 2-hydroxy-5-nitrobenzaldehyde
(2.17 g, 0.0130 mol) in ethanol (40 mL). Several drops of acetic acid were added to the flask, and the solution was refluxed for 4 hours. A yellow solid powder formed which was separated by filtration, washed with ethanol, and dried under oil-pump vacuum to give 204 (4.0 g, 72%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.52 (s, 1H), 11.84 (s, 1H), 8.55 (s, 1H), 8.51 (d, $J = 2.9$ Hz, 1H), 8.18 (dd, $J = 9.1$, 2.9 Hz, 1H), 7.53 (d, $J = 8.4$ Hz, 2H), 7.41 (d, $J = 8.4$ Hz, 2H), 7.10 (d, $J = 9.1$ Hz, 1H), 4.52 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 196.35, 162.55, 141.40, 139.98, 136.74, 131.36, 131.29, 127.31, 121.63, 120.27, 120.17, 117.01, 36.40. The FT-IR spectrum of compound 204 showed bands at 3039, 2281, 1630, 1571, 1512, 1342, 1187, 1101, 969, and 939 cm$^{-1}$. Positive DART mass: m/z= [M+H]$^+$ calcd. 425.9576 and found 425.9573.

5-4-vinylbenzyl-3-(2-hydroxy-5-nitrophenyl)methylene dithiocarbazate (205). A warm solution of 4-vinylbenzyl dithiocarbazate 200 (2.24 g, 0.0100 mol) in ethanol (100 mL) was added to a boiling solution of 2-hydroxy-5-nitrobenzaldehyde (1.67 g, 0.0100 mol) in ethanol (40 mL). Then a few drops of acetic acid were added, and the mixture was refluxed in an oil bath for 3 hours. A yellow solid powder formed which was separated by filtration, washed with ethanol, and dried under oil-pump vacuum to give 205. (2.98 g, 80%) $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.50 (s, 1H), 11.82 (s, 1H), 8.55 (s, 1H), 8.51 (d, $J = 2.9$ Hz, 1H), 8.26 – 8.06 (m, 1H), 7.50 – 7.36 (m, 4H), 7.09 (d, $J = 9.1$ Hz, 1H), 6.72 (dd, $J = 17.7$, 10.9 Hz, 1H), 5.82 (d, $J = 18.2$ Hz, 1H), 5.25 (d, $J = 11.0$ Hz, 1H), 4.52 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 196.55, 162.51, 141.31, 139.97, 136.53, 136.21, 136.12, 129.45, 127.24, 126.20, 121.68, 120.18, 116.98,
The FT-IR spectrum of compound 205 showed bands at 3641, 3516, 3086, 2970, 1624, 1597, 1508, 1047, 968, and 913 cm\(^{-1}\). Positive DART mass: m/z=[M+H]\(^+\) calcd. 374.0628 and found 374.0699.

**S-benzyl-3-(4-(diethylamino)-2-hydroxy)methylenedithiocarbazate (207)** A warm solution of S-benzyldithiocarbazate 196 (1.93 g, 0.0100 mol) in ethanol (20 mL) was added to a boiling solution of 4-(diethylamino)-2-hydroxy benzaldehyde (1.983 g, 0.01000 mol) in 30 mL of ethanol. Then a few drops of acetic acid were added, and the mixture was refluxed in the oil bath for 3 hours. A yellow solid powder formed which was separated by filtration, washed with ethanol, and dried under oil-pump vacuum to give 207 (3.0 g, 80.4%). \(^1\)H NMR (400 MHz, DMSO) \(\delta\) 13.19 (s, 1H), 10.10 (s, 1H), 8.32 (s, 1H), 7.42 (d, \(J=7.1\) Hz, 2H), 7.37 – 7.22 (m, 3H), 6.27 (d, \(J=8.9\) Hz, 1H), 6.09 (s, 1H), 4.50 (s, 2H), 3.33 (q, 4H overlap with H2O), 1.09 (t, \(J=5.8\) Hz, 6H). \(^13\)C NMR (101 MHz, DMSO) \(\delta\) 192.37, 159.70, 151.07, 147.26, 137.53, 137.00, 130.38, 129.16, 128.47, 127.17, 106.36, 104.33, 97.36, 43.64, 37.26, 12.32. The FT-IR cm\(^{-1}\) spectrum of compound 206 showed bands at 3415, 3105, 2972-2835, 1624, 1587, 1511, and 1024 cm\(^{-1}\). Positive DART mass: m/z=[M+H]\(^+\) calcd. 374.1355 and found 374.1610.

**S-4-bromobenzyl-3-(4-(diethylamino)-2-hydroxy)methylenedithiocarbazate (208).** A warm solution of 4-bromo benzyl dithiocarbazate 198 (2.77 g, 0.0100 mol) in ethanol (30 mL) was added to a boiling solution of 4-(diethylamino)-2-hydroxy benzaldehyde (1.93 g, 0.0100 mol) in 30 mL of ethanol. Then a few drops of acetic acid were added, and the mixture was refluxed in the oil bath for 3 hours. A yellow
solid powder formed which was separated by filtration, washed with ethanol, and
dried under oil-pump vacuum to give **208** (3.50 g, 77.6%). $^1$H NMR (400 MHz,
DMSO) δ 13.24 (s, 1H), 9.99 (s, 1H), 8.30 (d, $J$ = 14.2 Hz, 1H), 7.50 (dd, $J$ = 14.8,
7.8 Hz, 2H), 7.42 − 7.23 (m, 3H), 6.25 (dd, $J$ = 16.5, 7.8 Hz, 1H), 6.07 (d, $J$ = 13.8
Hz, 1H), 4.47 (s, 2H), 3.32 (q, $J$ = 16.3, 10.7 Hz, 4H), 1.25 − 0.88 (t, 6H). $^{13}$C NMR
(101 MHz, DMSO) δ 190.30, 157.65, 149.30, 145.62, 135.12, 129.55, 129.51,
128.59, 118.44, 104.24, 102.68, 95.37, 42.19, 34.55, 10.85. The FT-IR spectrum of
the compound **208** showed bands at 3114, 2975, 2933, 2869, 1622, 1580, 1511, 1240,
1092, 1031, and 956 cm$^{-1}$. Positive DART mass: m/z = [M+H]$^+$ calcd. 452.0460 and
found 452.0509.

*S*-4-vinylbenzyl-3-(4-(diethylamino)-2-hydroxy)methylenedithiocarbazate
(209). A warm solution of 4-vinyl benzyl dithiocarbazate **199** (2.24 g, 0.0100 mol)
in ethanol (30 mL) was added to a boiling solution of 4-(diethylamino)-2-hydroxy
benzaldehyde (1.93 g, 0.0100 mol) in 40 mL of ethanol. Then a few drops of acetic
acid were added, and the mixture was refluxed in an oil bath for 3 hours. . A yellow
solid powder formed which was separated by filtration, washed with ethanol, and
dried under oil-pump vacuum to give **209** (3.526 g, 88.37%) $^1$H NMR (400 MHz,
DMSO) δ 13.17 (s, 1H), 10.07 (s, 1H), 8.31 (s, 1H), 7.41 (dd, $J$ = 8.3 Hz, 4H), 7.31
(d, $J$ = 8.9 Hz, 1H), 6.72 (dd, $J$ = 17.6, 11.0 Hz, 1H), 6.26 (dd, $J$ = 8.9, 2.1 Hz, 1H),
6.08 (d, $J$ = 2.1 Hz, 1H), 5.81 (d, $J$ = 17.7 Hz, 1H), 5.25 (d, $J$ = 10.9 Hz, 1H), 4.50
(s, 2H), 3.33 (q, $J$ = 13.5, 6.5 Hz, 5H), 1.09 (t, $J$ = 7.0 Hz, 6H). $^{13}$C NMR (101 MHz,
DMSO) δ 192.19, 159.39, 151.05, 147.26, 136.74, 136.24, 136.09, 130.40, 129.41,
126.20, 114.33, 106.02, 104.34, 97.36, 43.95, 37.26, 3.95, 37.26, 12.62. The FT-IR spectrum of this compound 209 showed bands at 3236, 3104, 2974, 1627, 1579, 1096, 1023, 955, and 893 cm\(^{-1}\). The Positive DART mass: m/z= [M+H]\(^+\) was calculated 400.1512 and found 400.1479.

\textbf{\textit{S}-4-benzoic acid methyl ester-3-(4-(diethylamino)-2-hydroxy) methylene dithiocarbazate (210).} A warm solution of 4-benzoic acid methyl ester dithiocarbazate 200 (2.56 g, 0.0100 mol) in ethanol (30 mL) was added to a boiling solution of 4-(Diethylamino)-2-hydroxy benzaldehyde (1.93 g, 0.0100 mol) in 40 mL of ethanol. Then a few drops of acetic acid were added, and the mixture was refluxed in an oil bath for 3 hours. A yellow solid powder formed which was separated by filtration, washed with ethanol, and dried under oil-pump vacuum to give 210. (3.57 g, 82.8\%) \(^1\)H NMR (400 MHz, DMSO-d\(^6\)) \(\delta\) 13.24 (s, 1H), 10.05 (s, 1H), 8.35 (s, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 8.9 Hz, 1H), 6.27 (dd, J = 9.0, 1.8 Hz, 1H), 6.09 (d, J = 1.9 Hz, 1H), 4.61 (s, 2H), 3.85 (s, 3H), 3.34 (q, J = 6.9 Hz, 4H), 1.09 (t, J = 7.0 Hz, 6H). \(^{13}\)C NMR (101 MHz, DMSO-d\(^6\)) \(\delta\) 191.65, 165.90, 159.25, 150.83, 147.11, 143.12, 130.12, 129.25, 128.24, 106.00, 104.11, 96.89, 51.84, 43.71, 36.50, 12.39. The FT-IR spectrum of compound 210 showed bands at 3140, 2967, 2364, 1715, 1624, 1592, 1502, 1277, 1025, and 955 cm\(^{-1}\). The Positive DART mass: m/z= [M+H]\(^+\) was calculated 432.1410 and found 432.1208.
CHAPTER 3
Synthesis, Spectroscopic Characterization, and Antibacterial Activity of Vanadium Complexes

3.1. Introduction
The misuse of antibiotics has exacerbated the problem of bacteria that are resistant to antibiotics. Even without this misuse, bacteria can adapt themselves to become resistant to antibiotics by generating β-lactamase enzymes, modifying the active site of penicillin-binding proteins (PBPs), reducing the expression of outer membrane proteins (OMPs), and activating efflux pumps. Among these, the generation of β-lactamase enzymes is the most prevalent mechanism of bacterial resistance to antibiotics, and more than 850 β-lactamases have been identified.\(^1\) Based on the amino acid sequences of β-lactamases, these enzymes are divided into four molecular classes, A, B, C and D; this is known as Ambler’s classification.\(^95\) This has increased the fears and challenges involved in finding therapeutic alternatives especially for Gram-negative bacteria, in which most of the antibiotic and β-lactamase inhibitors are unable to cross cell membranes.\(^96\)

There has been a marked increase in studies of the structural, biological, and catalytic properties of vanadium in regard to its coordination chemistry. Homogeneous vanadium (IV and V) and their immobilized analogues catalyze organic reactions, for example, the hydroxylation of hydrocarbons, and the oxidation of alcohols to aldehydes and ketones.\(^97\)
One important mixed hard-soft nitrogen-sulfur ligand is dithiocarbazate, and small modifications in its derivative structures have been shown to have different bioactivity applications.\textsuperscript{88,89} For example, S-benzyl/S-methyl dithiocarbazate dioxo and oxovanadium(V) complexes with pyridoxal have been more effective than a commonly used drug against amoebiasis, metronidazole.\textsuperscript{92} As is described in Chapter 1, vanadium complexes have been tested as antibacterial agents against diverse types of bacteria and fungi that cause different kinds of diseases in humans, animals, and plants. However, the targets of these complexes whilst killing different types of bacteria or decreasing their growth, have not been discussed and the mechanisms involved are still not clear. In this chapter, we describe the synthesis of six vanadium(V) complexes which have fluorescence properties. The fluorescence properties of these complexes enable them to be used as fluorescent cellular imaging agents in living cells; confocal fluorescence microscopy is used to determine the target of these complexes in bacteria and for cell imaging studies.

