SYNTHESIS OF OCTADECANAMIDE CHALCONES, OCTADECANAMIDE PYRAZOLINES AND THEIR *IN-SILICO* AND *IN-VITRO* ANTIMICROBIAL ACTIVITY

by

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Declaration

I, **SHURHOVOLIE TSURHO**, hereby declare that the matter illustrated in this Thesis entitled **"SYNTHESIS OF OCTADECANAMIDE CHALCONES, OCTADECANAMIDE PYRAZOLINES AND THEIR *IN-SILICO* AND *IN-VITRO* ANTIMICROBIAL ACTIVITY"** submitted by me for the degree of Doctor of Philosophy in Chemistry is the result of investigations carried out byme in the Department of Chemistry, Nagaland University under the direct Supervision of **Dr. MADDELA PRABHAKAR**, Assistant Professor, Department of Chemistry, Nagaland University.

I further declare that in keeping with the general practice of reporting scientific observations, due acknowledgments have been made wherever the work described is based on the findings of other investigators and the contents of this thesis did not form the basis for award of any degree to me or to the best of my knowledge to anybody else.

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Certificate

It is to certify that the thesis entitled ***"Synthesis of Octadecanamide Chalcones, Octadecanamide Pyrazolines and Their In-Silico and In-Vitro Antimicrobial Activity"*** is based on the original work done by ***Mr. Shurhovolie Tsurho*** leading to advancement of knowledge in the field of Organic Chemistry and Medicinal Chemistry, under my direct supervision in the Department of Chemistry, Nagaland University, Lumami, Nagaland.

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Dedicated
to
Apuo & Azuo

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******

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(SHURHOVOLIE TSURHO)

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Abbreviations

AcOH	Acetic acid
Al_2O_3	Aluminum oxide
$AlCl_3$	Aluminium chloride
B_2O_3	Boric oxide
$Ba(OH)_2$	Barium hydroxide
BF_3	Boron trifluoride
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
$CDCl_3$	Deuterated chloroform
COX-2	Cyclooxygenase-2
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediamine tetraacetic acid
H_2SO_4	Sulfuric acid
HCl	Hydrogen chloride
HIV	Human immunodeficiency virus
JAK2	Janus kinase 2
JAK3	Janus kinase 3
KBr	Potassium bromide
KF	Potassium fluoride
KOH	Potassium hydroxide
LPS	Lipopolysaccharide
MABA	Meta-aminobenzoic acid
MgO	Magnesium oxide
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant Staphylococcus aureus
MVD	Molegro Virtual Docker
Na_2CO_3	Disodium carbonate
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance

NSAIDs	Nonsteroidal anti-inflammatory drugs
PARP	Poly (ADP-ribose) polymerase
$SOCl_2$	Thionyl chloride
TMS	Trimethylsilyl group
VEGFR-2	Vascular Endothelial Growth Factor Receptor-2

Chapter 1

Introduction

Abstract

In this chapter, the importance of the various natural and synthetic chalcones, pyrazoles, pyrazolines and their biological activities has been briefly discussed. The aim of the present investigation has been stated in the background of known chemistry of chalcones, pyrazoles, pyrazolines and their biological activities.

1.1 Historical Perspective and Chemistry of Chalcone

Chalcones represent key structural motifs of many important biologically active compounds, including synthetic and natural products.[1] The chemistry of chalcones has been an interesting field of study and has garnered phenomenal scientific interest over the years owing to their simple chemistry, ease of hydrogen atom manipulation, simple synthetic procedures and their promising pharmacological and biological activities.[2] The name 'chalcones' was coined by Kostanecki and Tambor.[3] The alternative names given to chalcones are benzyl acetophenone or benzylidene acetophenone, α-β-unsaturated ketones, 1,3-diphenyl-2-propen-1-ones, *α*-phenyl-*β*-benzoylethylene, *β*-phenylacrylophenone, phenyl styryl ketone, etc.[4] Chalcones form a major component of natural products with widespread distribution in vegetables, fruits, tea, spices, and soy-based foodstuff.[5] They belong to the largest class of plant secondary metabolites and are the biogenetic precursors of flavonoids and isoflavonoids, found in abundance in plants.[6] Chalcones are generally colored compounds because of the presence of chromophore and auxochrome.[7] Chalcone-containing plants have been used for centuries as traditional medicine in Asia, Africa and South America.[8] Chalcones and their analogs have been known to possess diverse pharmacological activities such as antibacterial, antifungal, anti-tuberculous, anti-tumor, anti-inflammatory, anti-diabetic activity, anti-leishmanial, antimalarial, antimitotic, antispasmolytic, antiinvasive activity, antioxidant.[9,10] Chemically, chalcones consist of open chain flavonoids in which two aromatic benzene rings, namely rings A and B, are linked by an aliphatic three-carbon α, β-unsaturated carbonyl system i.e., 1,3-diphenyl-2-propen-1-one derivative (Figure 1.1).[11] The two aromatic rings and the electrophilic α, β-unsaturated carbonyl system is in continuous conjugation. Stereo chemically, chalcones can

exist both in *trans* (*E*) and *cis* (*Z*) isomeric forms.[12] The *trans* isomer is the most thermodynamically stable form whereas the *cis* isomer is the most unstable due to steric effects between ring A and the carbonyl group (Figure 1.2).[2] The stability of the *trans*-isomer makes it the most predominant configuration among the chalcones.

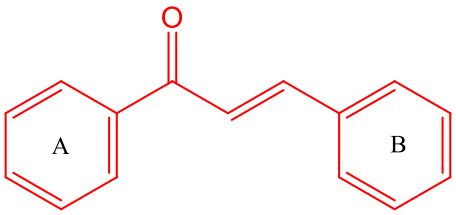


Figure 1.1. Structure of chalcone.

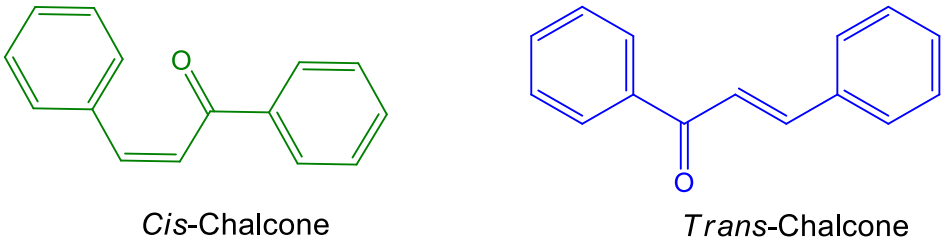
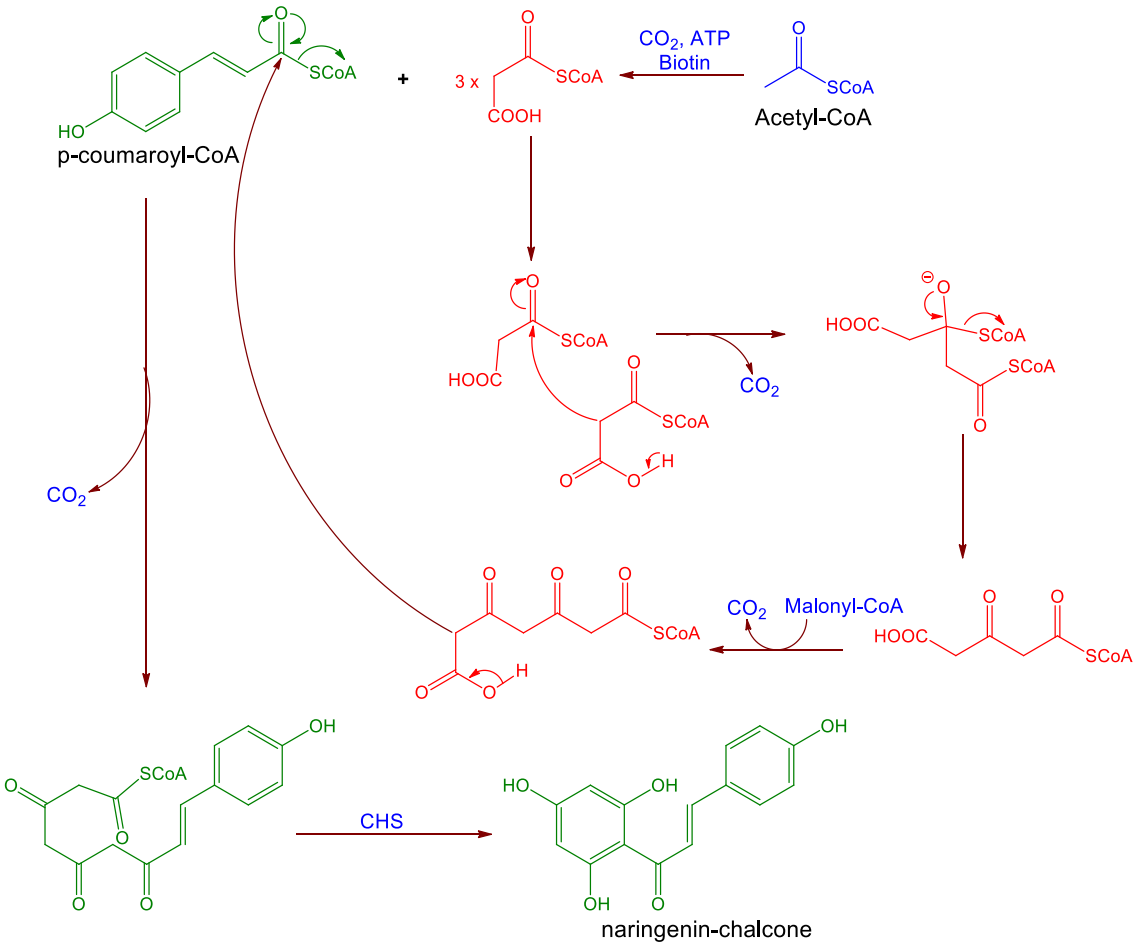


Figure 1.2. Chemical structure of *cis*-chalcone and *trans*-chalcone.

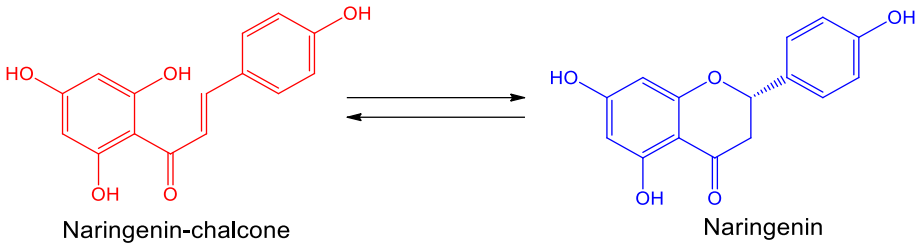
Generally, naturally occurring chalcones are common with substitutions such as hydroxyls, prenylation and methylation, but they also exist as dihydrochalcones, glycosides and dimers (bichalcones).[13] In the biosynthetic approach, the formation and metabolic transformation of naturally occurring chalcone is a convergence of two biogenesis pathways: the shikimic acid and malonate pathways.[14] The enzymes *chalcone synthase* and *chalcone isomerase* are the vital enzymes in these pathways and play a crucial role in the biosynthesis and biosynthetic transformations which leads to different flavonoids. Numerous studies on these pathways have enlightened researchers on the formation of the chalcones. In higher plants, chalcones are synthesized from one molecule of *p*-coumaril-CoA and three molecules of malonyl-CoA chalcones by the enzyme *chalcone synthase*. The amino acid L-phenylalanine formed in the shikimic acid pathway is converted to *p*-coumaril-CoA through the phenylpropanoid pathway, which results in the formation of the aromatic B-ring and the 3C bridge of chalcone (C_6-C_3-). The aromatic A-ring is formed after the condensation of three molecules of malonyl-CoA (-C_6).[15,16] Narginine chalcone (4,2',4',6'-tetrahydroxychalcone) is one of the synthesized forms of chalcone in citrus plants

and several other plants derived from one molecule of 4-coumaril-CoA and three molecules of malonyl-CoA catalyzed by the enzyme *chalcone synthase* (Scheme 1.1).[15,17]



Scheme 1.1. Biosynthesis of naringenin-chalcone.

Once synthesized, chalcones can be converted to naringenin catalyzed by the enzyme *chalcone isomerase*. Chalcone isomerase "Type 1" which is widely found in higher plants (except leguminous plants) produces the 5-hydroxyflavonone naringenin, which is the biosynthetic precursor of virtually all flavonoids (flavones, isoflavones, flavonols, condensed tannins and anthocyanins) (Scheme 1.2).[18,19]



Scheme 1.2. Naringenin-chalcone conversion to naringenin.

Chalcones are called minor flavonoids because of their restricted occurrence in nature. They are very important biosynthetic compounds because of being the precursor of almost all flavonoids. The important property that separates chalcones and dihydrochalcones from other flavonoids is that an open chain with three-carbon molecules binds to A and B rings instead of the C ring of flavonoids (Figure 1.3).[8] The α, β-unsaturated ketone moiety in chalcone is likely responsible for the versatile biological activities observed in chalcones. Because of their simple structure, reactivity and promising biological and pharmacological activities, there has been a growing interest in the development of chalcone derivatives. Studies have been focused on the development of new synthetic protocols and chalcone derivatives with more potent and efficient therapeutic properties.