Dioxovanadium(V) complexes $K\{\text{VO}_2\{S$-methyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate}\}$ \textsuperscript{211} and $K\{\text{VO}_2\{S$-benzyl-3-(2-hydroxy-5-nitrophenyl methylenedithiocarbazate}\}$ \textsuperscript{212} (Scheme 3.1) have been tested for antibacterial activity against \textit{Burkholderia pseudomallei} strains and \textit{Burkholderia mallei} strains. \textit{B. pseudomallei} strains are the gram-negative motile bacteria which cause melioidosis (Whitmore disease) and have shown resistance to antibiotics such as aminoglycosides, macrolides, polymyxins, β-lactam, and β-lactamase inhibitor by generating different classes of β-lactamases.\textsuperscript{98,99} In addition, \textit{B. mallei} strains are the
gram-negative nonmotile bacteria which cause glanders disease. These diseases are prevalent in Southeast Asia, northern Australia, Eastern Europe, North Africa, and the Middle East.\textsuperscript{100,101} There are three exposure modes for melioidosis and glanders: inhalation, cutaneous inoculation, and ingestion. The impact of these diseases is seen in horses, donkeys, and mules and thus transferred to the humans who have a direct relationship with these animals. The symptoms that accompany these diseases in humans include fever, headache, sore throat, ulceration in the nose, purulent fluid from the nose, pneumonia, and abscesses in the lungs. In advanced cases, they cause blood poisoning, festering blisters covering the body, abscesses in the muscles, and severe pneumonia, sometimes leading to death.\textsuperscript{102,101} These deaths are usually due to the difficulty in recognizing the symptoms and making a correct diagnosis. Inhalation causes acute infections of melioidosis which lead to a 20 to 50\% mortality rate.\textsuperscript{103} The progress of the disease based on the length of the exposure to the organism is shown in Figure 3.1. The pneumonic form of the disease develops rapidly and if untreated is always fatal. Even when treated with antibiotics for a week, with an apparent subsidence of the disease, there is often a relapse with fatal results. More research needs to be done to determine the ideal length of antibiotic treatment for full recovery.\textsuperscript{101} Many US soldiers were found to be infected with melioidosis after returning from the invasion of Vietnam.\textsuperscript{104} As early as the Civil War in the U.S., and again during both World Wars, \textit{B. mallei} was employed in biological warfare; there was even suspected use in Afghanistan by the former Union
Figure 3.1 Differences in exposure length leading to varying types of infection by *Burkholderia mallei* strains.\textsuperscript{101}
of Soviet Socialist Republics. Recently, the possible use of *B. pseudomallei* and *B. mallei* as bioweapons has reawakened interest in protecting humans and animals from these bacteria. In the following section, we will describe the synthesis, characterization, and antibacterial activities of vanadium complexes.

3.2. Synthesis and spectroscopic characterization of dioxovanadium(V) complexes 211-216.

Stoichiometric amounts of [VO(acac)₂] and the Schiff base ligand were dissolved in methanol. The solution was reflux for 2 h to give a clear brown solution then KOH added. The mixture was refluxed for an additional two hours to give a deep green solution. The solution was reduced to 75 ml under a flow of nitrogen gas. After two days in an aerobic atmosphere, yellow vanadium(V) crystals formed with good yield, which were collected by filtration, washed by cold methanol.

All of the vanadium complexes 211-216 were yellow solids and were stable in the solid state in a dark, anaerobic environment. However, the yellow solutions of vanadium(V) complexes are gradually changed to green with the exposure to visible light for around 2-3 months. This change in color reflects the reduction of vanadium(V) to vanadium (VI). All complexes were characterized by ¹H NMR, ¹³C NMR, FT-IR, UV-vis, and fluorescence. All of the characterization data is provided in the experimental section of this chapter. The ¹H NMR and ¹³C NMR spectral data of the vanadium complexes were recorded in DMSO-d⁶. The spectra of the free ligands showed two singlet peaks in the range of 13.58–13.46 and 11.86-11.82 ppm due to a phenolic proton (OH) and an (NH) proton, which are absent in
the spectra of the vanadium complexes, indicating the coordination of the phenolate oxygen atom with the vanadium ion. In addition, the change in the chemical shift of the CH=N azomethine proton from 8.55-8.57 ppm in the free ligands to 9.22-9.31 ppm in the vanadium complexes suggests the coordination of the azomethine nitrogen atom (CH=N) with the vanadium ion.

The $^{13}$C NMR spectra recorded for the complexes contain signals that match those of the carbon atoms of the molecules as shown in the experimental section. A large coordination shift was observed for the signal peaks of the phenolate carbon (C-O) and the enethiolate sulfur atom that shift from 158.7 to 169.9 and from 174.76 to 176.96, respectively, in the vanadium complexes.

The IR spectra (Table 3.1) of the Schiff base ligands showed low intensity broad bands in the range of 3381-3505 cm$^{-1}$ which overlap between two stretching bands $\nu$(OH) and $\nu$(N-H), which disappear in the vanadium complexes. The disappearance of these bands indicates that the Schiff base ligands coordinated to the vanadium ions in their deprotonated forms. In addition, both the $\nu$(C=O) and $\nu$(N=N) bands of free Schiff base ligands are shifted to low wavelengths in the IR spectra of the vanadium complexes, indicating the coordination of the vanadium ion with the ligand by an azomethine nitrogen atom. Furthermore, the band in the range of 897-945 cm$^{-1}$ provides for the coordination of the ligand by phenolate (C-O), and losing the $\nu$ (C=S) bands in all the vanadium complexes indicates the coordination of vanadium ion with the Schiff base ligands via the thiolate groups. provides for the coordination of the ligand by phenolate (C-O), and losing the $\nu$ (C=S) bands in all the vanadium
Scheme 3.1 Synthesis of dioxovanadium(V) complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_1$</th>
<th>Complex</th>
<th>$R_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>195</td>
<td>CH$_3$</td>
<td>211</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>196</td>
<td>Bn</td>
<td>212</td>
<td>Benzyl</td>
</tr>
<tr>
<td>197</td>
<td>$p$-NO$_2$ Bn</td>
<td>213</td>
<td>$p$-NO$_2$ benzyl</td>
</tr>
<tr>
<td>198</td>
<td>$p$-Br Bn</td>
<td>214</td>
<td>$p$-Br benzyl</td>
</tr>
<tr>
<td>199</td>
<td>$p$-vinyl Bn</td>
<td>215</td>
<td>$p$-vinyl benzyl</td>
</tr>
<tr>
<td>200</td>
<td>$p$-COOMe Bn</td>
<td>216</td>
<td>$p$-COOMe benzyl</td>
</tr>
</tbody>
</table>

i) [VO(acac)$_2$], KOH, MeOH, reflux 4h.
Table 3.1. Characteristic infrared frequencies (cm⁻¹) of the ligands and vanadium complexes.

<table>
<thead>
<tr>
<th>Ligand/Complex</th>
<th>ν(NH)/ν(OH)</th>
<th>ν(N=C)</th>
<th>ν(V-N)</th>
<th>ν(V-O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>201</td>
<td>3419</td>
<td>1632</td>
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<td>-</td>
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<td>540</td>
<td>897</td>
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<tr>
<td>204</td>
<td>3381</td>
<td>1608</td>
<td>-</td>
<td>-</td>
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<td>214</td>
<td>-</td>
<td>1596</td>
<td>509</td>
<td>897</td>
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<td>205</td>
<td>3505</td>
<td>1623</td>
<td>-</td>
<td>-</td>
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<tr>
<td>215</td>
<td>-</td>
<td>1596</td>
<td>516</td>
<td>890</td>
</tr>
</tbody>
</table>
complexes indicates the coordination of vanadium ion with the Schiff base ligands via the thiolate groups.

The UV/V spectroscopic data of the ligands and their vanadium complexes were recorded in methanol are presented in Table 3.2. All of the vanadium complexes display a medium electronic spectral at 366-381 nm, which can be attributed to LMCT arising from the phenolate oxygen to an empty d-orbital of the vanadium(V) center. After coordination, this band is shifted to a higher wavelength according to literature data. The blue-shift in this band is due to the action of the methanol in coordination with the vanadium. All the complexes showed a medium to strong electronic spectral band at λ =311 nm which also can be assigned to the π- π* transition. In addition, no d-d bands were anticipated in these complexes, because vanadium(V) complexes are d⁰ electron configurations. The emission spectra for some ligands and vanadium complexes were recorded in methanol at the same concentration. All ligands and complexes excited at 400 nm. As shown in Figure 3.3 a-c, the ligands show a stronger intensity of fluorescence in comparison with their complexes under the same conditions. As a result of the decrease in the electrons on the ligand, there is a weak intensity of the band which corresponds to the complexes. Furthermore, the vanadium complexes are soluble in water and can be excited around 430, making them appropriate for confocal fluorescence microscopy.