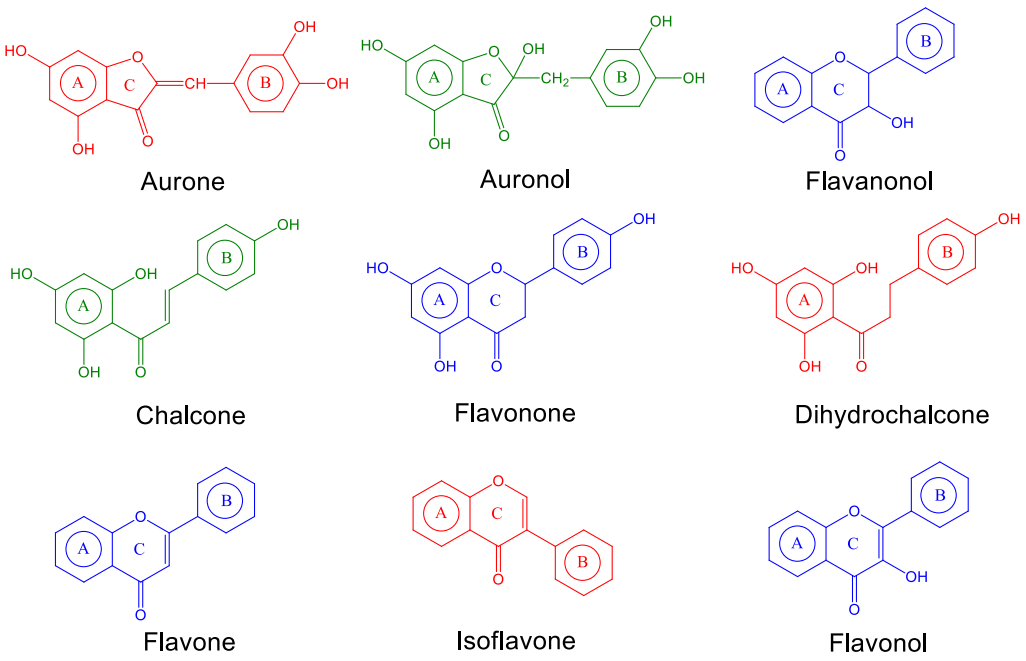
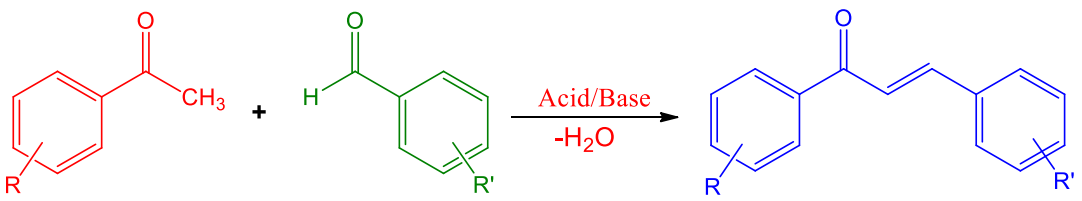


Figure 1.3. Structures of some flavonoid species.

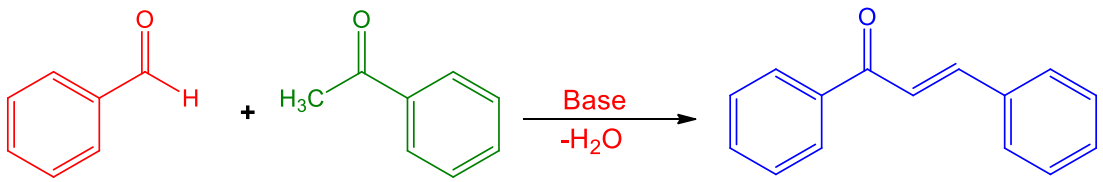
1.2 Synthetic Preparation of Chalcones

Although chalcones are available naturally, the synthetic preparation of chalcones offers wider scope towards yield and purity. The development of newer efficient and simple synthetic protocols is being investigated worldwide. Chalcones possess a modest privileged/scaffold simple moiety and therefore it has been used as a template for a variety of substitutions with a simple and easy method of synthesis.[20] Generally, chalcones are synthesized through condensation reactions by using acid or base catalysis (Scheme 1.3). Among the variety of methods available for chalcone synthesis, the most convenient method is the classical Claisen-Schmidt condensation of equimolar quantities of substituted acetophenone with substituted aldehydes in the presence of aqueous-alcoholic alkali. The alkali concentration usually ranges between 10 and 60 % and the reactions are carried out at room temperature for several hours.[21]



Scheme 1.3. Synthesis of Chalcones by Claisen-Schmidt condensation.

Another prominent classical method used for the preparation of chalcone is the Aldol condensation reaction between benzaldehyde and acetophenone in the presence of a base (Scheme 1.4).[22] In the initial step, acetophenone is treated with a base similar to KOH, which converts it into a more active enolate form. It is then reacted with benzaldehyde which forms an intermediate and upon heating loses a molecule of water to form chalcone.



Scheme 1.4. Formation of Chalcones by Aldol condensation.

Chalcones have also been synthesized using non-conventional methods such as Suzuki reaction,[23] Friedel Crafts reaction,[24] direct cross coupling,[25] Heck reaction[26] and Julia-Kocienski reaction (Figure 1.4).[27] Recently, various studies have been focused on the synthesis of chalcone using a facile and eco-friendly approach such as grinding,[28]

microwave irradiation,[29] ultrasonic irradiation[30] and solvent-free synthesis method.[31] Besides, various modified methods for the synthesis of chalcones have been reported using various catalysts such as $SOCl_2$, KF/natural phosphate, natural phosphate lithium nitrate, Na_2CO_3, acyclic acidic ionic liquid, high-temperature water, boron trifluoride-etherate,[32] KF-Al_2O_3,[33] zeolites and hydrotalcites,[34] organolithium,[35] zinc oxide[36] and alumina etc.[37] The broad pharmacological application of chalcones, their simple chemistry and ease of synthesis have attracted the attention of many pharmacologists, researchers and chemists to design and develop newer synthetic protocols for the preparation of chalone.

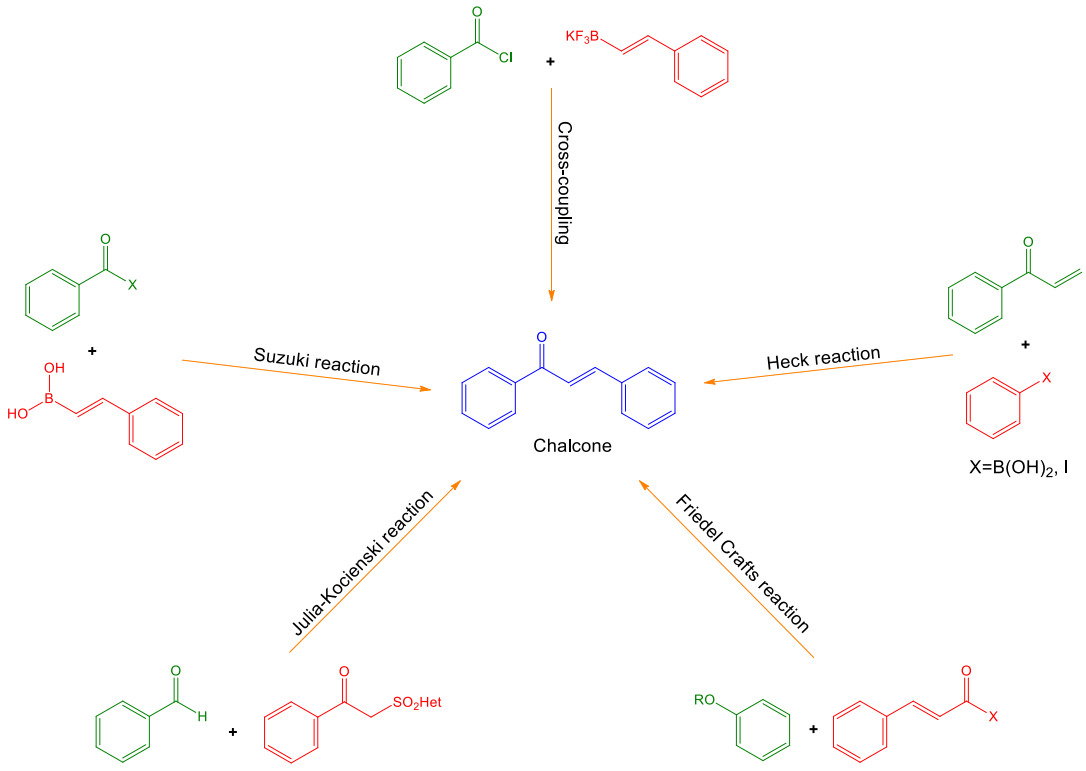


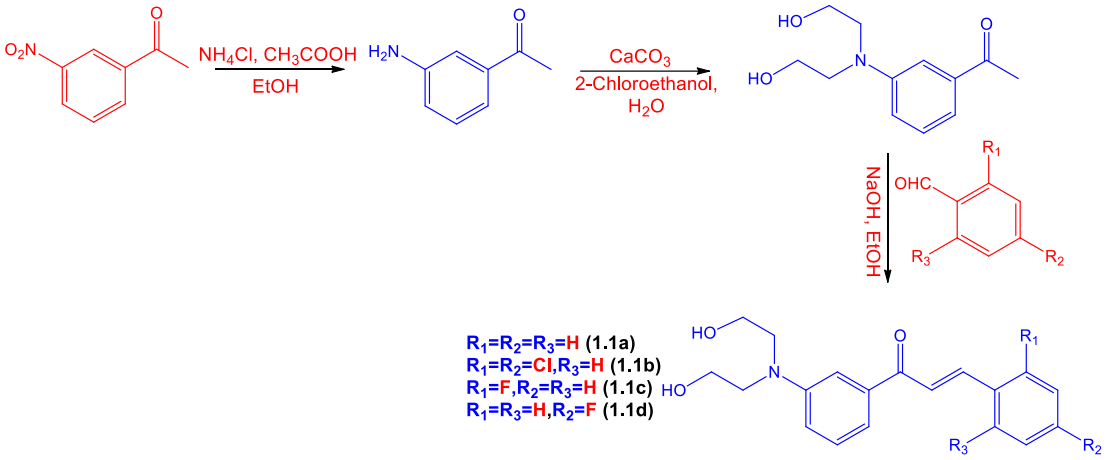
Figure 1.4. Synthesis of chalcone using well-known non-classical reaction methods.

1.3 Chalcone Synthesis and Biological Importance

1.3.1 Chalcone with Anti-microbial Activity

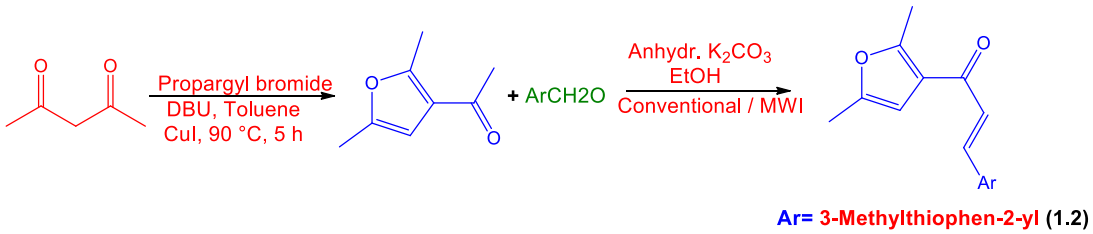
The increased resistance to existing antibiotics has led to the search for newer and novel antimicrobial agents. In this regard, Chalcones has generated great interest for possible therapeutic agents due to their simple chemistry, ease of synthesis and abundance in plants.[38] Over the years, numerous studies have been focused on the synthesis and development of novel chalcone derivatives against various infectious microbial agents. For example, Xianwen Fang *et al.* in **2014** synthesized a series of new 3-[N, N-bis(2-

hydroxyethyl)-amino]-chalcone derivatives and all the newly synthesized compounds were tested for their antimicrobial activity. The study reveals that compounds **1.1a**, **1.1b**, **1.1c** and **1.1d** shows potent activity against *Candida albicans* with MIC values of 32 lg/Ml (Scheme 1.5).[39]



Scheme 1.5. Synthesis of chalcone derivatives.

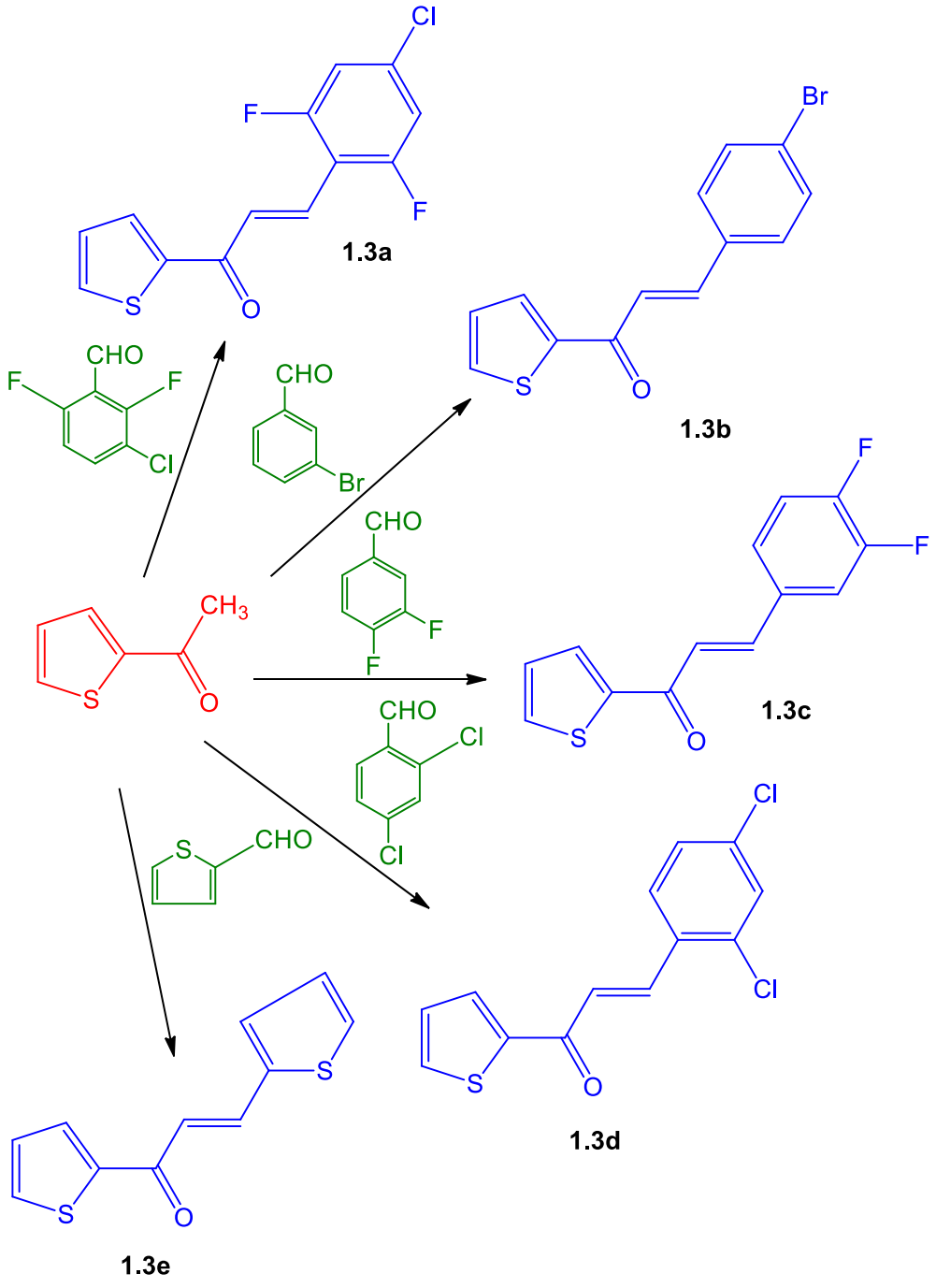
S. Kulathooran and collaborators in **2014** synthesized some novel heterocyclic chalcone derivatives using anhydrous potassium carbonate by classical as well as microwave irradiation method (Scheme 1.6). Compound **1.2** incorporated with methyl-substituted thiophene derivative was observed to be the most effective of all against *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Rhizopus arrhizus* and *Candida albicans* except *Aspegillus niger*.[40]



Scheme 1.6. Synthesis of heterocyclic chalcone derivatives.