The X-ray diffraction result of complex 212, which was recrystallized by keeping a saturated ethanolic solution of the crude 212 at 5 °C for seven days or by the slow
Table 3.2  Electronic spectroscopic data of ligands and vanadium complexes in ethanol

<table>
<thead>
<tr>
<th>Ligand/Complex</th>
<th>$\lambda_{\text{max}}, \text{nm} (\varepsilon, \text{mol}^{-1} \text{ cm}^{2})$</th>
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</thead>
<tbody>
<tr>
<td>201</td>
<td>305 (84875), 345 (109624), 426 (7181)</td>
</tr>
<tr>
<td>211</td>
<td>310 (24178), 363 (12020)</td>
</tr>
<tr>
<td>202</td>
<td>305 (84876), 347 (120201), 432 (7158.9)</td>
</tr>
<tr>
<td>212</td>
<td>313 (109556), 363 (53337)</td>
</tr>
<tr>
<td>203</td>
<td>298 (102046), 346 (117698), 429 (5805.5)</td>
</tr>
<tr>
<td>213</td>
<td>311 (105548), 380 (45889)</td>
</tr>
<tr>
<td>204</td>
<td>303 (64637), 347 (89196), 432 (3912.1)</td>
</tr>
<tr>
<td>214</td>
<td>311 (105644), 380 (45623)</td>
</tr>
<tr>
<td>205</td>
<td>298 (105789), 347 (142660), 430 (7885)</td>
</tr>
<tr>
<td>215</td>
<td>311 (97273), 380 (40250)</td>
</tr>
<tr>
<td>216</td>
<td>310 (97273), 381 (40250)</td>
</tr>
</tbody>
</table>
Figure 3.2  UV-Vis spectra of vanadium complexes 211, 212, 213, 214, 215 and 216.
Figure 3.3a  Emission spectra of 203 and 213.

Figure 3.3b  Emission spectra of 204 and 214.
Figure 3.3 c Emission spectra of 205 and 215.
evaporation of aqueous solutions at room temperature, is shown in Figure 3.4 and Table 3.2. The five-coordinated vanadium complex has a distorted square pyramidal geometry (τ = 0.257), with the following angles with O(21) as the apex of the pyramid: O(21)-V-S(2) = 105.137°, O(21)-V-N(12) = 101.787°, O(21)-V-O(20) = 108.578° and O(21)-V-O(22) = 107.086°; the following bond lengths were obtained: V-S(1) = 2.367 Å, V-N(12) = 2.203Å, V-O(20) = 1.924Å, V-O(22) = 1.640Å and V-O(21) = 1.612Å. The distortion comes from the steric demands of the narrow angles of the bicyclic structure constituted by the five and six membered chelate rings. This result indicates that the Schiff base 202 is coordinated to the vanadium ion as a tridentate ligand via a thiolate sulfur atom, an azomethine nitrogen atom, and phenoxyl oxygen. In the same kind of vanadium(V) complexes which contain ONS as tridentate Schiff base ligands, the distance of V=O =1.615Å for both oxo groups indicating that the V=O groups are not involved in the hydrogen bonding and other weak intermolecular contacts.111 Furthermore, the bond lengths V-S(1) = 2.367 Å, V-N(12)= 2.203Å, V-O(20) = 1.924Å agree with those found by other researchers.109

3.3 Antimicrobial activity of 211 and 212

The vanadium complexes 211 and 212 were screened for antimicrobial activity against *Burkholderia mallei* and *Burkholderia pseudomallei* strains by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and the results summarized in Table 3.3. Ceftazidime, a cephalosporin antibiotic, was used as the reference drug in this study due to its efficacy in treating glanders and melioidosis.112
Figure 3.4  ORTEP representation of 212 (Figure provided by the Naval Research Laboratory, Washington, D.C.).
Table 3.2. Selected bond lengths (Å) and bond angles (°) for complex 212

<table>
<thead>
<tr>
<th>Bond or Angle</th>
<th>Distances (Å)</th>
<th>Angles (°)</th>
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</thead>
<tbody>
<tr>
<td>O(20)-V(1)-S(1)</td>
<td>143.470</td>
<td>V(1)-O(20) 1.924</td>
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<tr>
<td>O(21)-V(1)-O(22)</td>
<td>107.086</td>
<td>V(1)-O(21) 1.612</td>
</tr>
<tr>
<td>O(21)-V(1)-O(20)</td>
<td>108.578</td>
<td>V(1)-O(22) 1.640</td>
</tr>
<tr>
<td>O(21)-V(1)-S(1)</td>
<td>105.137</td>
<td>V(1)-N(12) 2.203</td>
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<tr>
<td>O(21)-V(1)-N(12)</td>
<td>101.787</td>
<td>V(1)-S(1) 2.367</td>
</tr>
<tr>
<td>O(21)-V(1)-O(22)</td>
<td>107.086</td>
<td>V(1)-O(20) 1.924</td>
</tr>
<tr>
<td>O(22)-V(1)-O(20)</td>
<td>95.675</td>
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<tr>
<td>O(20)-V(1)-N(12)</td>
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<tr>
<td>O(22)-V(1)-S(1)</td>
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<tr>
<td>O(22)-V(1)-N(12)</td>
<td>149.947</td>
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Table 3.3  Antibacterial activities of complexes 211 and 212 against *B. pseudomallei*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ceftazidime</th>
<th>211</th>
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<tr>
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<td>1</td>
<td>64</td>
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<tr>
<td>316c</td>
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<td>64</td>
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<td>E203</td>
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<td>NCTC4845</td>
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<td>4</td>
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<tr>
<td>STW115-2</td>
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<td>4</td>
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<td>112c</td>
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<td>296</td>
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<td>423</td>
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<td>64</td>
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<tr>
<td>465a</td>
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<tr>
<td>644</td>
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<td>730</td>
<td>2</td>
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<tr>
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<tr>
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<tr>
<td>E304</td>
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</tr>
<tr>
<td>576a</td>
<td>2</td>
<td>64</td>
<td>32</td>
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</table>
The results show that the antibacterial activity of complex 212 against *B. pseudo* strains is higher than that with *B. mallei* strains. In addition, complex 211 exhibited more potent antibacterial activity against all strains of bacteria than complex 211 except E40 and 275 bacterial strains, where both of the vanadium complexes showed the same minimum inhibitory concentration (MIC) value. In some strains, the MIC values of complex 212 are only 2-fold higher than the control (Ceftazidime), which is promising for preliminary data, with the synthesis of some benzyl derivative vanadium complexes as the next step in this work. The aim of synthesizing vanadium complexes with benzyl derivatives is to evaluate if there is any increase in antibacterial activity of these complexes with substitutions of nitro, bromo, methyl ester and vinyl in the benzyl ring.

3.4. Experimental

3.4.1. General data

All $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer and referenced to internal tetramethylsilane (TMS). IR spectra were recorded on a Nicolet Magna FT-IR spectrometer, UV-Vis spectra were recorded on a Hewlett Packard 8453 diode array UV-Vis spectrophotometer, and fluorescence spectra were recorded on a Horiba Spex Fluoro Max-3 spectrophotometer. The crystal structure for complex $\text{K[VO}_2\{S\text{-benzyl-3-(2-hydroxy-5-nitrophenyl) methyl enedithiocarbazate)}\}$ 212 was provided by the Naval Research Laboratory, Washington, D.C.
All chemicals and solvents were used as received without further purification: Potassium hydroxide (Fisher); vanadyl acetylacetonate (ACROS Organics); ethanol, methanol (VWR); and DMSO-d$_6$ (Cambridge Isotope Laboratories).

**Synthesis of K[VO$_2${S-methyl-3-(2-hydroxy-5-nitrophenyl) methylene dithiocarbazate}]$]^{211}$**  
A 250 mL round-bottom flask was charged with methyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate $^{201}$ (0.271 g, 1.00 mmol), methanol (120 mL), and a stirrer bar. Then a solution of VO(acac)$_2$ (0.265 g, 1.00 mmol) in methanol (30 ml) was added. The flask was heated to 70 °C using an oil-bath, and the mixture was refluxed for 3 hours. After 3 hours, KOH (0.0673 g, 1.20 mmol) was added, and a brown solution was formed. Reflux was continued for 2 hours. The solution was transferred to an Erlenmeyer flask and reduced to 75 ml under a flow of nitrogen gas. After two days in an aerobic atmosphere, yellow crystals formed, which were collected by filtration, washed by cold methanol, and dried over anhydrous sodium sulfate to give $^{216}$ (0.22 g, 56%). $^1$H NMR (400 MHz, DMSO) δ 9.22 (s, 1H), 8.74 (d, $J = 3.0$ Hz, 1H), 8.21 (dd, $J = 9.3$, 3.0 Hz, 1H), 6.96 (d, $J = 9.3$ Hz, 1H), 2.55 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 176.96, 158.70, 137.80, 136.45, 130.55, 128.15, 120.56, 119.17, 15.79.

**Synthesis of K[VO$_2${S-benzyl-3-(2-hydroxy-5-nitrophenyl) methylene dithiocarbazate}]$]^{212}$**  
A 500 mL round-bottom flask was charged with S-benzyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate $^{202}$ (0.184 g, 0.467 mmol), methanol (450 mL), and a stirrer bar. Then a solution of VO(acac)$_2$ (0.124 g, 0.467 mmol) in methanol (30 ml) was added. The flask was heated to 70 °C using an oil-
bath, and the mixture was refluxed for 3 hours. After 3 hours, KOH (0.315 g, 0.560 mmol) was added, and a brown solution was formed. Reflux was continued for 2 hours. The solution was transferred to an Erlenmeyer flask and reduced to 75 ml under a flow of nitrogen gas. After two days in an aerobic atmosphere, yellow crystals formed, which were collected by filtration, washed by cold methanol, and dried over anhydrous sodium sulfate to give 212 (0.7 g, 75%). \(^1\)H NMR (400 MHz, DMSO) \(\delta 9.31 \,(s,\,1\,H),\, 8.79 \,(d,\,J = 3.0\,Hz,\,1\,H),\, 8.22 \,(dd,\,J = 9.3,\,3.0\,Hz,\,1\,H),\, 7.48 \,(d,\,J = 7.2\,Hz,\,2\,H),\, 7.34 \,(t,\,J = 7.4\,Hz,\,2\,H),\, 7.26 \,(t,\,J = 7.3\,Hz,\,1\,H),\, 6.97 \,(d,\,J = 9.2\,Hz,\,1\,H),\, 4.42 \,(s,\,2\,H). \(^{13}\)C NMR (101 MHz, DMSO) \(\delta 175.72,\, 169.99,\, 159.14,\, 137.94,\, 137.77,\, 130.77,\, 129.30,\, 128.51,\, 128.36,\, 127.20,\, 120.71,\, 119.29,\, 36.56.