Musthafa Yaseen Mowlana and Abdul Jamal Abdul Nasser in **2014** synthesized Thiophene chalcones from acetylthiophene with substituted aromatic aldehydes by Claisen-Schmidt condensation and further evaluated the synthesized compounds for their antibacterial and antifungal activity against *Klebsiella aerogenes*, *Proteus Vulgaris*, *Mucor racemosus*, *Aspergillus flavous* and *Aspergillus fumigatous* respectively. The result after

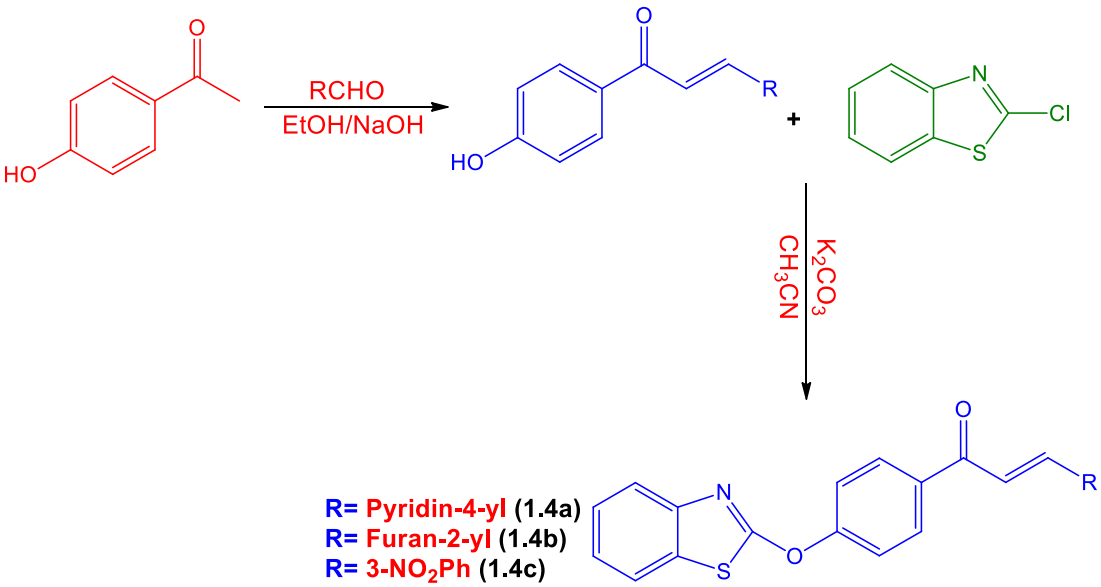
screening shows that all the derivatives **1.3a-e** compounds exhibit good activity (Scheme 1.7).[41]



Scheme 1.7. Synthesis of thiophene-chalcone derivatives.

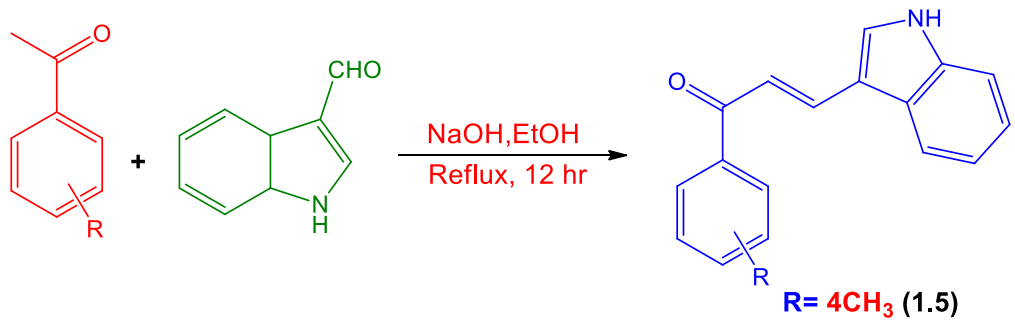
Chalcone derivatives containing a benzothiazole scaffold were synthesized by Yihui Wang and co-workers in **2019** and were evaluated for their antibacterial activity against *Xanthomonas oryzae pv. Oryzae* (Xoo), *Xanthomonas axonopodis pv. Citri* (Xac),

Ralstonia solanacearum (Rs) and bismerthiazol was used as reference drug. The study reported that compounds **1.4a**, **1.4b** and **1.4c** exhibited the most potent antibacterial activity (Scheme 1.8).[42]



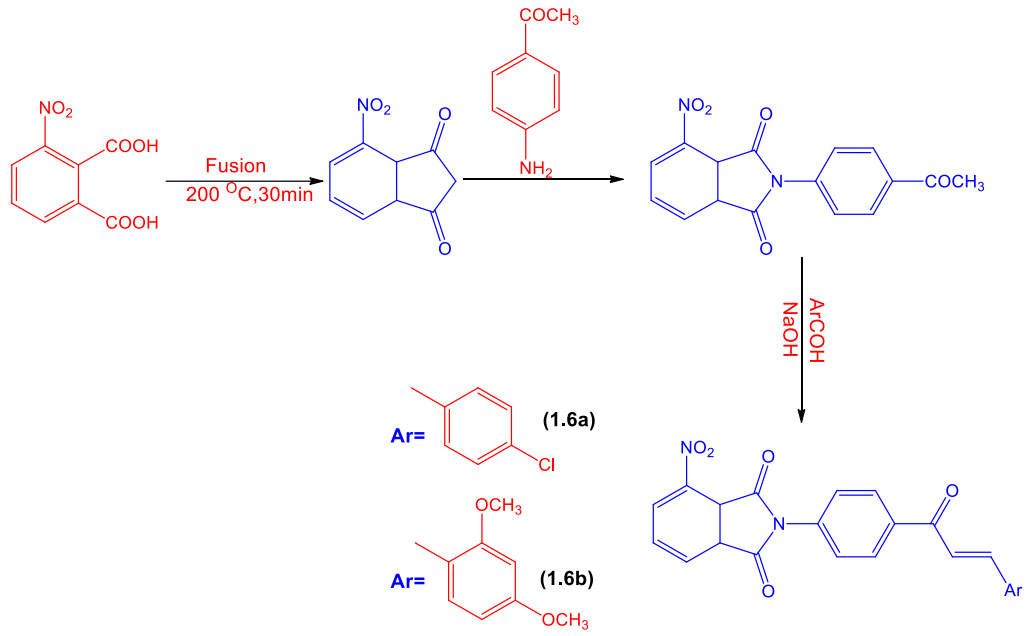
Scheme 1.8. Synthesis of chalcone derivatives containing benzothiazole scaffold.

Rizwan Arif *et al.* in **2020** synthesized some indole-chalcone derivatives by Claisen-Schmidt condensation reaction in basic conditions (Scheme 1.9). Their antimicrobial properties were evaluated against gram-positive bacteria like *Staphylococcus pneumoniae* and *Enterococcus faecalis* and gram-negative bacteria like *Salmonella enterica*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. The study reported that compound **1.5** showed potent activity against *E. coli* with MIC 125 mg/mL as well as moderate activity against against *P. aeruginosa* and *S. pneumoniae* with MIC 250 mg/mL.[43]



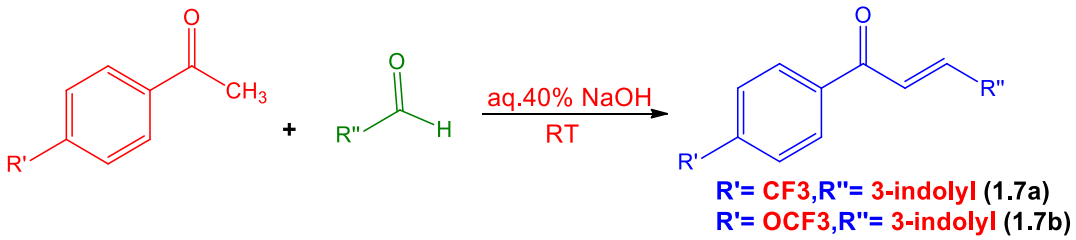
Scheme 1.9. Synthesis of indole chalcone derivatives.

In **2021**, Luma S. Ahamed and co-workers synthesized a series of novel 3-nitro phthalimide-chalcone derivatives by solvent-free methods (Scheme 1.10) which were screened for their antifungal activity against *candida albicans* and also antibacterial activity against *Escherichia coli* and *staphylococcus aureus*. The newly synthesized compounds **1.6a** and **1.6b** showed better activity than their reference drugs Cephalexin and Fluconazole respectively.[44]



Scheme 1.10. Solvent-free synthesis of phthalimide chalcone derivatives.

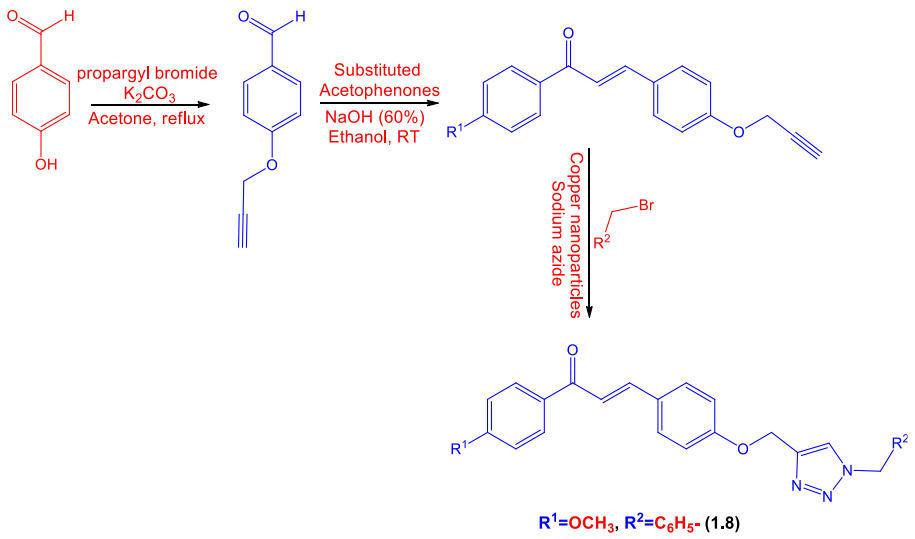
Surendra Babu Lagu and co-workers in **2020** synthesized a series of chalcones bearing trifluoromethyl and trifluoromethoxy substituent. All the newly synthesized compounds were screened for their antibacterial and antifungal activity where among all the compounds **1.7a** and **1.7b** manifest potential activities and also show non-toxic on the human liver cell lines (L02). It was also observed that compounds bearing electron-withdrawing nitro and the chloro substituents at the ortho or the meta position also showed good antimicrobial activity (Scheme 1.11).[45]



Scheme 1.11. Synthesis of fluorinated indole chalcone derivatives.