**Synthesis of K[VO\(_2\)(S-4-nitrobenzyl-3-(2-hydroxy-5-nitrophenyl)methylenedithiocarbazate)]**(213). A 500 mL round-bottom flask was charged with S-4-nitrobenzyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate 203 (0.786 g, 0.00200 mol), methanol (450 mL), and a stirrer bar. Then a solution of VO(acac)\(_2\) (0.5303 g, 0.002000 mol) in methanol (30 ml) was added. The flask was heated to 70 °C using an oil-bath, and the mixture was refluxed for 3 hours. After 3 hours, KOH (0.1346 g, 0.002400 mol) was added, and a brown solution was formed. Reflux was continued for 2 hours. The solution was transferred to an Erlenmeyer flask and reduced to 75 ml under a flow of nitrogen gas. After two days in an aerobic atmosphere, yellow crystals formed, which were collected by filtration, washed by cold methanol, and dried over anhydrous sodium sulfate to give 213 (0.716 g, 70%), \(^1\)H NMR (400 MHz; DMSO): \(\delta 9.31 \,(s,\,1\,H),\, 8.79 \,(d,\,J = 3.0\,Hz,\,1\,H),\, 8.24 \,(d,\,J =
3.0 Hz, 1H), 8.20 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.7 Hz, 2H), 6.96 (d, J = 9.4 Hz, 1H), 4.51 (s, 2H). $^{13}$C NMR (400 MHz; DMSO): δ 174.8, 169.9, 159.4, 146.57, 146.40, 137.8, 130.6, 128.3, 123.4, 120.6, 119.1, 35.6.

Synthesis of $\text{K[VO}_2\{\text{S-4-bromobenzyl-3-(2-hydroxy-5-nitrophenyl)methyl enedithiocarbazate}\}\}$ (214). A 500 mL round-bottom flask was charged with S-4-bromobenzyl-3-(2-hydroxy-5-nitrophenyl)methylenedithiocarbazate 204 (0.8526 g, 0.00200 mol), methanol (450 mL), and a stirrer bar. Then a solution of VO(acac)$_2$ (0.5303 g, 0.00200 mol) in methanol (30 ml) was added. The flask was heated to 70 °C using an oil-bath, and the mixture was refluxed for 3 hours. After 3 hours, KOH (0.1346 g, 0.002400 mol) was added, and a brown solution was formed. Reflux was continued for 2 hours. The solution was transferred to an Erlenmeyer flask and reduced to 75 ml under a flow of nitrogen gas. After two days in an aerobic atmosphere, yellow crystals formed, which were collected by filtration, washed by cold methanol, and dried over anhydrous sodium sulfate to give 214 (0.97 g, 89.7%).

$^1$H NMR (400 MHz, DMSO) δ 9.30 (s, 1H), 8.78 (d, J = 2.7 Hz, 1H), 8.22 (dd, J = 9.2, 2.7 Hz, 1H), 7.52 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 6.96 (d, J = 9.3 Hz, 1H), 4.38 (s, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 175.24, 169.87, 159.15, 137.81, 137.53, 131.45, 131.20, 130.67, 128.27, 120.61, 120.13, 119.13, 35.66.

Synthesis of $\text{K[VO}_2\{\text{S-4-vinyl-benzyl-3-(2-hydroxy-5-nitrophenyl)methyl enedithiocarbazate}\}\}$ (215). A 500 mL round-bottom flask was charged S-4-vinylbenzyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate 205 (0.3659 g, 0.001380 mol), methanol (450 mL), and a stirrer bar. Then a solution of VO(acac)$_2$
(0.5303 g, 0.002000 mol) in methanol (30 ml) was added. The flask was heated to 70 °C using an oil-bath, and the mixture was refluxed for 3 hours. After 3 hours, KOH (0.0931 g, 0.00166 mol) was added, and a brown solution was formed. Reflux was continued for 2 hours. The solution was transferred to an Erlenmeyer flask and reduced to 75 ml under a flow of nitrogen gas. After two days in an aerobic atmosphere, yellow crystals formed, which were collected by filtration, washed by cold methanol, and dried over anhydrous sodium sulfate to give 215 (0.97 g, 89.7%).

Yield (0.54 g, 79.3%); \(^1\)H NMR (400 MHz, DMSO) \(\delta\) 9.31 (s, 1H), 8.79 (d, \(J = 3.0\) Hz, 1H), 8.23 (dd, \(J = 9.3, 3.0\) Hz, 1H), 7.44 (d, \(J = 2.7\) Hz, 4H), 6.97 (d, \(J = 9.3\) Hz, 1H), 6.71 (dd, \(J = 17.6, 11.0\) Hz, 1H), 5.81 (d, \(J = 16.9\) Hz, 1H), 5.24 (d, \(J = 11.0\) Hz, 1H), 4.40 (s, 2H). \(^{13}\)C NMR (101 MHz, DMSO) \(\delta\) 175.43, 169.78, 158.96, 137.73, 137.30, 136.17, 135.88, 130.57, 129.38, 128.16, 126.07, 120.52, 119.08, 114.07, 36.15.
Part (II): Synthesis of 9-CD$_3$-11-cis-Retinal for the Investigation of the Activation Mechanism of GPCR Rhodopsin by Solid-State $^2$H NMR Spectroscopy
ABSTRACT


a dissertation by

Samira M. Faylough

Research Advisor: Nasri Nesnas, Ph.D.

Rhodopsin is a G-protein-coupled receptor (GPCR) responsible for vision in vertebrates, and in recent years, it has become possible to view the structures which represent the active conformation of rhodopsin by means of X-ray techniques. Enabling the study of the membrane proteins in a native-like membrane environment, obtaining structural information such as bond orientations, and facilitating experiments to detect dynamic information which cannot be obtained with other biophysical methods are considered the advantages of $^2$H NMR methods. In this study, we synthesized 9-CD₃-11-cis-retinal and applied solid-state $^2$H NMR spectroscopy to study the structure and orientation of the retinal ligand in the active state of rhodopsin at Dr. Michael F. Brown’s lab in the Department of Physics, University of Arizona, Tucson, Arizona in collaboration with Dr. Nasri Nesnas, in the Department of Chemistry, Florida Institute of Technology, Melbourne, Florida.
CHAPTER 4

Synthesis of 11-trans and 11-cis Retinal

4.1. General introduction

The human’s eye structure contains the lens, iris, cornea, pupil, retina, vitreous humor, optic disk and optic nerve as shown in Figure 4.1. The retina is located at the back of the eye, and it is light-sensitive tissue, turning light into electrical impulses and sending them to the brain by the optic nerve. Therefore, the retina is counted as the part of the central nervous system.\textsuperscript{113, 114}

![Figure 4.1. Structure of the human eye and absorption spectra of cone cells](image)

The retina has two kinds of photoreceptor cells, cone and rod cells, and there are 120 million rods and about 6 million cones in the human retina.\textsuperscript{115} Also, the rod cells in the retina respond to a single photon of light, so the rod cells are very sensitive to light at low levels and responsible for black and white vision,\textsuperscript{116} while there are three kinds of cone cells which are concentrated in the retina and absorb at 450 nm (blue),...
530 nm (green), and 560 nm (red) as shown in Figure 4.1. It is indeed surprising that the maximum absorption of visual pigments ranges from 350nm to 680nm when realizing that this wide range is due merely to the interaction between the retinal receptors and the chromophore. Consequently, the cone cells have lower light sensitivity but provide color sensitivity.

The unique structures of the rods and cones are shown in Figure 4.2. The slender diameter of rod cells is 1-3 µm with a length of 40-50 µm, and the rod outer segment contains a stack of thousands of lipid bilayer disks covered in a sack of plasma membrane. These disks are not connected to the ciliary plasma membrane. The cones, on the other hand, have a shorter outer segment with their disks connected to the membrane continuously over the entire length of the outer segment.\textsuperscript{115}

![Figure 4.2. Rod and cone photoreceptors\textsuperscript{115}](#)
4.1.1. Rhodopsin

Rhodopsin is a protein which is expressed in the outer segments of the rod in vertebrate and invertebrate photoreceptors and is responsible for the initial steps in the vision process by detection of the photons by the rod cells.\textsuperscript{117, 118, 119} It is a member of the G protein-coupling receptor (GPCR) family, and it contains the protein called opsin as well as retinal, which is linked to it by a protonated Schiff base (PSB) as shown in Figure 4.3. The retinal undergoes an isomerization process from the 11-cis retinal to the all-trans retinal configuration when cells absorb light, and this is considered the first step in the photo-activation process.\textsuperscript{120}

\textbf{Figure 4.3.} 11-cis retinal, opsin, and rhodopsin

By using X-ray techniques, the structure of inactive bovine rhodopsin has been determined.\textsuperscript{121, 122} The molecular weight of bovine rhodopsin is 40 KDa and includes an apoprotein consisting of 348 amino acids and retinal chromophore.\textsuperscript{123, 124} The rhodopsin polypeptide chain side spans the membrane of the disk, folding into seven transmembrane $\alpha$-helices with the amino terminus on the intradiscal side and the carboxyl terminus on the cytoplasmic side of the disk membrane. The amino acid
Lys which is linked to the chromophore is transformed inside H7 in all pigments as shown in Figure 4.4.125, 126, 127

In the retinal binding pocket, the Trp265, Gla122, and Phe261 cover the β-ionone ring of the retinal chromophore while in the middle, between the retinal ring and the Schiff base linkage to Lys296, the side chain of Trp265 is located. In addition, down from the retinylidene group and close to both the β-ionone ring the tmethyl group of C13, there is the indole ring of Trp265. In their ground state, the amino acids Thr118 and Tyr268 are found near the center of the retinal, with the side chain of Glu122 located close to the β-ionone ring. Glu183 makes a close water-mediated approach to the retinal. When the protein is in its activated form, at the other end of the chromophore the Schiff base is protonated, and Glu113 serves as a counterion.121, 128

Figure 4.4. Amino acid sequence of bovine rhodopsin125
4.1.2. The visual transduction of Rh

In the photosensitivity in insects, crustacea, arthropods, cephalopods, and vertebrates, 11-cis retinal works as a chromophore. Visual transduction has been studied in photoreceptor rod cells, and under conditions of low light, rod cells are responsible for vision.\textsuperscript{129} When rhodopsin is exposed to light, the 11-cis retinal converts to an all trans-retinal form which results in a high level of strain on the opsin protein. A new conformation, called metarhodopsin II, is produced, which interacts with a G-protein in the disk membranes, known as transducin. A \( \alpha \)-subunit of transducin binds GTP, thus activating cGMP phosphodiesterase, which converts cGMP to GMP. When it is inactive, sodium ions are allowed to enter, and the pores in the membrane of the rod cells bind cGMP. When activated by light, cGMP is degraded, leading to the closure of the sodium pores, and causing the hyperpolarization of the membrane. A nerve impulse is triggered by the polarized rod cell through the synaptic terminal to other cells in the retina, which the brain interprets as visual stimulus.