1.3.2 Chalcone with Anti-Cancer Activity

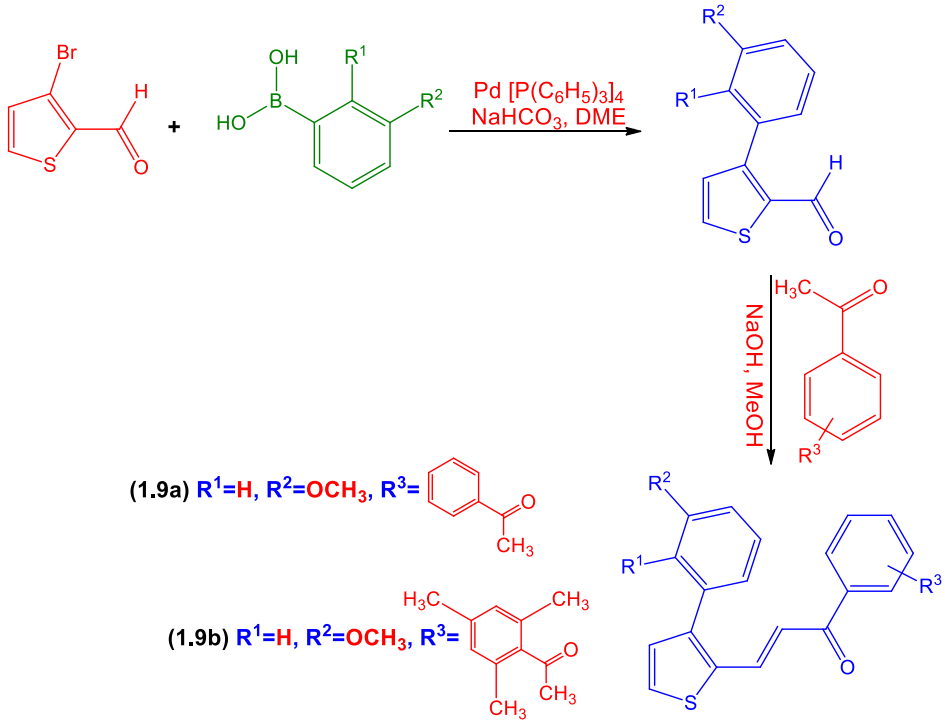
Cancer is one of the leading causes of death worldwide. Although there has been increasing growth in the development of new drugs and new targets, it is still responsible for millions of deaths across the globe. The incidence of drug-resistant cancers and the low specificity of anticancer agents have been the main hurdles in the control and treatment of cancer.[46] Thus, it is the need of the hour to design and develop novel anticancer agents with high efficiency. Chalcones remained a fascination among researchers owing to their structural heterogeneity and ability to act on various drug target.[47] They are known to possess a wide variety of biological activities including anticancer activity. Numerous studies have reported the use of chalcone as a potential template for the development of novel anticancer agents**.** Pinki Yadav and co-workers in **2017** synthesized a series of novel chalcone linked-1,2,3-triazoles by base-catalyzed Claisen-schimdt condensation. The newly synthesized chalcone linked-1,2,3-triazoles were evaluated and exhibited good anticancer activity. They reported that compound **1.8** was the most active of all where it induced apoptosis and G2/S arrest and also triggered mitochondrial potential loss in pancreatic cancer MIA-Pa-Ca-2 cells (Scheme 1.12).[48]



Scheme 1.12. Synthesis of chalcone linked-1,2,3-triazoles derivatives.

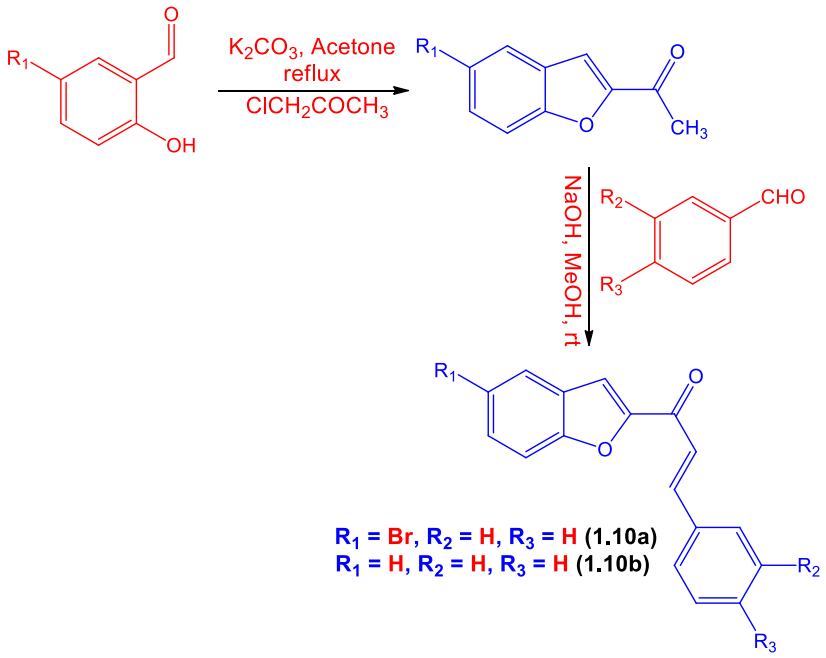
V. Venkataramireddy *et al.* in **2016** synthesized and evaluated a series of new 3-aryl thiophene-2-aryl and heteroaryl chalcones. A good number of compounds showed excellent activity of which the most potent anti-tumor activity was exhibited by compound **1.9a** with IC50 value of 21 µg/mL and compound **1.9b** exhibited IC50 of 22.8 µg/mL which also

indicate the potential cytotoxic properties against human colon cancer cells (HCT-15) (Scheme 1.13).[49]



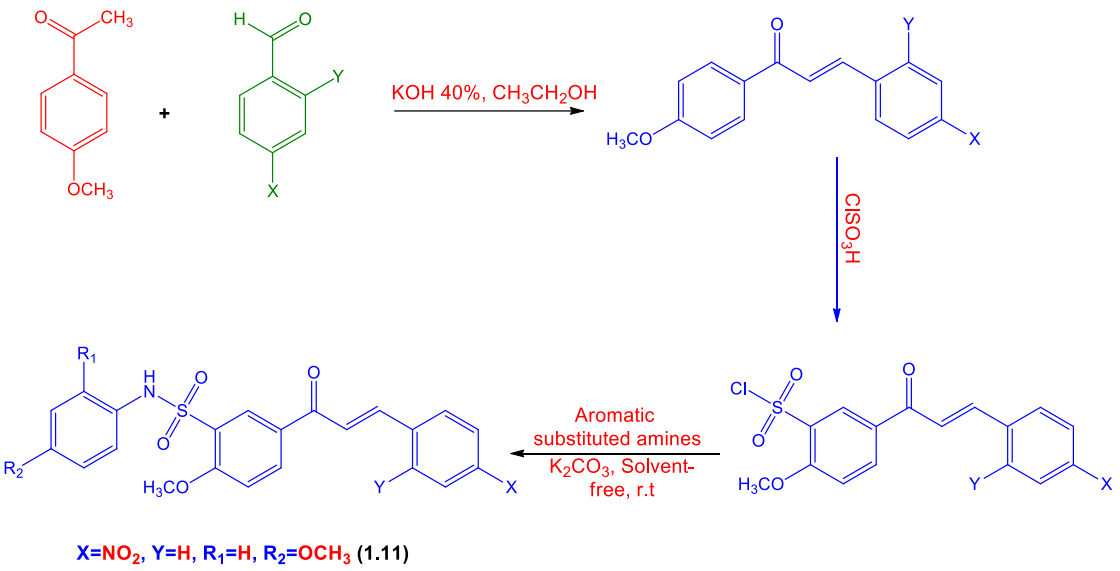
Scheme 1.13. Synthesis of aryl/heteroaryl chalcone derivatives.

Demet Coskun and co-workers in **2016** synthesized several new benzofuran substituted chalcone derivatives and evaluated their anticancer activity where compounds **1.10a** and **1.10b** exhibited the highest potency against MCF-7 and PC-3 cell lines (Scheme 1.14).[50]



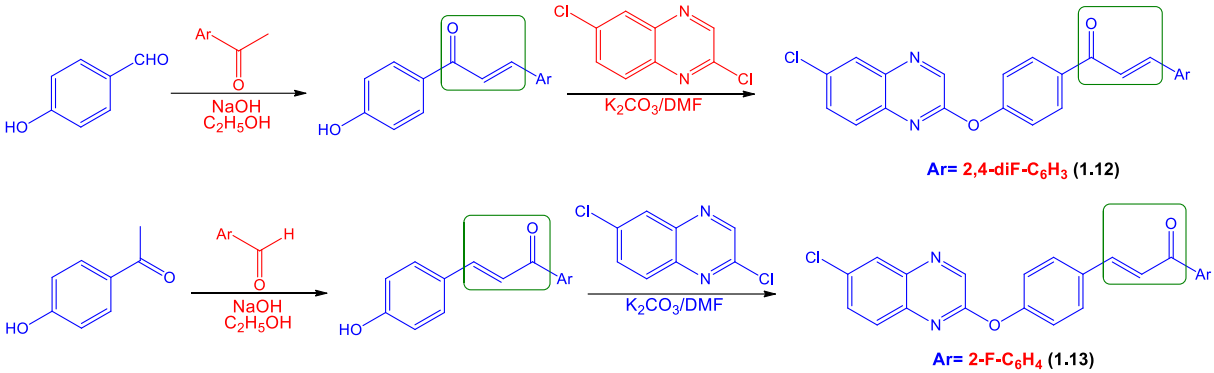
Scheme 1.14. Synthesis of benzofuran substituted chalcone derivatives.

Ahmad Pesaran Seiied Bonakdar *et al.* in **2017** synthesized some new chalcone sulfonamide derivatives and screened for anticancer activity. Compound **1.11** exhibit the most potent anticancer activity of all against human breast cancer cell line (MCF-7) (Scheme 1.15).[51]



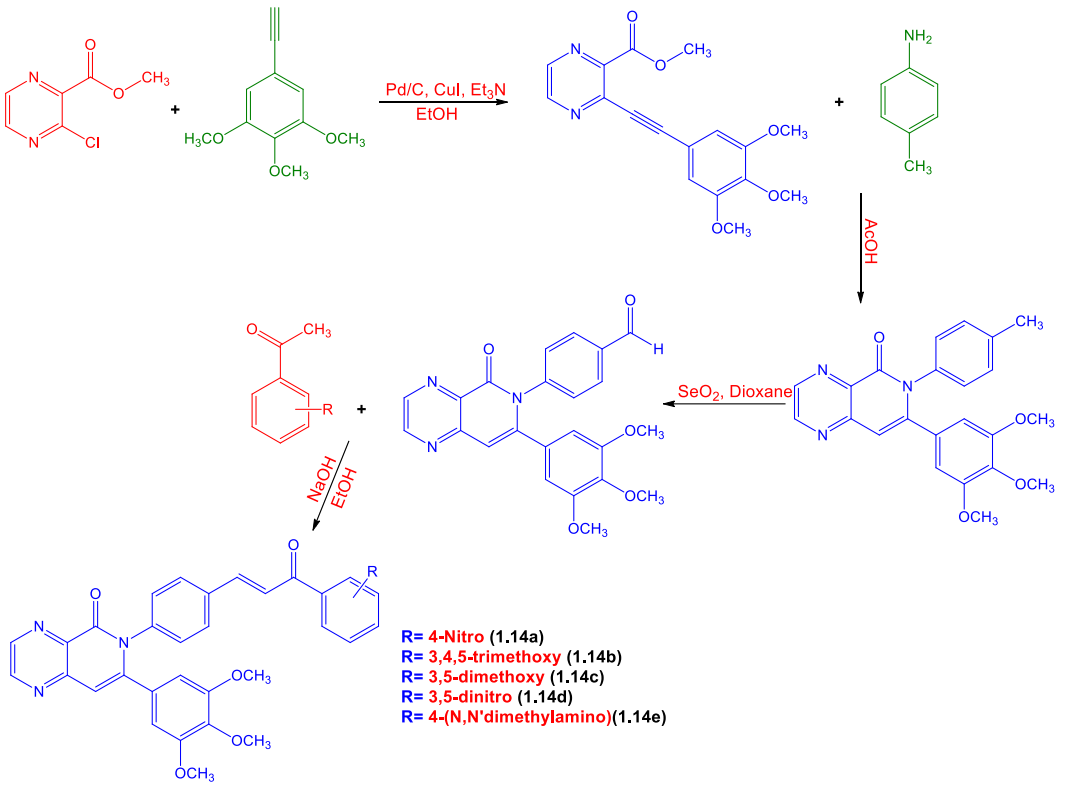
Scheme 1.15. Synthesis of chalcone-sulfonamide derivatives.

In **2021**, Xiaoyun Maa and co-workers synthesized two novel series of quinoxaline chalcone conjugates by aldol condensation. Three cancer cell lines were evaluated in vitro such as prostatic hyperplasia epithelial cell (BPH-1), human breast cancer cell line (MCF-7), and neuron-like rat pheochromocytoma cell line (PC12). From the result obtained, it was observed that compound **1.12** exhibits potent activity against BPH-1 and MCF-7 with IC50 values of 10.4 and 9.1 µM while compound **1.13** shows potent activity against PC12 with IC50 values of 16.4 µM (Scheme 1.16).[52]



Scheme 1.16. Synthesis of quinoxaline-chalcone conjugates.

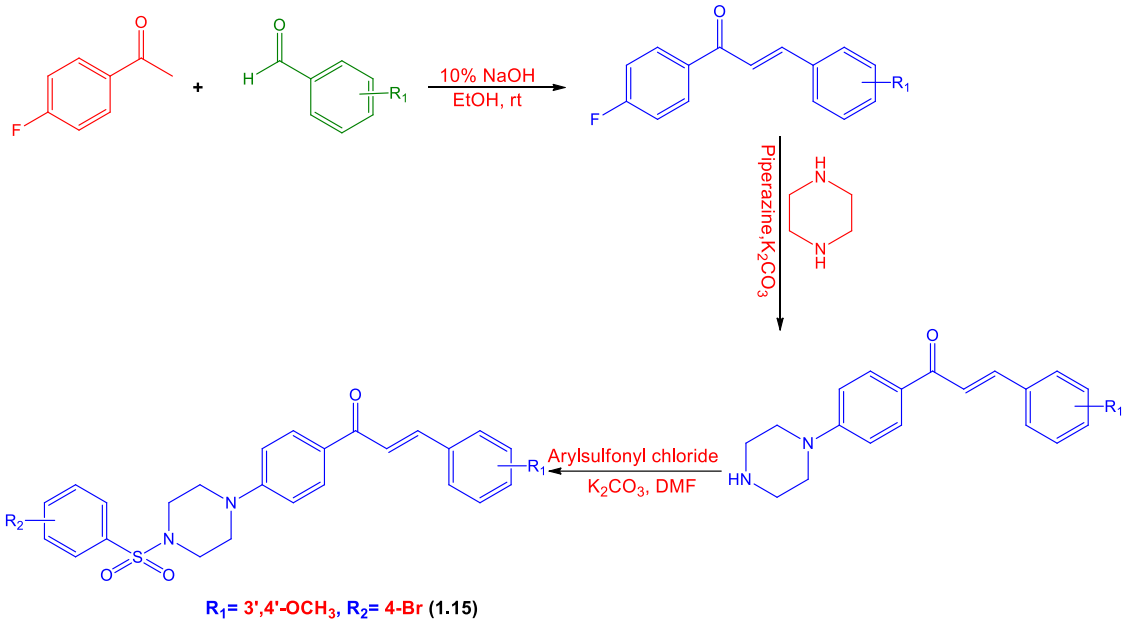
Dandamudi Srilaxmi *et al.* in **2021** synthesized a number of new chalcone linked pyrido[4,3-b]pyrazin-5(6H)-one derivatives and evaluated their anticancer activity by employing MTT assay against human cancer cell lines such as MCF-7 (breast cancer), Colo-205 (colon cancer), A2780 (ovarian cancer), A-549 (lung cancer) and Du-145 (prostate cancer). The results reveal that compounds **1.14a**, **1.14b**, **1.14c**, **1.14d** and **1.14e** exhibit potent anticancer activity (Scheme 1.17).[53]



Scheme 1.17. Synthesis of pyrido[4,3-b] pyrazin-5(6H)-one linked chalcone derivatives.

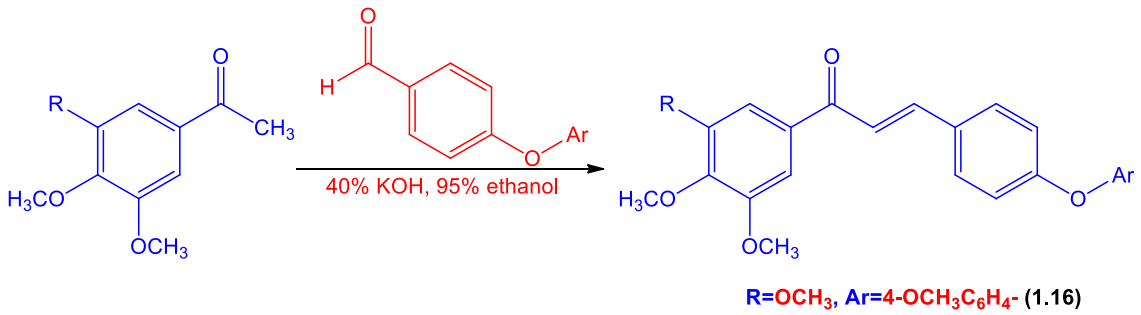
1.3.3 Chalcone with Anti-Inflammation Activity

Anti-Inflammation refers to the ability of a substance or treatment that reduces the inflammation or swelling caused by the biological response of body tissues to harmful stimuli such as pathogens or irritants.[54] Chalcones are known to have tremendous potential of exhibiting anti-inflammatory activity and many studies have reported the development of chalcone moieties with anti-inflammatory activity. Jingfen Li and co-workers in **2017** synthesized a series of novel chalcone derivatives containing aryl-piperazine or aryl-sulfonyl-piperazine fragment (Scheme 1.18). The novel chalcone derivatives were tested for their anti-inflammatory activity where compound **1.15** shows the best activity and was also observed that it can significantly inhibit the release of LPS-induced IL-6 and TNF-α by RAW264.7 macrophages in a dose-dependent manner.[55]



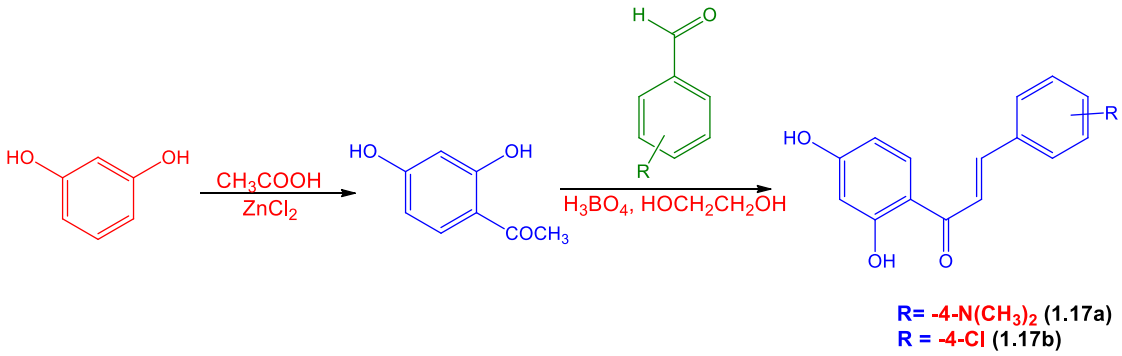
Scheme 1.18. Synthesis of chalcone derivatives as anti-inflammatory agents.

In **2021**, Soha H Emam *et al.* synthesized a number of methoxylated phenyl-based chalcones and studied their anti-inflammatory activity. The result reveals that out of all the newly synthesized chalcones derivatives, compound **1.16** shows the highest activity by inhibiting nitric oxide concentration in LPS-induced RAW264.7 macrophages with IC50 at 11.2 µM (Scheme 1.19).[56]



Scheme 1.19. Synthesis of methoxyphenyl chalcone derivatives.

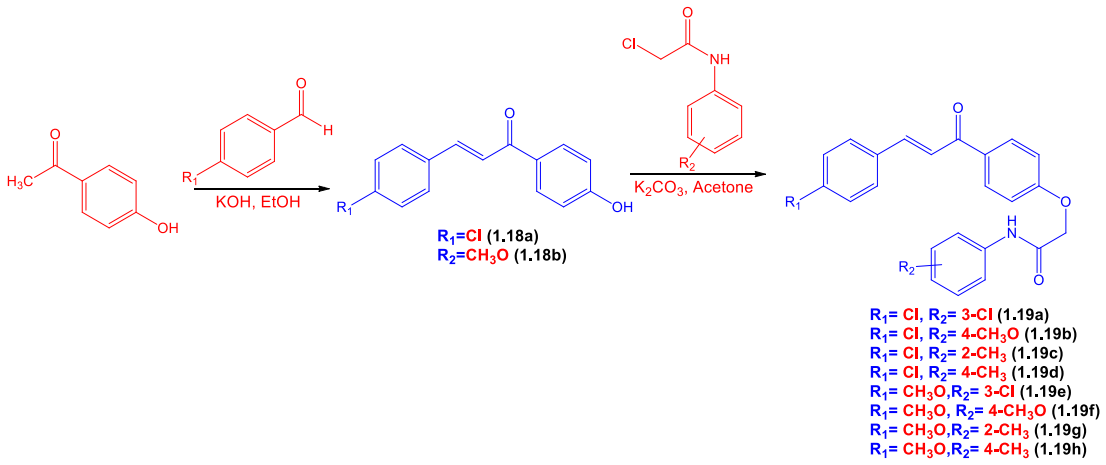
Xue-Wu Zhang and collaborators in **2010** synthesized a series of chalcone derivatives. The newly synthesized chalcone derivatives were then evaluated for their anti-inflammatory activity where compounds **1.17a** and **1.17b** show good activity (Scheme 1.20) comparable to that of reference drug ibuprofen.[57]



Scheme 1.20. Synthesis of substituted chalcone derivatives.

1.3.4 Chalcone with Anti-oxidant Activity

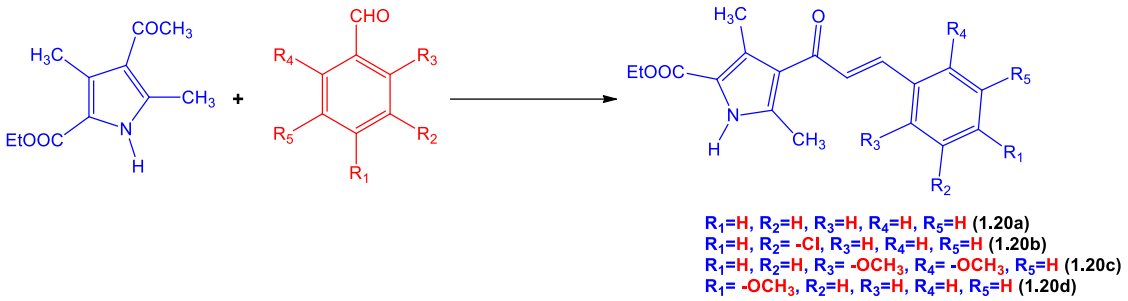
Naturally occurring chalcones as well as their synthetic analogues have intrigued researchers due to their interesting and wide spectrum of biological and pharmacological activities, including antioxidant activity. Cong Tien Nguyen and co-workers in **2021** synthesized some chalcone containing N-Arylacetamide group and screened for their antioxidant activity by DPPH radical scavenging method. The result obtained from the screening of compounds **1.19a-h** at a concentration of 10.0 µg/mL shows equivalent activity to that of reference drug ascorbic acid at a concentration of 6.0-8.0 µg/mL while compounds **1.18a** and **1.18b** show higher activity than ascorbic acid at same concentration (Scheme 1.21).[58]



Scheme 1.21. Synthesis of chalcone derivatives containing N-arylacetamide group.

In **2021**, Poonam Rawat *et al.* synthesized pyrrole-chalcones derivatives and studied their free radical scavenging activity by DPPH method. The studies exhibit that compounds **1.20a**, **1.20b**, **1.20c** and **1.20d** shows better free radical scavenging than the standard BHT

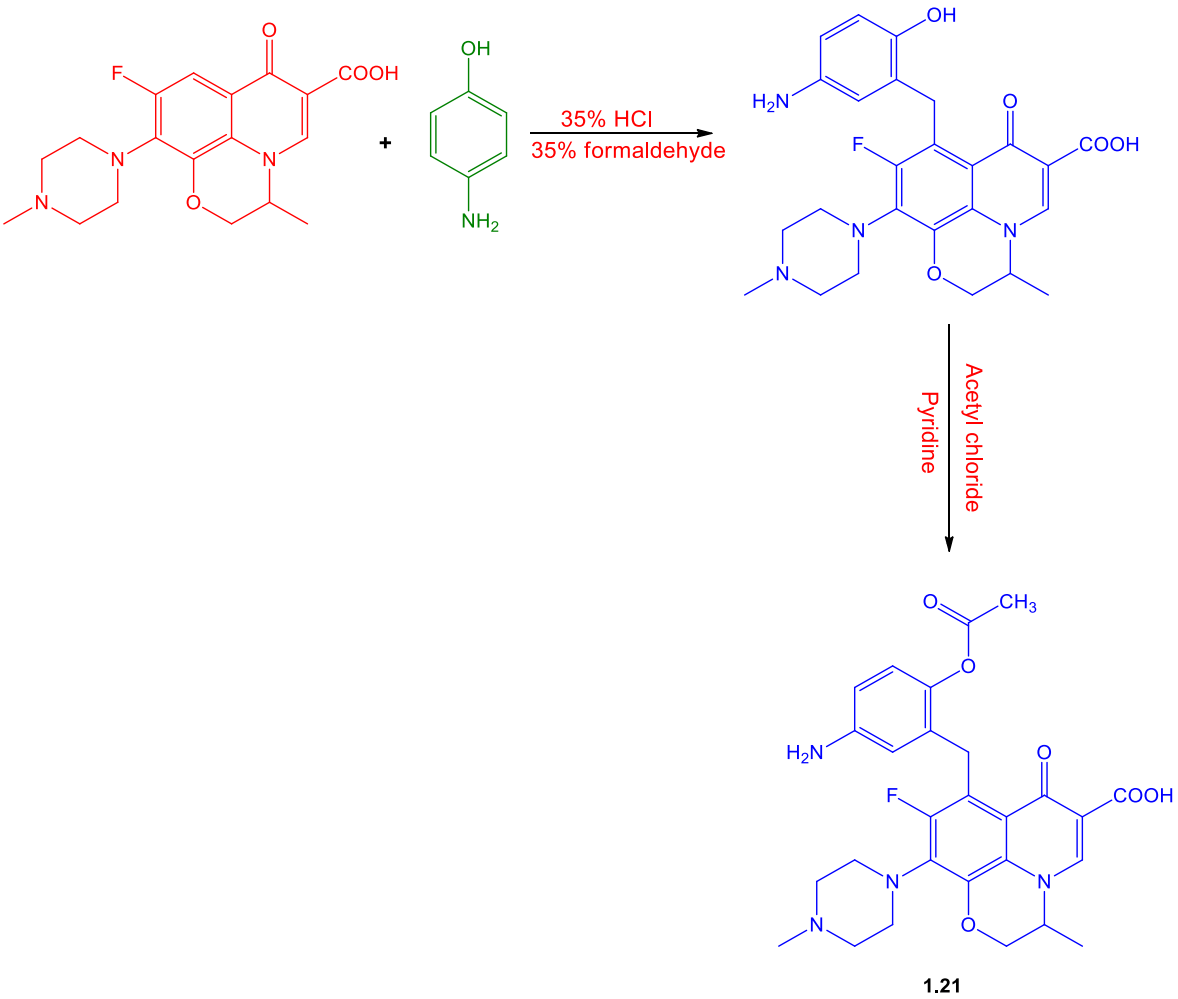
while compounds **1.20a**, **1.20b** and **1.20d** shows better Fe^{2+} ion chelating activity than standard EDTA and Total reductive capability than standard BHA (Scheme 1.22).[59]



Scheme 1.22. Synthesis of pyrrole-chalcone derivatives.