All-trans retinal that results from photon isomerization is released from metarhodopsin II, after which it binds to CRALBP, and then reduces back to retinol in the neural retina’s Müller cells. The retinol is then transported by the IRBP into the RPE by way of the interphotoreceptor matrix and enters back into the cycle. It is noteworthy that retinal is active in no other cell types, even where other retinoids are very active.\textsuperscript{130, 131}
As shown in Figure 4.5, 11-cis-retinal is isomerized to trans isomer when irradiated with 500 nm light, forming the first intermediate photo-Rh in the low femto-sec time scale, the only light-triggered change occurring in rhodopsin. Changes to batho-Rh and others that take place subsequently are caused by the relaxation of the protein, thus relieving the high strain due to the “trans” 11-ene of the chromophore/protein complex. The strain energy, which is estimated to be 30-36 kcal/mol, is reflected in the red-shifted absorption maxima of photo-Rh (555 nm) and batho-Rh (535 nm). After the temperature of the batho-Rh has been raised above -140°C, the chromophore is able to rotate around the 11-12 single bond, forming lumirhodopsin with a retro 11-12-s-tram structure followed by meta-I Rh. At the meta-II stage, the extramembrane loop conformation on the cytoplasmic side brings about the excitation of the G protein, leading to a series of enzymatic reactions.  

Figure 4.5. Cycle of retinal and rhodopsin
It is evident that the metarhodopsin II in the photoreceptor membrane causes electrical changes in this membrane, which result in nerve impulse generation.\textsuperscript{134,135}

It is not yet known why 11-cis-retinal was naturally selected to be used as the chromophore for sensing light in visual pigments. The hybrid quantum mechanics/molecular mechanics (QM/MM) method has been used to study 7-cis, 9-cis, 11-cis and 13cis-retinal isomers (Figure 4.6) in the visual pigments of a variety of vertebrates and invertebrates, to determine their stability, structure, spectroscopy, and energetics. The electrostatic interaction between retinal and opsin is shown during the dark state to prefer 11cis-retinal over other cis-isomers, while 9-cis-retinal is found to be only slightly higher in energy than 11-cis-retinal in all of the pigments, providing strong evidence of the presence in nature of 9-cis-rhodopsin.\textsuperscript{136}

![Figure 4.6. Structure of retinal isomers](image)

100
Due to their important roles in biological processes such as cell growth and differentiation, embryonic development, reproduction, the immune response, and vision, retinoids, including vitamin A and its structural and functional analogues, have been studied intensively.\textsuperscript{137}

4.2. Synthesis of retinal

Natural pigments provide several target molecules which are activated by the absorption of a quantum of light in the course of biological processes.\textsuperscript{134} The ability to synthesize the analogues of 11-cis-retinal is of utmost importance in the study of rhodopsin on a bioorganic level, because this is the photoreceptor in visual transduction; however, the instability of 11-cis-retinoids makes their synthesis complicated.\textsuperscript{138} While many reports about the synthesis of retinal and its derivatives exist, only a few of them are in regard to the synthesis of stereoselective cis-retinal. Moreover, there are many challenges making the synthesis of a stereoselective cis-retinal difficult, including problems with the synthesis of the starting materials, the low stereoselectivity of the product, and the low yield.

4.2.1. Synthesis of 11-trans retinal

Valla et. al have synthesized 11-trans retinal in six steps. In the first step, the Knoevenagel condensation of β-ionone with cyanoacetic acid leads to nitrile 1, and to avoid a mixture of nitriles, the use of piperidine and benzene were necessary in this step. The nitrile 1 was reduced by DIBAL-H to give (β-ionylidene)acetaldehyde 2 which condensed with methyl cyanobutenoate to form a 75% yield of C20 a-cyano acid 3. Then decarboxylation of 3 in piperidine/pyridine gave retinonitrile 4 which
was reduced with DIBAL-H to form retinal 5 as shown in Scheme 4.1. In steps two and three, the pure sample of the (all-E)-1 and (all-E)-2 were obtained by CC (SiO₂, CH₂Cl₂) while CC (silica gel, CH₂Cl₂ was used to purify (all-E) in other steps.¹³⁹

![Scheme 4.1. Synthesis of 11-all trans retinal](image)

4.2.2. Synthesis of 11-cis-retinal

4.2.2.1. Preparation of 11-cis-retinal by semi-hydrogenation

HWE coupling of β-ionone 6 with dimethyl (3-trimethylsilyl-2-propynyl) phosphonate, which was prepared by the reacting of (3-bromo-1-propyn-1-yl) trimethylsilylane with dimethyl hydrogen phosphonate, formed 99% of the alkyne 7 (5:1E:Z at C9). Then compound 7 was deprotected with tetrabutylammonium fluoride (TBAF) to yield acetylene 8. The palladium coupling of 8 with vinyl iodide
gave 9 (91%) which was deprotected with tetrabutylammonium fluoride (TBAF) to form 10 (99%). Compound 10 was reduced with Cu/Ag-activated Zn dust to give 11 (Z:E 13:1 at C11). 11-cis retinol was oxidized by tetrapropylammonium perruthenate (TPAP)/4-methylmorpholine N-oxide (NMO) to form a quantitative yield of 11-cis-retinal 12 as shown in 4.2.\(^{138}\)

Scheme 4.2. Synthesis of 11-cis-retinal by semi-hydrogenation.
4.2.2.2. Preparation of 11-cis-retinal by Hiyama Cross-Coupling Reaction\textsuperscript{137, 140}

The stability, low toxicity, and low price of the silicon starting materials have made them more attractive to traditional organometallic donors. The Hiyama cross-coupling reaction has been used to synthesize 11-cis-retinal as shown in Scheme 4.3.

![Scheme 4.3. Hiyama Cross-Coupling reaction to synthesize 11-cis-retinal.](image)

In the first step, the metalation of enzyme 13 with chloromethylsilacyclobutane, benzyldimethylchlorosilane, or chlorodiisopropylsilane in the presence of BuLi as a base gave 14 c-e, which was converted to coupling partners (1Z,3E)-Dienylsilanes 15 c-e by reacting with Cp\textsubscript{2}Zr(H)Cl and pentane (Scheme 4.4). The Hiyama cross-coupling of trienyl iodide with (1Z,3E)-dienylsilanes 15 c-e in the presence of Pd\textsubscript{2}(dba)\textsubscript{3} afforded retinyl ethers 17 as pure isomers. However, the coupling of triflate (1Z,3E)-dienylsilanes 16 c-e failed and the only products isolated from the reaction mixtures were the starting materials. The deprotection of retinyl ethers 17 by TMSCl afforded trans-retinol and 11-cisretinol 18 which were oxidized by BaMnO\textsubscript{4} to obtain 11-cis-retinal 12 with a 90% yield (Scheme 4.4).
4.2.2.3. Preparation of 11-cis-retinal by using the Peterson reaction and the Emmons-Horner reaction.

The Peterson reaction has been used to synthesize the first stereoselective 11Z-retinal from the β-ionylideneacetaldehyde-tricarbonyliron complex as shown in Scheme 4.5. First, β-ionylideneacetaldehyde-tricarbonyliron complex 20 was obtained by the treatment of β-ionone-tricarbonyliron complex with lithium...
acetonitrile followed by its reduction by DIBAL. Then compound 20 reacted with the lithium enolate of ethyl trimethylsilylacetoacetate to give the 11Z-isomer 21 which reacted with triphenylstannylmethyllithium to form a high yield of C18-ketone-tricarbonyliron complex 23 which reacted with diisopropyl cyanomethyl phosphonate (The Emmons-Horner reaction) to form 24. Then 24 was transformed to 11Z-retinal 12 by reaction with CuCl2 and then reduction by DIBAL.\textsuperscript{141,142}

\begin{align*}
\text{Me}_{3}\text{SiCH}_{2}\text{CO}_{2}\text{Et} & \quad \text{LDA, -78°C} \\
\text{EtO}_{2}\text{C} & \quad \text{Fe(CO)}_{3} \\
\text{Ph}_{3}\text{SnCH}_{2}I/\text{n-BuLi} & \quad 79\% \\
\text{H}_{3}\text{CO}_{2} & \quad \text{Fe(CO)}_{3} \\
\text{CuCl}_{2} & \quad 72\% \\
\end{align*}

Scheme 4.4. Preparation of 11-cis-retinal by using the Peterson reaction and the Emmons-Horner reaction.

The mechanism of the Peterson reaction to form stereoselective 11Z-retinal from the \(\beta\)-ionylideneacetaldehyde-tricarbonyliron complex has not yet been explained.
However, there are two forms from the six transition states, 21a and 22a, that are appropriate due to both of them not having steric repulsion between the 9-methyl group and the ethyl enolate as present in the other transition states. In addition, in comparing 21a to 21b, the former has little interaction between the diene-tricarbonyliron complex and the trimethylsilyl group compared with the latter. As a result, the 21a was the transition state preferred to form the Z-olefin by syn elimination from the β-hydroxysilyl adduct.\textsuperscript{141, 142}

![21a and 21b](image)

4.2.2.4. Synthesis of 11-cis retinal by Suzuki-Miyaura reaction or stannanes (a Stille reaction).

The (11Z)-retinal 12 has been synthesized by Uenishi et al. where aldehyde 27 was reacted with carbon tetrabromide and triphenylphosphane to form an 88% yield of compound 28. The stereoselective hydrogenolysis of 28 with tributyltin hydride and catalytic [Pd(PPh\textsubscript{3})\textsubscript{4}] formed 86% of compound 29. The Suzuki coupling reaction of compound 29 with 26, in the presence of cat.[Pd(PPh\textsubscript{3})\textsubscript{4}], Ag\textsubscript{2} CO\textsubscript{3}, and KOH formed 77% of compound 30 which was deprotected with tetrabutylammonium
fluoride and oxidized with BaMnO₄ to form (11Z)-retinal. Compound 26 was obtained from regio- and stereoselective addition reactions of tributylstannyl cuprate with 2-buten-1-ol to form (E)-3-tributylstannyl-2-buten-1-ol 25. Compound 25 protected with t-BuMe₂SiCl (TBDMSCl) and treated with boronic acid gave 57% of compound 26 (Scheme 4.5). Also, a palladium-catalyzed coupling reaction of enol triflates and tributylstannylolefins has been used to synthesize 13-demethyl or 13-substituted all-E- and 9Z-retinoic acids with good yields.

Scheme 4.5. Synthesis of 11-cis retinal by Suzuki-Miyaura reaction or stannanes (a Stille reaction).

4.2.3. Synthesis of deuterated 11-cis-retinals

Synthesis of 11-Z-[5-CD₃]-retinal, 11-Z-[9-CD₃]retinal, and 11-Z-[13-CD₃]-retinal²,³
18-Trideutero-49A and 19-trideutero-11-cis-retinal 49B were obtained by a Wittig reaction between (β-ionyldieneethyl)triphenylphosphoniumbromide and (E)-4-acetoxy-2-methyl-crotonaldehyde 48 to form retinyl acetates which were reacted with KOH to give retinols; then these retinols oxidized with MnO2 to form a mixture of cis-and trans-retinal. High performance liquid chromatography HPLC was used to separate and purify 18-Trideutero-49A and 19-trideutero-11-cis-retinal 4B as shown in Scheme 4.6.


Compound 42A was synthesized in 11 steps starting with the formulation of 2-Methylcyclohexanone 31 by ethyl formate to give compound 32 which methylated with methyl iodide to give compound 33. Compound 33 was converted into 34 by reacting with sodium hydroxide and then methylated with trideuteromethyl iodide to
form ketone 36. Diol 37 was obtained by the treatment of compound 35 with a Grignard reagent of 3-butyne-2-ol and was then acetylated with acetic anhydride to form 37. The dehydration of compund 37 by phosphoryl chloride and pyridine gave acetate 38 which, when treated with LiAIH, gave 39. Compound 39 was oxidized with MnO₂ to form 40 which was treated with a Grignard reagent of vinylmagnesium bromide to give 41. Compound 41 reacted with triphenylphosphonium bromide to give 42 and 43.

Scheme 4.7. Synthesis compounds 42 and 43.
give 42 A. On the other hand, Compound 42 B was synthesized by a base-catalyzed hydrogen exchange in D$_2$O/pyridine to give compound 43 which was treated with a Grignard reagent of vinylmagnesium bromide; then the product reacted with triphenylphosphonium bromide to give 42 B as shown in Scheme 4.7. In addition, (E)-4-acetoxy-2-methyl-crotonaldehyde 48 was obtained by the oxidization of compound 45 with m-chloroperbenzoic acid to form epoxide 46 which is converted by copper(II) chloride and lithium chloride to aldehyde 47. Then compound 47 was reacted with potassium acetate to give acetate 48 as shown in Scheme 4.8.


In addition, the Ito procedure, which uses the diene tricarbonyliron complex has been used to synthesize 11-Z-[5-CD3]-retinal, 11-Z-[9-CD3]retinal, and 11-Z-[13-CD3]-retinal. The regioselectively deuterated β-ionone 40 was synthesized by the reacting of 2,2-dimethylcyclohexanone with CD$_3$I in the presence of lithium diisopropylamide (LDA) as a base to give 80% of compound 51 which, when treated with LDA and N-phenylbis(trifluoromethanesulfonylimide) (NPhTf$_2$) formed 65% of
The Heck reaction of triflate 52 with methylvinylketone and PdCl$_2$(PPh$_3$)$_2$ in DMF gave [5-CD$_3$]-β-ionone 40. While the regioselectively deuterated β-ionone 43 was obtained by treatment of the β-ionone with bromine and NaOH to form 53 which reacted with methoxymethylamine hydrochloride in the presence of DMAP and EDC to give the Weinreb amide 54. The compound 54 was treated with deuterated methylmagnesium iodide to give [9-CD$_3$]-β-ionone 43 in good yield (Scheme 4.9).


The [5-CD$_3$]- and [9-CD$_3$]-β-ionones were treated with triirondodecacarbonyl (Fe$_3$(CO)$_{12}$) to form the diene/tricarbonyliron complex 55 which alkylated with acetonitrile in the presence of LDA and then was reduced by diisobutylaluminum hydride (DIBAL) to give the (E)-conjugated aldehyde 57. The Peterson olefination
Scheme 4.10. Synthesis of 11-Z-[5-CD₃]-retinal, 11-Z-[9-CD₃]retinal of Z-ester 57 with ethyl trimethylsilylacetate formed 58 which was treated with isopropylmagnesium bromide and methoxymethylamine to give Weinreb amide 59 which was alkylated with methyl lithium to form 60. The Horner–Emmons reaction of 60 with diisopropyl cyanomethylphosphonate and sodium hydride formed 61. The demetalation of 61 with copper(II) dichloride gave 62 which was reduced with
DIBAL to form 11-Z-[5-CD3]-retinal 49A or 11-Z-[9-CD3]-retinal 49B as shown in Scheme 4.10.\textsuperscript{145}

The Weinreb amide 63 was alkylated with CD3MgI to give 64. The Horner–Emmons reaction of 64 with diisopropyl cyanomethylphosphonate and sodium hydride formed 65 which demetalated with CuCl\textsubscript{2} to give 66 which was reduced with DIBAL to form 11-Z-[13-CD3]-retinal 67.\textsuperscript{145}

Scheme 4.11. Synthesis of 11-Z-[13-CD\textsubscript{3}]-retinal.

Furthermore, there are some studies that aim to examine and understand both isomer structure and the role of the methyl groups in 11-cis-retinal in visual pigment. For example, the monodemethylated forms of 11-cis demethylretinal 68 and 69 (Figure
4.7) show that the methyl groups in C1 are more important for pigment formation than the methyl group in C5. In addition, the extra methyl group at C18 (70) caused a rise in the stability of the retinal in the visual pigment complex due to the increased level of hydrophobic interactions.146

Figure 4.7. Structure of compounds 68-70.

In addition, a study of the capability of (9Z)- and (11Z)-8-Methylretinals to bind with opsin showed the bond to be extremely strained because of the steric clashes of retinal substituents with the receptor in the protein opsin.147 In another study, the 11-cis-9-demethylretinal failed to yield the biochemically active form of rhodopsin (meta II) upon photoisomerization.148 Also, the incorporation of (11Z)-3-diazo-4-oxoretinal 71 with rhodopsin formed the diazoketo rhodopsin which upon bleaching, gave rise to intermediates bathoRh, lumi-Rh, meta-Rh, and meta-II-Rh at lower temperatures, and the change that happens at the batho-Rh to lumi-Rh stage is considered the major conformation leading to visual transduction.149
5.1. Preparation of 9-CD₃-11-cis-retinal by semi-hydrogenation

In this chapter we synthesized 9 CD₃-11-cis-retinal by the semi-hydrogenation of 11-cis-retinol precursors with Cu/Ag-activated zinc dust, as shown in Scheme 5.1. The Horner–Wadsworth–Emmons (HWE) coupling of 9-CD₃-β-Ionone 72 with dimethyl (3-trimethylsilyl-2-propynyl) phosphonate 74, which was prepared by the reacting of (3-bromo-1-propyn-1-yl) trimethylsilane with dimethyl hydrogen phosphonate, formed 94% of the alkyne 75 (5:1 E:Z at C9). Then compound 75 was deprotected with tetrabutylammonium fluoride (TBAF) to yield acetylene 76. Hydromagnesiation of 2-butynyl alcohol with isobutylmagnesium bromide in the presence of Cp₂TiCl₂ as a catalyst was then quenched with iodine to give 78 which was followed by the protection of alcohol with tributyldimethylsilyl chloride to give 79. The palladium coupling of 76 with vinyl iodide 79 afforded 98 (91%) which was deprotected with tetrabutylammonium fluoride (TBAF) to form 80 (83%). Compound 80 was reduced with Cu/Ag-activated Zn dust to give 81 (Z:E -13:1 at C11). 11-cis retinol was oxidized by MnO₂ to yield of 11-cis-retinal 83 as shown in Scheme 5.1.
Scheme 5.1. Synthesis of 9-CD$_3$-11-cis-Retinal.
5.2. Activation Mechanism of GPCR Rhodopsin by Solid-State \(^2\)H NMR Spectroscopy

The intermediates in the rhodopsin photoisomerization process, which include bathorhodopsin (Batho), lumirhodopsin, and metarhodopsin (Meta I, and Meta II), are characterized by a UV-visible absorption spectrum. In addition, the deprotonation of the Schiff base coupled to the protonation of the counterion at Glu113 causes the large shift in absorption from 480 nm in Meta I to 380 nm in Meta II.\(^{150}\) Comparison of the dark ground state with the structural information on the Meta II intermediate is necessary to understand the Rh active state. The mechanism by which rhodopsin is light activated is made understandable by learning more about the stereochemistry and orientation of the retinal chromophore in its photointermediate states.\(^{151}\)

The X-ray structures of rhodopsin have been discovered recently.\(^{152,153}\) These reported crystal structures of opsin regenerated with all \textit{trans} retinal have shown flipped orientation of all the \textit{trans} retinal chromophore in the active Meta-II state, which includes alteration in the roll angle of the \(\beta\)-ionone ring and the polyene chain in the active Meta-II state, compared to the inactive dark state.\(^{154-155}\) In the lumi and Meta-I states, there is a modification of the orientation of both the C9- and the C13-Me groups on the polyene chain, so that they point towards the extracellular side rather than pointing towards the cytoplasmic side as they do in the active Meta II state.\(^{156,157-158}\) In contrast, other X-ray crystallography\(^{153,159}\) results harmonize with \(^{13}\)C dipolar assisted rotational resonance (DAAR) NMR studies,\(^{160,161}\) revealing that
the retinal roll angle was unchanged in the active state. $^{13}$C (DAAR) NMR studies did not indicate a retinal flip about its axis, but only displacement toward helix H5. Based on this difference, more data is desired to resolve the retinal structure in the Meta-II state. In this study, solid-state $^2$H NMR spectroscopy is used to study the structure and orientation of the 9-CD$_3$-11-cis-retinal ligand in the active state of rhodopsin and to compare its results to both 5-CD$_3$-11-cis-retinal and 13-CD$_3$-11-cis-retinal. This work was done by Dr. Michael F. Brown’s group in the Department of Physics at the University of Arizona in collaboration with Dr. Nasri Nesnas in the Department of Chemistry at the Florida Institute of Technology.

Information from recent studies shows that the retinal chromophore is highly distorted in the regions of both the β-ionone ring and the C$_{11}=$C$_{12}$ double bond, and from photocrosslinking studies, the activation of the photoreceptor occurs from the release of the β-ionone ring from the retinal binding pocket in association with helical movements.$^{162,163}$

Solid-state NMR spectroscopy is the characteristic tool for investigating the properties of molecules in the solid state, liquid crystals, and many supramolecular biological assemblies.$^{164}$

In this study, Rhodopsin was regenerated with retinal which contained $^2$H-labeled methyl groups in the positions C5-, C9-, and C13-Me and was then recombined into phospholipid bilayers. These were then placed on glass slides using a spin-dry centrifugation technique and were bleached and trapped in their active state; $^2$H NMR
spectra of the samples were measured at different orientations with respect to the magnetic field.

Two structures with no steric clashes in the binding pocket of the active rhodopsin were discovered, one of which has a great similarity in conformation to that of the “flipped” X-ray structures. Although the other structure has the same position of the β-ionone ring, the polyene chain and β-ionone ring are not flipped and the orientation of the chromophore is similar to that of the dark state.