1.3.5 Chalcone with Anti-tubercular Activity

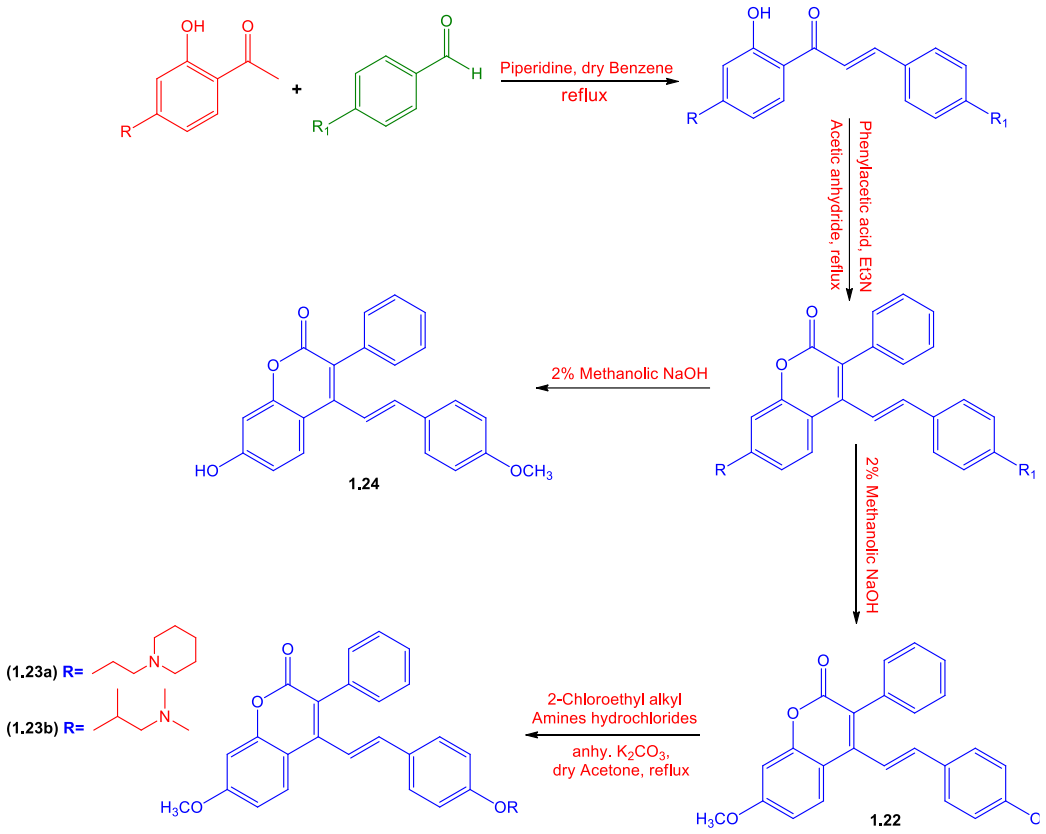
Tuberculosis (TB) is a devastating chronic necrotizing infectious disease with a wide variety of manifestations caused by *Mycobacterium tuberculosis*.[60] It is one of the top causes of death worldwide, claiming the lives of approximately two million people every year.[61] This alarming scenario calls for an urgent need to develop newer, safer and potent antituberculosis drugs for effective therapy. Numerous attempts have been made over the years to design and develop chalcone moieties with anti-tubular activity. Ramakrishna Chintakunta and Venkata Subbareddy Gopireddy in **2021** synthesized some Ofloxacin chalcone conjugates and their antitubercular activity was evaluated using MABA method. Out of all compound **1.21** exhibit the most efficient at 3.12 µg/ml (Scheme 1.23).[62]



1.21

Scheme 1.23. Synthesis of Ofloxacin chalcone conjugates.

Imran ahmad *et al.* in **2013** synthesized Lipophilic chalcones and their conformationally restricted analogues. Antitubercular activity against Mycobacterium tuberculosis H37Rv strain was evaluated where compounds **1.22**, **1.23a**, **1.23b** and **1.24** were active. There in vitro cytotoxicity in a non-cancerous human epithelial kidney cell line (HEK-293) was also conducted and was found that compounds **1.23a** were approximately 2.85 times more selective towards tubercular versus healthy cells while compounds **1.22** was 16 times more selective (Scheme 1.24).[63]



Scheme 1.24. Synthesis of lipophilic chalcone derivatives.

1.4 Historical Perspective & Chemistry of Pyrazole

Heterocyclic compounds are a unique and extraordinarily important class of compounds that constitute more than half of all known organic compounds. They belong to the natural product family and over the years have gained much importance in the field of medicinal chemistry because of their presence in a variety of pharmacologically active moieties.[64] Pyrazoles are the most popular five-membered heterocyclic compounds containing two nitrogen heteroatoms. The reason for their popularity and importance is due to their usefulness in the field of drug research and agricultural research.[65] The general structure of pyrazole is shown in Figure 1.5.

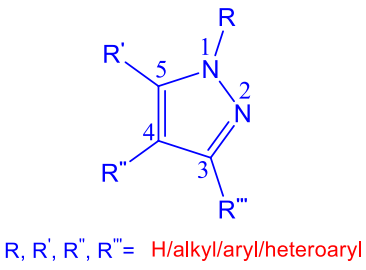


Figure 1.5. The general structure of pyrazole.

The term Pyrazole was first coined by a German scientist named Ludwig Knorr in the year 1883.[66] Ludwig knorr first synthesized pyrazole by reacting ethyl acetoacetate with phenylhydrazine which yielded 1-phenyl-3-methyl-5-pyrazolone (Figure 1.6).[67]

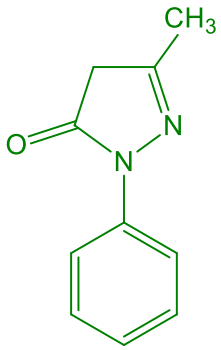
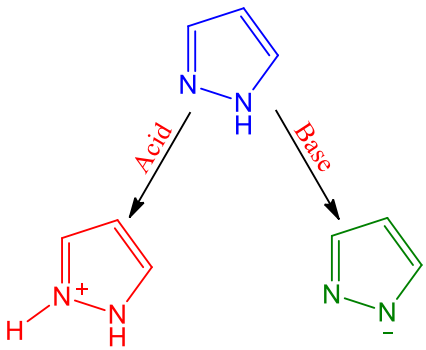


Figure 1.6. Structure of 1-Phenyl-3-methyl-5-pyrazolone.

Pyrazoles are composed of three carbon atoms and two nitrogen in the adjacent position.[66] In the two nitrogen atoms present in the pyrazole, the N1 (nitrogen atom 1) is "pyrrole-like" due to unshared electrons conjugation with the aromatic system whereas N2 (nitrogen atom 2) is "pyridine-like" since the unshared electrons are not compromised with resonance like that of a pyridine system. Therefore, due to this very reason between two nitrogen atoms can react with both acids and bases (Scheme 1.25).[68]



Scheme 1.25. Cations and anions produced from pyrazole in presence of acid and base.

The first reported natural pyrazole 1-pyrazolyl-alanine (or β-(1-pyrazolyl)alanine) (Figure 1.7) was isolated from the seeds of watermelon in 1959.[69]

Figure 1.7. Structure of natural pyrazole 1-pyrazolyl-alanine.

The pyrazoles molecules are aromatic due to their planar conjugated ring structures with six delocalized π-electrons (**1.25a**). The aromaticity arises from the four π-electrons as well as the unshared pair of electrons on the -NH nitrogen. Pyrazole exists in two forms namely pyrazolines which is the partially reduced form and is basic in nature (1.**25b** or **1.25c**) whereas the completely reduced form is pyrazolidine (**1.25d**) (Figure 1.8).[70]

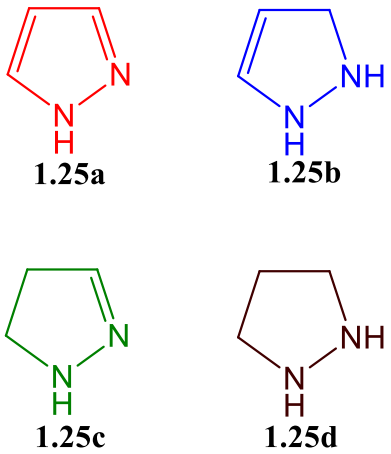
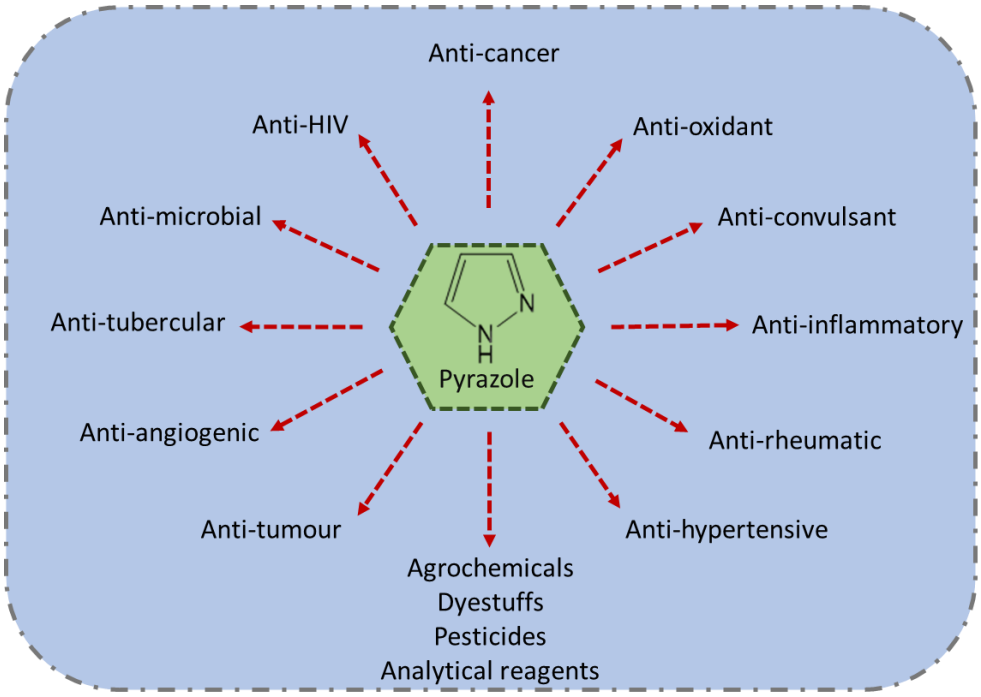


Figure 1.8. Structure of pyrazole class.

1.4.1 Pyrazole Role in Medicinal and Agricultural Application



Pyrazole moieties form the basis of many pharmacologically active compounds and display a broad spectrum[71] of chemical, biological, medicinal and agricultural applications.[72] Pyrazoles are reported to possess a vast biological activity such as anticancer,[73-75] antileukemia,[76] anti-HIV,[77,78] antioxidant,[79] antimicrobial,[80] antifungal,[81] analgesic,[82,83] antiviral,[84,85] anti-hyperglycemic activity,[86] hypoglysimic,[87] anticonvulsant, antihistaminic,[88] anti-inflammatory,[89] antitumor,[90] antipyretic, antidepressant, antirheumatic, antidiabetic,[91] protein kinase C inhibitor,[92] inhibition of cyclooxygenase-2, antiangiogenic, antipyretic, antihypertensive, antiplatelet, nitric oxide synthase (NOS),[93] antiangiogenic agents, A3 adenosine receptor antagonists, neuropeptide YY5 receptor antagonists, kinase inhibitor for the treatment of type 2 diabetes, hyperlipidemia, thrombopiotinmimetics.[94] Pyrazole also has a long history in the agrochemical industry.[95] As pesticides, they are used as insecticides, fungicides and herbicides.[96-98] Other application includes dyestuffs, analytical reagents and agrochemicals.[99,100]

1.4.2 Pyrazole Synthesis and Biological Importance

Pyrazoles are well-known examples of N-containing heterocycles and they are versatile lead compounds in pharmaceuticals owing to their wide spectrum of biological activities. They comprise a wide range of both synthetic and natural products that exhibit

innumerable biological, chemical, agrochemical and pharmacological properties. Due to their diverse range of therapeutic properties, these compounds have been widely used in drug development research and as a result, various protocols have been designed and developed for the synthesis of these pyrazole moieties. Some of the commercially available pyrazole moiety (Figure.1.9) are:- Celecoxib which functions as an anti-inflammatory agent and potent COX-2 inhibitor; Rimonabant that acts as cannabinoid receptor and is used for obesity; Novalgin as antipyretic and analgesic; Ramifenazone, Lonazolac and Rimonabant are NSAIDs; Pyrazofurin is potential of antiviral activity; Zaleplon functions as a sedative and hypnotic; Sildenafil utilized for primary pulmonary hypertension.[101,102]

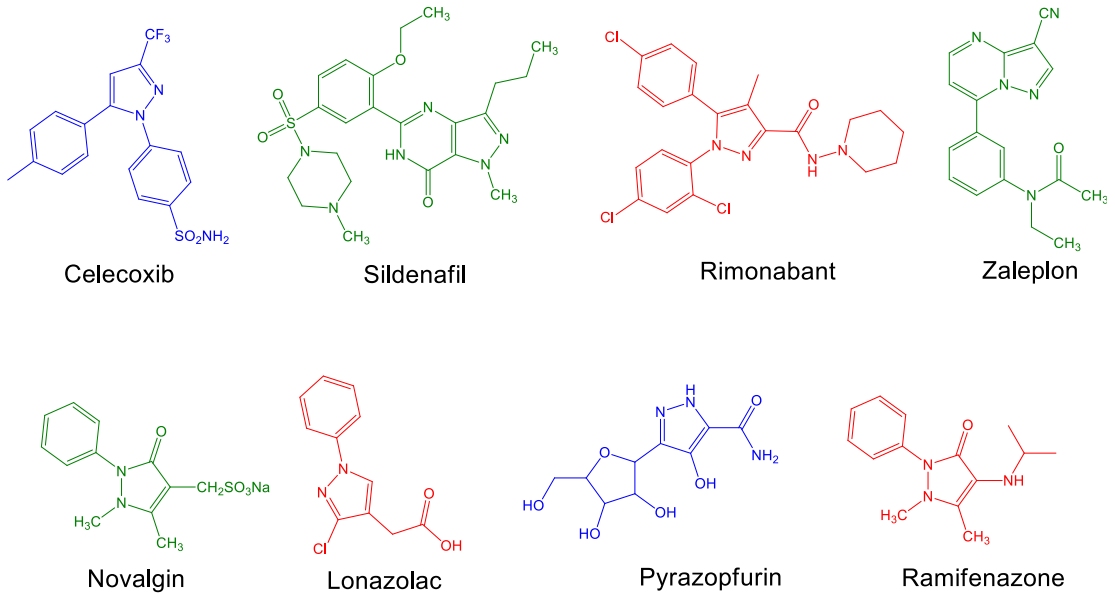


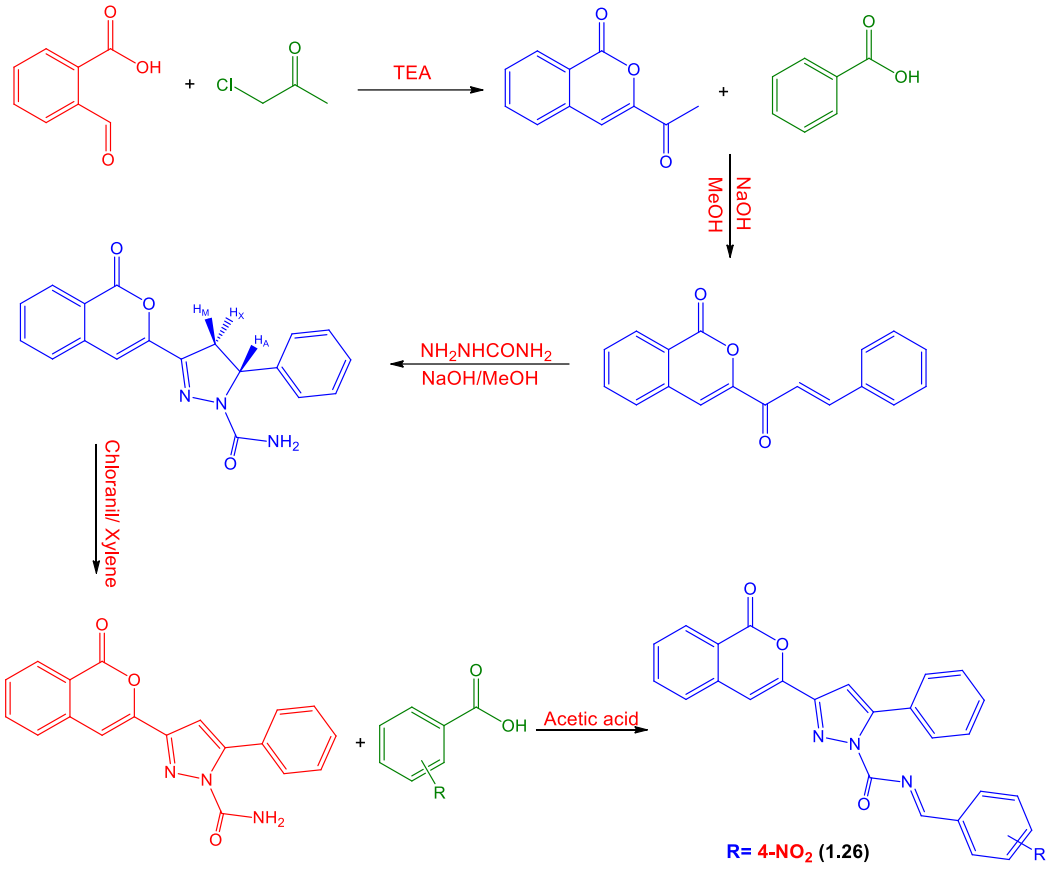
Figure 1.9. The structures of some drugs bearing the pyrazole moiety

Some examples of pyrazole moiety associated with a number of biological activities were examined from the already existing literature and are described below.

1.4.2.1 Pyrazole with Anti-microbial Activity

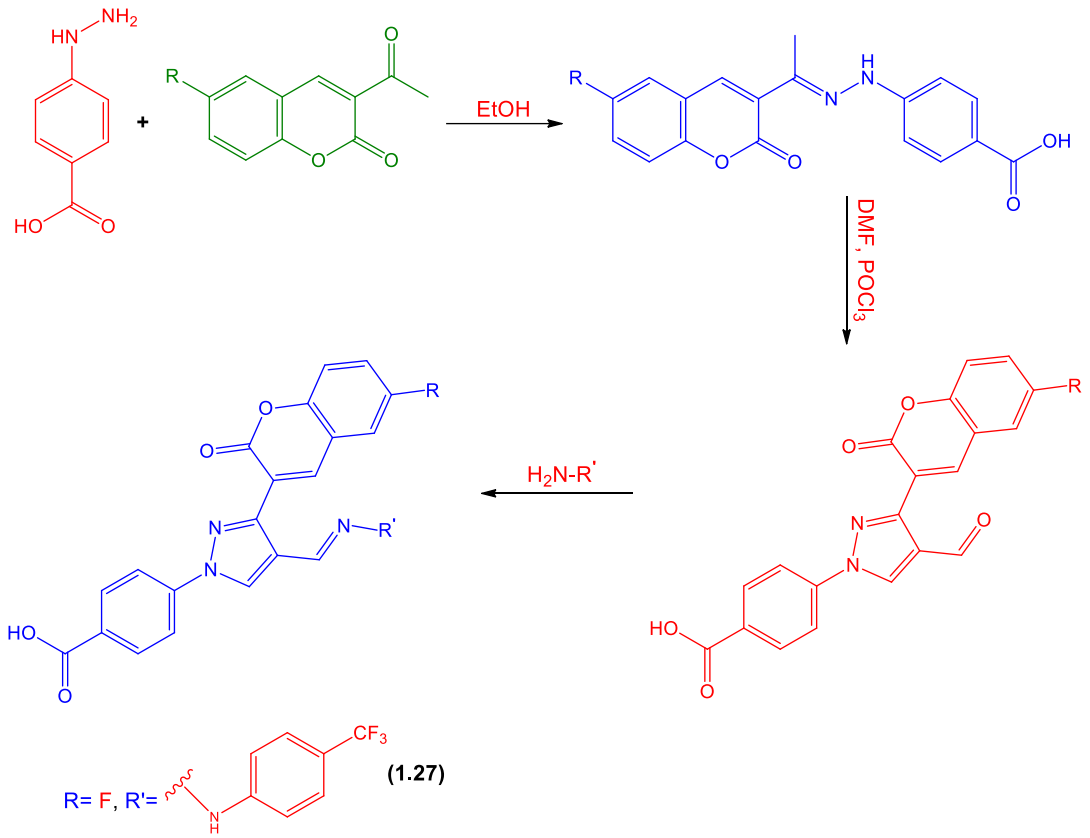
Antimicrobial agents are compounds that inhibit the growth of microorganism or kill them. The incidence of disease caused by different microorganisms such as bacteria, virus, fungi etc. has increased over the years, posing a tremendous threat to human health. Although a large number of antimicrobial agents are available, their efficacies are threatened by the emergence and increase in the number of resistant microorganisms, generating a substantial need for the synthesis of new classes of antimicrobial agents. Pyrazole and its derivatives have been found to possess various antibacterial, antifungal and

antiviral properties. Guda Mallikarjuna Reddy and co-workers in **2020** developed a series of new tri-substituted pyrazole derivatives. Their antibacterial and antifungal activities were evaluated and were found out that compound **1.26** bearing nitro-substitution shows higher antimicrobial activity while amino attached tri-substituted pyrazole do not exhibit any activity against all the tested microbial strains (Scheme 1.26).[103]



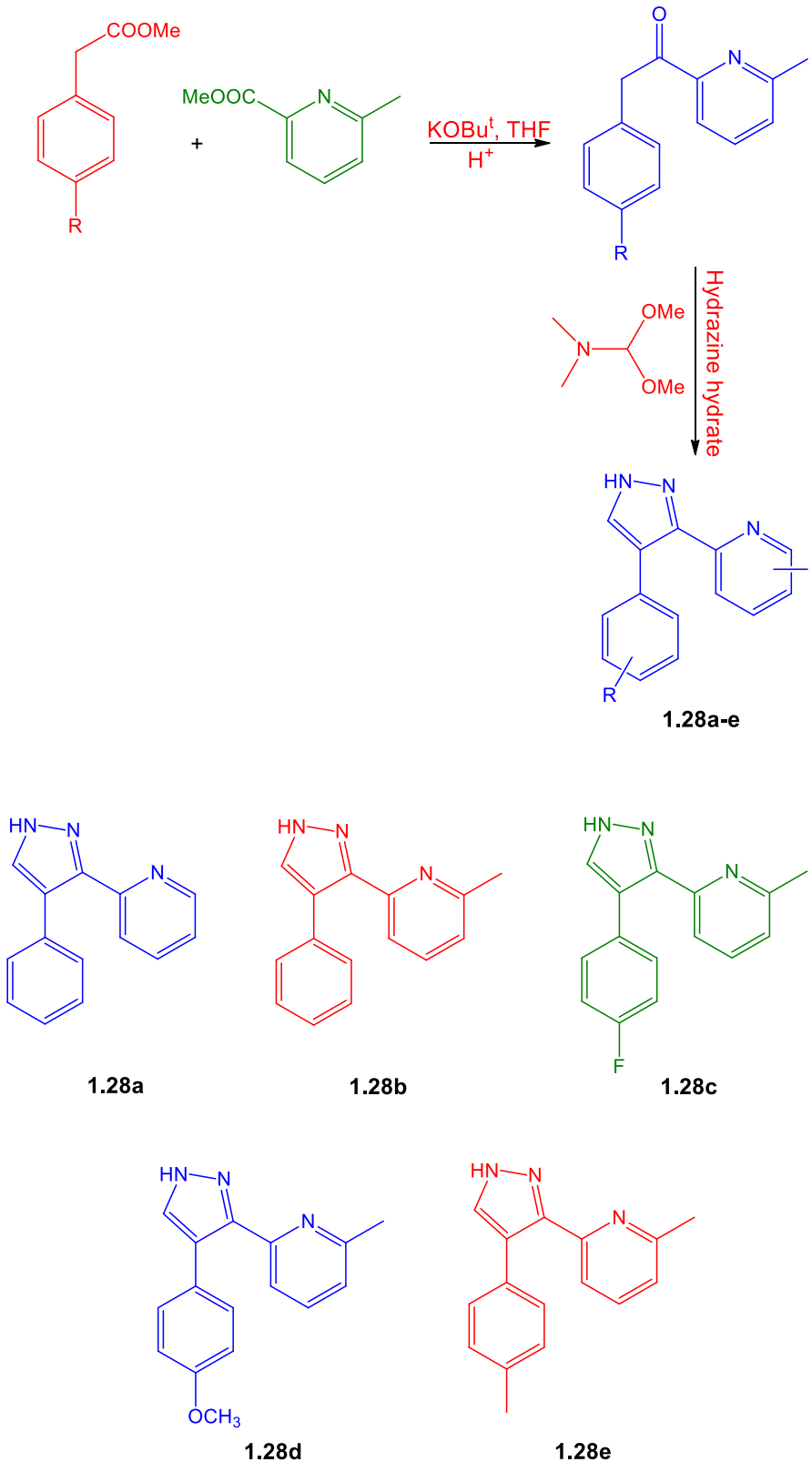
Scheme 1.26. Synthesis of tri-substituted pyrazole derivatives.

A series of new coumarin-substituted pyrazole derivatives were synthesized by Rawan Alnufaie *et al.* in **2020** and their antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) was studied. Some of the compounds showed potent activity against MRSA with some MIC value as low as 1.56 µg/mL. The study also reported that compound **1.27** exhibited mild toxicity comparing the IC50 against HEK293 cells to that of MIC against bacteria. Overall in the study reported, the fluoro-substituted compounds are more superior to the hydroxy-substituted compounds (Scheme 1.27).[104]



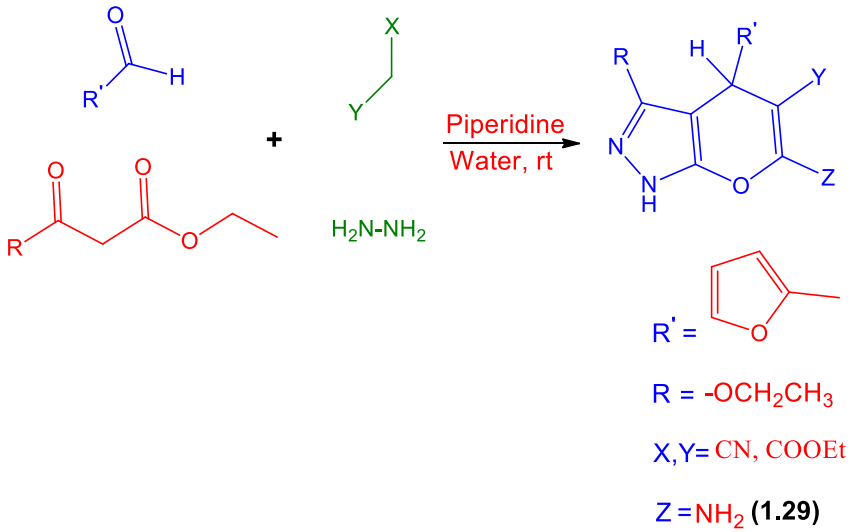
Scheme 1.27. Synthesis of coumarin-substituted pyrazole-derivatives.