The question remains as to how it is possible to observe different structures when using different methods and how the contradictory data can be reconciled. The presence of both retinal orientations may be possible, with the prevailing and observable structure in a particular experiment decided by the differing system properties and preparation conditions in the studies being discussed; in the work being studied here, after opsin crystals were soaked with all trans retinal, the rhodopsin mutant was photo-bleached and then crystallized, the expressed rhodopsin was photoactivated in detergent micelles, and the rhodopsin was regenerated with ²H-labeled retinal and studied in lipid bilayers. It is unlikely that any of the methods where only a single orientation is seen can totally eliminate the existence of the other retinal orientation. A further question would deal with the importance of the retinal orientation or roll angle in the activation of rhodopsin, since the same β-ionone ring position and a similar angle of the retinal tilt are provided with both orientations.
5.4. Conclusions

In this Chapter, we synthesized 9 CD$_3$-11-cis-retinal by semi-hydrogenation of 11-cis-retinol precursors with Cu/Ag-activated zinc dust and applied solid-state $^2$H NMR spectroscopy to study the structure and orientation of the retinal ligand in the active state of rhodopsin. $^2$H NMR and linear dichroism (LD) data show two possible structures for the binding pocket of the active state of rhodopsin. The first structure conforms closely to the X-ray structures with a β-ionone ring and a polyene chain flipped around the long axis of the retinal. The second structure, with the same position of the β-ionone ring, is not flipped and has an orientation similar to that of the dark state. While it is not possible to establish conclusively the presence of the second retinal conformation in the Meta II state from the $^2$H NMR and LD results, partially due to the issue of even parity, it is consistent with $^{13}$C DARR NMR data and CD studies of locked retinoids.

5.5. Experimental

5.5.1. General data

Reactions were conducted under N$_2$ using a standard Schlenk line. All compounds were characterized by $^1$H, and $^{13}$C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer and referenced to internal tetramethylsilane (TMS).

All chemicals were used as received without further purification: Potassium hydroxide, and silver nitrate (Fisher); β-ionone, 3-(Trimethylsilyl)propargyl bromide, isobutylmagnesium bromide, tert-butylchlorodiphenylsilane, sodium deuteroxide, tert-butyllithium, copper(I) iodide, isopropylamine, manganese dioxide,
triethylamine, 4-dimethylaminopyridine, and zinc (ACROS Organics); 2-butyne-1-
ol(Chem Impex Int'L Inc); and bis(Triphenylphosphine)palladium (II) chloride
(Oakwood Products); tetra-n-butylammonium fluoride, and copper(II) acetate (Alfa
Aesar). dichloromethene, hexane, ethyl acetate and THF (VWR); and CDCl₃ and
C₆D₆ (Cambridge Isotope Laboratories). 11-cis-retinal is an unstable compound, so
we stored it in benzene at -78°C.

**Synthesis of 9-CD₃-β-Ionone (72).** A 25 mL round-bottom flask was charged
with β-ionone (1.0 mL, 0.0050 mol), sodium deuteroxide solution (4 mL) and a
stirrer bar. Then the heterogeneous mixture was chilled to 0 °C using an ice-bath
and stirred for 10 hours under N₂ gas. After 10 hours, the mixture was extracted
with CH₂Cl₂ (5 mL) and the CH₂Cl₂ solvent was removed under reduced pressure to
give 72 (0.95 g, 98%). ¹H NMR (400 MHz, Chloroform-d) δ 7.27 (d, J = 15.7 Hz, 1H),
6.11 (d, J = 16.4 Hz, 1H), 2.07 (t, J = 6.0 Hz, 2H), 1.76 (s, 2H), 1.63 (p, J = 6.3 Hz, 3H),
1.55 – 1.40 (m, 2H), 1.07 (s, 6H).

**Synthesis of dimethyl (3-trimethylsilyl-2-propynyl)phosphonate (74).** A 25
mL round-bottom flask was charged with diethyl phosphonate (3.0 mL, 24 mmol),
anhydrous THF (6 mL), and a stirrer bar. The solution was chilled to 0 °C using an
ice-bath and stirred for 10 mins under N₂ gas. Then a solution of sodium
bis(trimethylsilyl) amide 2 M in THF(10 mL, 24 mmol) was added. The solution
was stirred for one hour then treated with 3-bromo-1-trimethylsilylprop-1-yne (3.24
mL, 20.0 mmol) in anhydrous THF (6 mL) and stirred at the same temperature for 4
hours. The mixture was diluted with H2O (20 mL), then extracted with CH2Cl2 (2×20 mL) and washed with (1.5 mL HCl 2M, 1.5 mL H2O). The extract was concentrated under reduced pressure and purified by column chromatography (silica gel, hexane/EtAc, 1:1) to give 74 (5.83 g, 98%) 1H NMR (400 MHz, CDCl3) δ 4.35–3.97 (m, 4H), 2.81 (d, J = 22.3 Hz, 2H), 1.36 (t, J = 7.1 Hz, 6H), 0.16 (s, 9H). 13C NMR (101 MHz, CDCl3) δ 95.91 (d, J = 13.8 Hz), 88.09 (d, J = 8.8 Hz), 63.23 (d, J = 6.7 Hz), 20.23 (s), 18.80 (s), 16.58 (d, J = 6.0 Hz).

Synthesis of 1,3,3-trimethyl-2-[(1E,3E)-3-methyl-d3-6-(trimethylsilyl)-1,3-hexa-dien-5-yn-1-yl]cyclohexene (75). A 25 mL round-bottom flask was charged with dimethyl (3-trimethylsilyl-2-propynyl)phosphonate 74 (0.124 g, 0.500 mmol), anhydrous THF (3 mL), and a stirrer bar. The solution was chilled to 0 °C using an ice-bath and stirred for 10 mins under N2 gas. The solution was treated with nBuLi (0.36 mL, 0.57 mmol) to give a red solution and the reaction mixture was stirred at room temperature for one hour min. After one hour 9-CD3-β-Ionone 72 (0.0488 g, 0.250 mmol) in anhydrous THF (3 mL) was added. The mixture was stirred for an additional 24 h, and was then quenched with aqueous NH4Cl and extracted with ether (2×15 mL). The organic phases were washed with saturated NaCl and dried over anhydrous Na2SO4. The product was purified by column chromatography (silica gel, hexanes/CH2Cl2, 1:1) to give 75 (0.117 g, 94%; 3:1 E:Z at C9). This compound was used directly in the following reaction. 1H NMR (400 MHz, Chloroform-d) δ 6.90 (d, J = 16.4 Hz, 1H), 6.36 (d, J = 16.6 Hz, 1H), 6.26 (d, J = 15.9 Hz, 1H), 6.07 (d, J = 16.1 Hz, 1H), 5.43 (s, 1H), 5.35 (s, 1H), 2.08-2.06 (m, 2H), 2.01-1.97 (m, 2H),
1.77 (s, 3H), 1.68 (s, 3H), 1.65 – 1.56 (m, 2H), 1.05-1.46 (m, 2H), 1.08 (s, 6H), 1.00 (s, 6H), 0.21 (s, 9H), 0.19 (s, 9H).

Synthesis of 1,3,3-Trimethyl-2-[(1E,3E)-3-methyl-d3-1,3-hexadien-5-yn-1-yl]cyclohexene (76). A 25 mL round-bottom flask was charged with compound 75 (0.237 g, 0.820 mmol), anhydrous THF (3.6 mL), and a stirrer bar. The solution was treated with tetrabutylammonium fluoride nBu4NF (3.29 mL; 1 M in THF; 3.28 mmol) and stirred for 2 hours. After 2 hours the reaction mixture was quenched with aqueous NH4Cl (15mL) and extracted with Et2O (2×15 mL). The organic layer was washed with saturated aqueous solution NaCl (10 mL) and dried over anhydrous Na2SO4. The extract was concentrated under reduced pressure and purified by column chromatography (silica gel, hexane/ CH2Cl2, 1:1) to give 76 (5.83 g, 98%, 3:1 E:Z at C9). 1H NMR (400 MHz, Chloroform-d) δ 6.81 (d, J = 16.3 Hz, 1H), 6.34 (d, J = 16.9 Hz, 1H), 6.27 (d, J = 16.0 Hz, 1H), 6.09 (d, J = 16.4 Hz, 1H), 5.40 (s, 1H), 5.33 (s, 1H), 3.28 (s, 1H), 3.16 (s, 1H), 2.03-1.97 (m, 4H), 1.75 (s, 3H), 1.69 (s, 3H), 1.66-1.57 (m, 4H), 1.50-1.42 (m 4H), 1.04 (s, 6H), 1.01 (s, 6H).

Synthesis of (E)-3-iodobut-2-en-1-ol (78). A 250 mL round-bottom flask was charged with isobutylmagnesium chloride (27 mL, 0.054 mmol) and a stirrer bar under N2 gas and cooled to 0 °C using an ice-bath. Titanocene dichloride (281mg, 0.00113 mmol) was added, and the mixture was stirred for 10 min at 0 C. Then 2-butyn-1-ol (1.68 mL, 0.0230 mmol) in ether (6 mL) was added, and the mixture was stirred for an additional 24 h during which time it warmed to room temperature.
After 24 h, the reaction mixture was then cooled to -78 °C and iodine (10.29 g, 0.04000 mmol) was added. The mixture was stirred overnight while it was gradually warming to room temperature. The reaction was quenched by saturated aqueous sodium thiosulfate. (2×15 mL) and extracted with ethyl acetate (3×10 mL). The organic layer was washed with brine (2×15) and dried over magnesium sulfate and ethyl acetate was removed by rotary evaporation. The product was purified by column chromatography (silica gel, hexanes/CH₂Cl₂, 1:1) to give 78 (2.055 g, 45%).

\[ ^1H \text{ NMR (400 MHz, CDCl}_3 \text{)} \delta 6.41 (t, J = 7.7 Hz, 1H), 4.10 (d, J = 6.8 Hz, 2H), 2.46 (s, 3H), 1.43 (s, 1H). \]

\[ ^{13}C \text{ NMR (101 MHz, CDCl}_3 \text{)} \delta 139.70, 98.58, 59.97, 28.03. \]

**Synthesis of 1,1'-(1,1-dimethylethyl)[[(2E)-3-iodo-2-buten-1-yl]oxy]silylene] bis [benzene] (79)**. A 25 mL round-bottom flask was charged with compound 78 (1.98 g, 0.0100 mol), CH₂Cl₂ (10 mL) and a stirrer bar. The solution was stirred for 10 mins in an ice bath. under N₂ gas. Then TBDPSCl (2.8 mL, 0.011 mol) were added and the mixture was stirred at 0 °C for 3 h and then quenched by the addition of a sodium bicarbonate solution. (2×15 mL). The organic layers were washed with brine and dried over sodium sulfate, and the CH₂Cl₂ solvent was removed by rotary evaporation. The product was purified by silica gel flash chromatography (hexane) to give silyl ether 79 (4.30 g, 98%).

\[ ^1H \text{ NMR (400 MHz, Chloroform-d)} \delta 7.69 (d, J = 6.7 Hz, 4H), 7.43 (q, J = 8.1, 6.6 Hz, 6H), 6.37 (t, J = 6.1 Hz, 1H), 4.14 (d, J = 6.4 Hz, 2H), 2.20 (s, 3H), 1.06 (s, 9H). \]
**Synthesis of compound (80).** A 25 mL round-bottom flask was charged with compound 79 (63.6 mg, 0.145 mmol), iPrNH$_2$ (0.5 mL), and a stirrer bar. The solution was stirred for 10 mins at room temperature under N$_2$ gas, then tetrakis(triphenylphosphine)palladium (1.945 mg, 0.001000 mmol) was added and the solution was stirred at the same temperature for 5 min. Then CuI (1.4 mg, 0.0070 mmol) was added. After 5 min, compound 76 (26 mg, 0.12 mmol) was added, and the reaction mixture was stirred at room temperature for 7 h. The iPrNH$_2$ was removed under reduced pressure; then the reaction mixture was dissolved in Et$_2$O (7 mL), and extracted with aqueous NH$_4$Cl (7 mL) and saturated aqueous solution NaCl (10 mL) and dried over anhydrous Na$_2$SO$_4$. The extract was concentrated under reduced pressure and purified by column chromatography (silica gel, haptane) to give 80 (29 mg, 83%, 3:1 E:Z at C9).

**Synthesis of compound (81).** A 25 mL round-bottom flask was charged with compound 80 (0.182 g, 0.346 mmol), anhydrous THF (3.6 mL), and a stirrer bar. The solution was treated with tetrabutylammonium fluoride nBu$_4$NF (1 mL; 1 M in THF; 3.28 mmol) and stirred for 2 hours. After 2 hours the reaction mixture was quenched with aqueous NH$_4$Cl (10 mL) and extracted with Et$_2$O (2×15 mL). The organic layer was washed with a saturated aqueous solution NaCl (10 mL) and dried over anhydrous Na$_2$SO$_4$. The extract was concentrated under reduced pressure and purified by column chromatography (silica gel, 10% EtAc:hexane) to give 81 which was used directly for the next step.
**Synthesis of 9-CD$_3$-11-cis-retinal (83).** A 25 mL round-bottom flask was charged with Zn dust, distilled H$_2$O (40 mL), and a stirrer bar. Argon was bubbled through the mixture for 15 min. Then Cu(OAc)$_2$ (0.75 g) was added and the solution was stirred for 15 mins. After 15 mins AgNO$_3$ (0.75 g) was added to the mixture and stirred for 30 min. Then the activated Zn was filtered and washed with H$_2$O and then transferred directly to a 25 mL round-bottom flask containing a solvent (12.5 mL H$_2$O/17.5 mLMeOH). Then compound 81 (170 mg, 0.410 mmol) was added to this mixture and stirred in the dark at room temperature 24 h. The Zn dust was then filtered through Celite and washed with Et$_2$O and H$_2$O. The organic layer was washed by NaCl and dried over anhydrous Na$_2$SO$_4$. The organic solvent was removed under reduced pressure to give 82. Then 25 mL round-bottom flask was charged with compound 82 (0.2133g, 0.0007400 mol), CH$_2$Cl$_2$ (1.5 mL) and MnO$_2$ (0.170 g, 0.00196 mol). The mixture was stirred at 0°C under N$_2$ gas for 2h hours. After two hours the mixture was filtered and CH$_2$Cl$_2$ solvent was removed by rotary evaporation. The product was purified by column chromatography (silica gel, 20% EtAc: Hexane) to give 83 (5.2 mg83%). $^1$H NMR (400 MHz, Benzene-$d_6$) δ 9.91 (d, J = 7.8 Hz, 1H), 6.59 (d, J = 12.6 Hz, 1H), 6.44 – 6.28 (m, 2H), 6.23 (d, J = 15.9 Hz, 1H), 6.11 (d, J = 6.9 Hz, 1H), 5.59 (d, J = 13.1 Hz, 1H), 1.98 – 1.85 (m, 2H), 1.76 (s, 3H), 1.69 (s, 3H), 1.61 – 1.50 (m, 2H), 1.50 – 1.41 (m, 2H), 1.07 (s, 6H).
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Appendix
Figure 6.1 $^1$H NMR spectrum of $S$-methyl hydrazinecarbodithioate (195).

Figure 6.2 $^{13}$C NMR spectrum of $S$-methyl hydrazinecarbodithioate (195).
Figure 6.3  $^1$H NMR spectrum of $S$-benzyl hydrazinecarbodithioate (196).

Figure 6.4  $^{13}$C NMR spectrum of 4-nitrobenzyl hydrazinecarbodithioate (197).
Figure 6.5  \( ^1 \)H NMR spectrum of S-methyl 3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate (201).

Figure 6.6  \( ^{13} \)C NMR spectrum of S-methyl 3-(2-hydroxy-5-nitrophenyl) methylenedithio carbazate (201).
Figure 6.7  $^1$H NMR spectrum of $S$-benzyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate (202).

Figure 6.8  $^{13}$C NMR spectrum of $S$-benzyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate (202).
Figure 6.9 \(^1\)H NMR spectrum of S-4-bromobenzyl-3-(2-hydroxy-5-nitrophenyl)methylenedithiocarbazate (204).

Figure 6.10 \(^{13}\)C NMR spectrum of S-4-bromobenzyl-3-(2-hydroxy-5-nitrophenyl)methylenedithiocarbazate (204).
Figure 6.11  $^1$H NMR spectrum of S-4-vinylbenzyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate (205).

Figure 6.12  $^{13}$C NMR spectrum of S-4-vinylbenzyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate (205).
Figure 6.13  $^1$H NMR spectrum of $S$-benzyl-3-(4-(diethylamino)-2-hydroxy)methylene dithiocarbazate (206).

Figure 6.14  $^{13}$C NMR spectrum of $S$-benzyl-3-(4-(diethylamino)-2-hydroxy)methylene dithiocarbazate (206).
Figure 6.15 $^{13}$C NMR spectrum of S-4-bromobenzyl-3-(4-(diethylamino)-2-hydroxy) methylenedithiocarbazate (207).

Figure 6.16 $^1$H NMR spectrum of S-4-vinylbenzyl-3-(4-(diethylamino)-2-hydroxy) methylenedithiocarbazate (208).
Figure 6.17  $^{13}$C NMR spectrum of S-4-vinylbenzyl--3-(4-(diethylamino)-2-hydroxy) methylenedithiocarbazate (208).

Figure 6.18  $^1$H NMR spectrum of S-4-benzoic acid methyl ester-3-(4-(diethyl amino)-2-hydroxy)methylene dithiocarbazate (209).
Figure 16.19 $^{13}$C NMR spectrum of $S$-4-benzoic acid methyl ester-3-(4-(diethyl amino)-2-hydroxy)methylene dithiocarbazate (209).

Figure 6.20 $^1$H NMR spectrum of $K[VO_2\{S$-methyl-3-(2-hydroxy-5-nitrophenyl) methylene dithiocarbazate$\}]$ (211).
Figure 6.21 $^{13}$C NMR spectrum of K[VO$_2${S-methyl-3-(2-hydroxy-5-nitrophenyl) methylenedithiocarbazate}] (211).

Figure 6.22 $^1$H NMR spectrum of K[VO$_2${S-benzyl-3-(2-hydroxy-5-nitrophenyl) methylene dithiocarbazate}] (212).
Figure 6.23  $^{13}$C NMR spectrum of K[VO$_2$\{S-benzyl-3-(2-hydroxy-5-nitrophenyl) methylene dithio carbazate}\}] (212).

Figure 6.24  $^1$H NMR spectrum of K[VO$_2$\{S-4-bromobenzyl-3-(2-hydroxy-5-nitrophenyl)methyl enedithiocarbazate}\}] (214).
Figure 6.25  $^{13}$C NMR spectrum of K[VO$_2$\{(S-4-bromobenzyl-3-(2-hydroxy-5-nitrophenyl)methyl enedithiocarbazate)}\]} (214).

Figure 6.26  $^1$H NMR spectrum of K[VO$_2$\{(S-4-vinyl-benzyl-3-(2-hydroxy-5-nitrophenyl)methyl enedithiocarbazate)}\]} (215).
Figure 6.27 $^{13}$C NMR spectrum of K[VO$_2${(S-4-vinyl-benzyl-3-(2-hydroxy-5-nitrophenyl)methyl enedithiocarbazate)}] (215).

Figure 6.28 $^1$H NMR spectrum of 9-CD$_3$-β-Ionone.
Figure 6.29  $^1$H NMR spectrum of dimethyl (3-trimethylsilyl-2-propynyl)phosphonate

Figure 6.30  $^{13}$C NMR NMR spectrum of dimethyl (3-trimethylsilyl-2-propynyl)phosphonate
Figure 6.31 $^1$H NMR spectrum of 1,3,3-trimethyl-2-[(1$E$,3$E$)-3-methyl-d$_3$-6-(trimethylsilyl)-1,3-hexa-dien-5-yn-1-yl]cyclohexene and 1,3,3-trimethyl-2-(1$E$,3$Z$)-3-methyl-d$_3$-6-(trimethylsilyl)-1,3-hexa-dien-5-yn-1-yl]cyclohexene (75).
Figure 6.32 $^1$H NMR NMR spectrum of 1,3,3-Trimethyl-2-[(1$E$,3$E$)-3-methyl-d3-1,3-hexadien-5-yn-1-yl]cyclo- hexene and 1,3,3-Trimethyl-2-[(1$E$,3$Z$)-3-methyl-d3-1,3-hexadien-5-yn-1-yl]cyclo- hexene (76).

Figure 6.33 $^1$H NMR spectrum of ($E$)-3-iodobut-2-en-1-ol (78).
Figure 6.34  $^{13}$C NMR spectrum of (E)-3-iodobut-2-en-1-ol (78).

Figure 6.35  $^1$H NMR spectrum of 1,1'-[(1,1-dimethylethyl)[(2E)-3-iodo-2-buten-1-yl]oxy] silylene] bis [benzene] (79).
Figure 6.36  \(^1\)H NMR spectrum of compound 80.

Figure 6.37  \(^1\)H NMR spectrum of 9-CD3-11-cis-Retinal (83).