In **2020**, Ganesh Akula *et al.* synthesized several novel 3,4-substituted pyrazoles derivatives. The newly synthesized derivatives were screened for their antimicrobial activity against Gram-positive bacteria like *S. aureus* and *B. subtilis* as well as Gram-negative bacteria *P.aeruginosa* and *E.coli*. Antifungal activity was also tested against *Candida albicans* and *Aspergillus niger*. From the final result, it was observed that compounds **1.28a**, **1.28b**, **1.28c**, **1.28d** and **1.28e** exhibited potent activity towards antimicrobial strains (Scheme 1.28).[105]



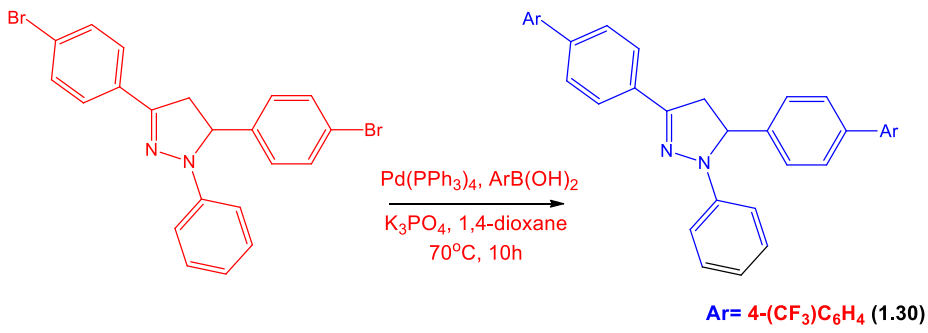
Scheme 1.28. Synthesis of 3,4-disubstituted pyrazole derivatives.

Pyrano[2, 3-c]pyrazoles derivatives were synthesized in the year **2021** by Samy A. El-Assaly and collaborators which were further evaluated for their antimicrobial activity. Most of the newly synthesized compounds show a good antibacterial activity of which compound **1.29** was the most promising of all. Henceforth, a molecular docking study was also performed and the result reveals that compound **1.29** exhibits potential binding affinity against penicillin-binding protein (Scheme 1.29).[106]



Scheme 1.29. One-pot synthesis of pyrano[2,3-*c*]pyrazoles.

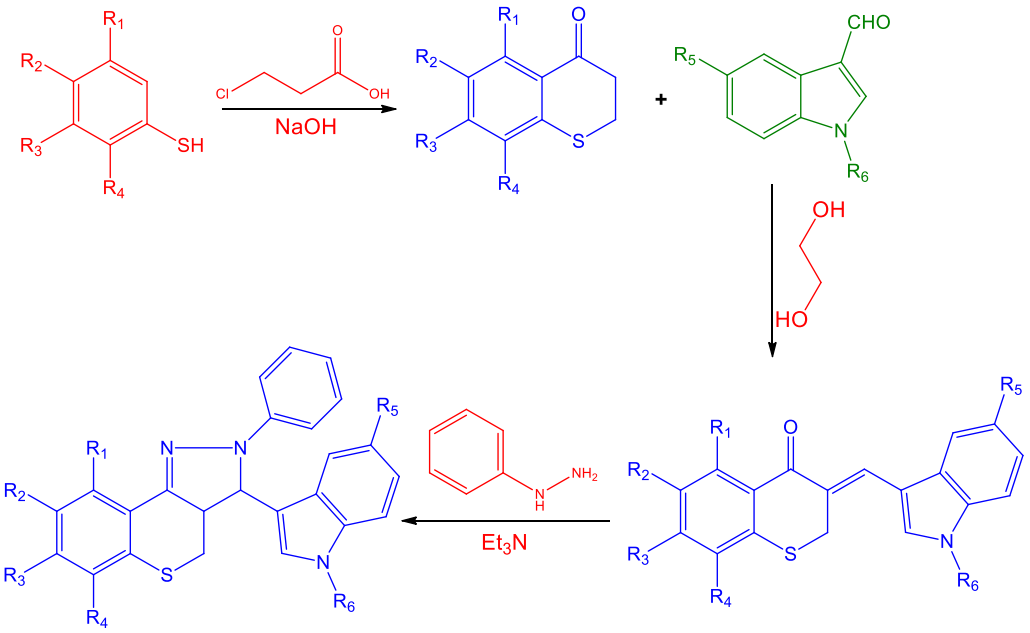
In **2021**, Diana Khaled Karim *et al.* reported the first palladium-catalyzed coupling reactions of 1, 3, 5-triphenyl pyrazoline achieved by Suzuki-Miyaura reactions. The new pyrazoline derivatives were evaluated for their antibacterial activity against four bacterial strains namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. They reported that compound **1.30** exhibited the highest activity among all as well as more significantly active than the reference drug Trimethoprim (Scheme 1.30).[107]



Scheme 1.30. Synthesis of 3,5-bis(biphenyl)-1-phenyl pyrazoline.

1.4.2.2 Pyrazole with Anti-cancer Activity

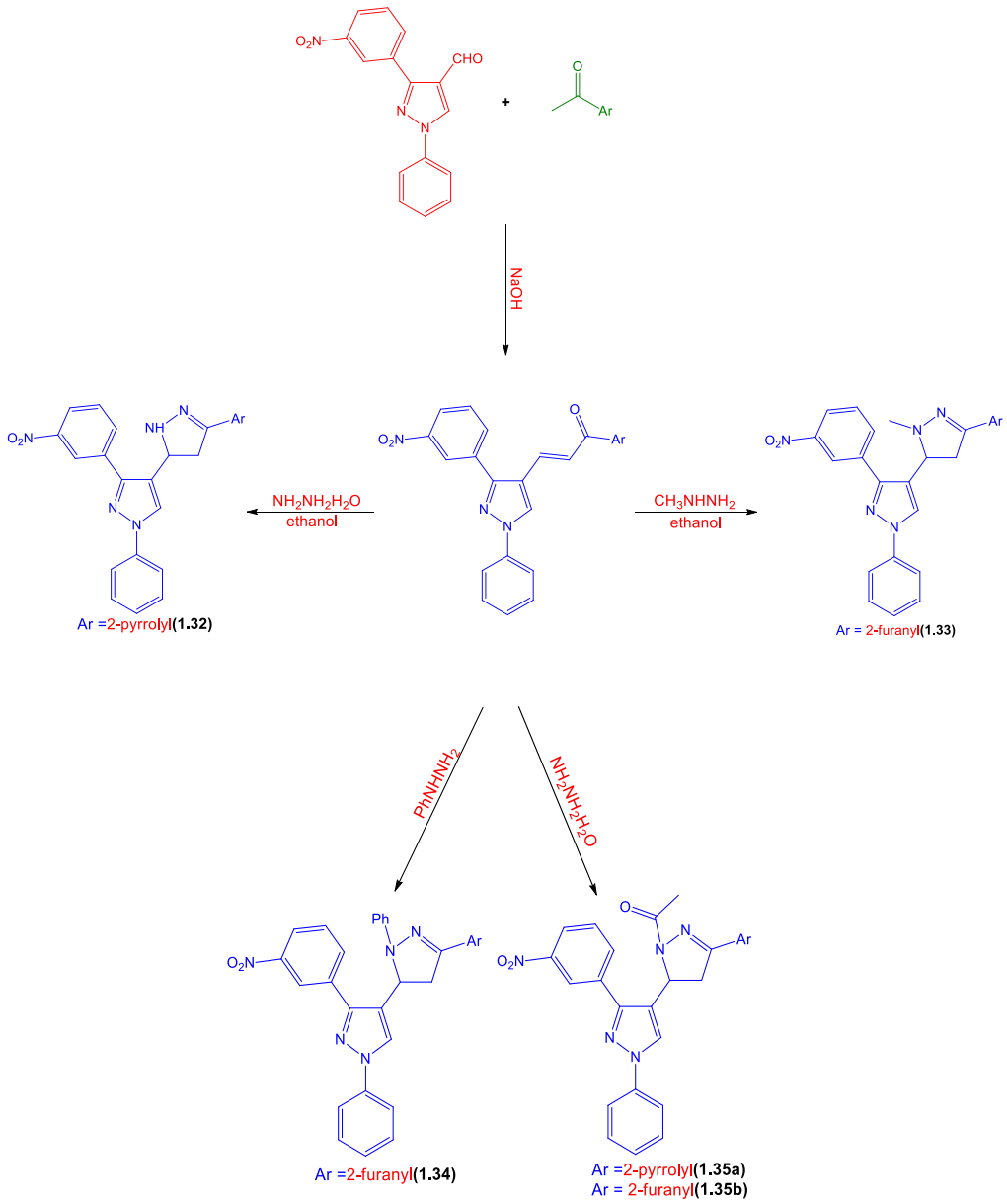
Cancer is a fatal disease characterized by abnormal cell growth with the potential to invade or spread to other parts of the body. It is one of the most frightening diseases and the second most leading cause of human death worldwide.[108] Cancer treatment generally includes chemotherapy, radiation therapy, and surgery. However, the successful applications of these treatment procedures are often limited in most cases due to their high systemic toxicity, resistance during treatment and adverse side effects[109]As such, the design and development of non-conventional, safe and efficient chemical classes of agents are the main goals of modern-day medicinal chemistry. Pyrazoles constitute an important class of compounds displaying anticancer activity, and in recent years, many studies have been devoted to designing new and potent anticancer drugs containing pyrazole moiety. Pyrazoline derivatives containing indole scaffold were synthesized by Yali Song and co-workers in **2020** and their antiproliferative activity was evaluated against four human cancer cell lines such as MGC-803, MCF-7, Bel-7404, Hela and a normal cell line L929. Compounds **1.31a** and **1.31b** shows potent anticancer activity against MGC-803 with IC50 values of 15.43 μM and 20.54 μM respectively by inducing G2/M cell cycle arrest and apoptosis (Scheme 1.31).[110]



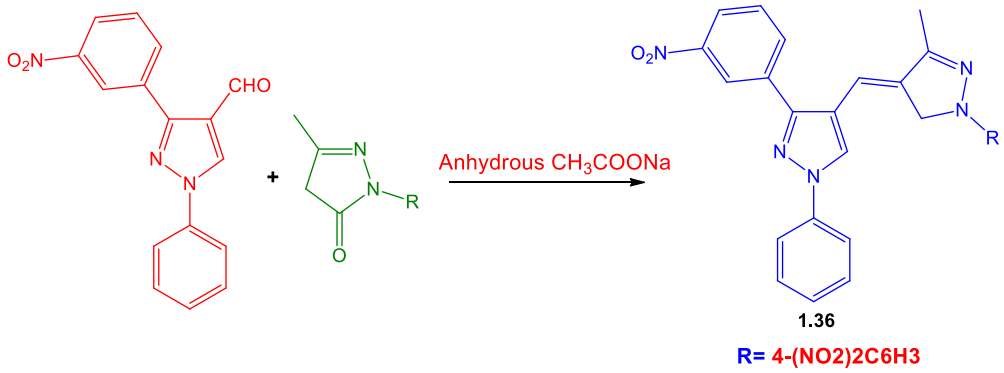
R1=H, R2=F, R3=H, R4=H, R5=H, R6=H (1.31a)
R1=H, R2=Cl, R3=H, R4=Cl, R5=H, R6=H (1.31b)

Scheme 1.31. Synthesis of pyrazoline containing indole skeleton.

In the year **2020**, Dina H. Dawood *et al.* evaluated a series of new pyrazole derivatives and investigated for breast cancer MCF-7 activity. Compounds **1.32**, **1.33**, **1.34**, **1.35a**, **1.35b** and **1.36** shows activity with IC50 values ranging from 16.50-26.73 µM. They were further studied for their VEGFR-2 inhibitory activity where the compounds **1.33**, **1.34**, **1.35a**, **1.35b** and **1.36** exhibit potential inhibitory efficiency versus VEFGR-2 kinase % inhibition range from 70 to 79 %. Compound **1.36** was also studied for flow cytometry analysis and reveals that **1.36** prompted pre-G1apoptosis and cell growth cessation at G2/M phase where it stimulates apoptosis *via* caspase-3 activation (Scheme 1.32 and Scheme 1.33).[111]

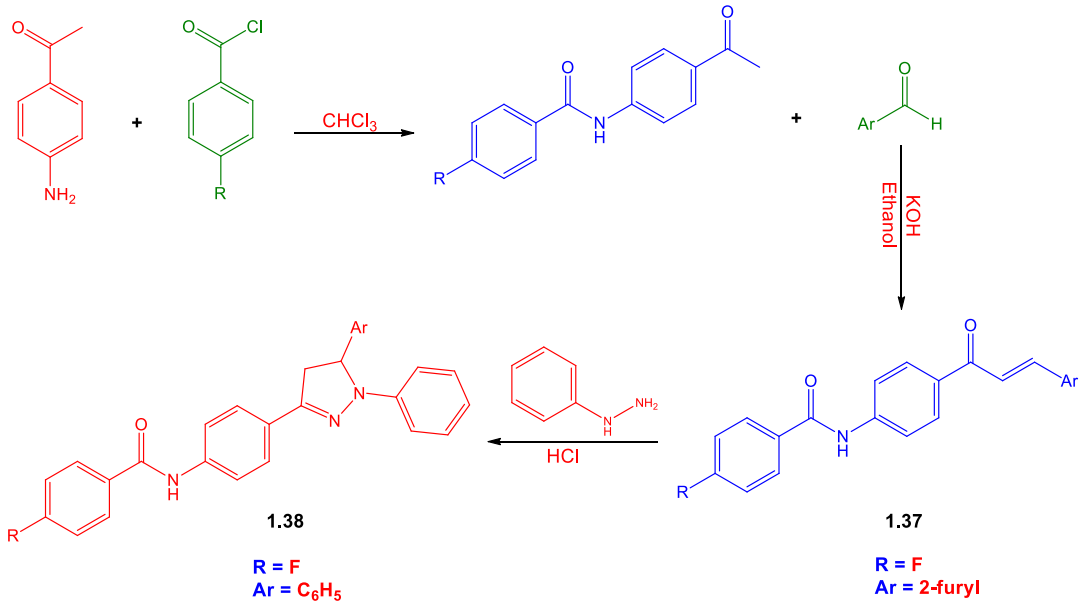


Scheme 1.32. Synthesis of substituted pyrazoline-pyrazole derivatives.



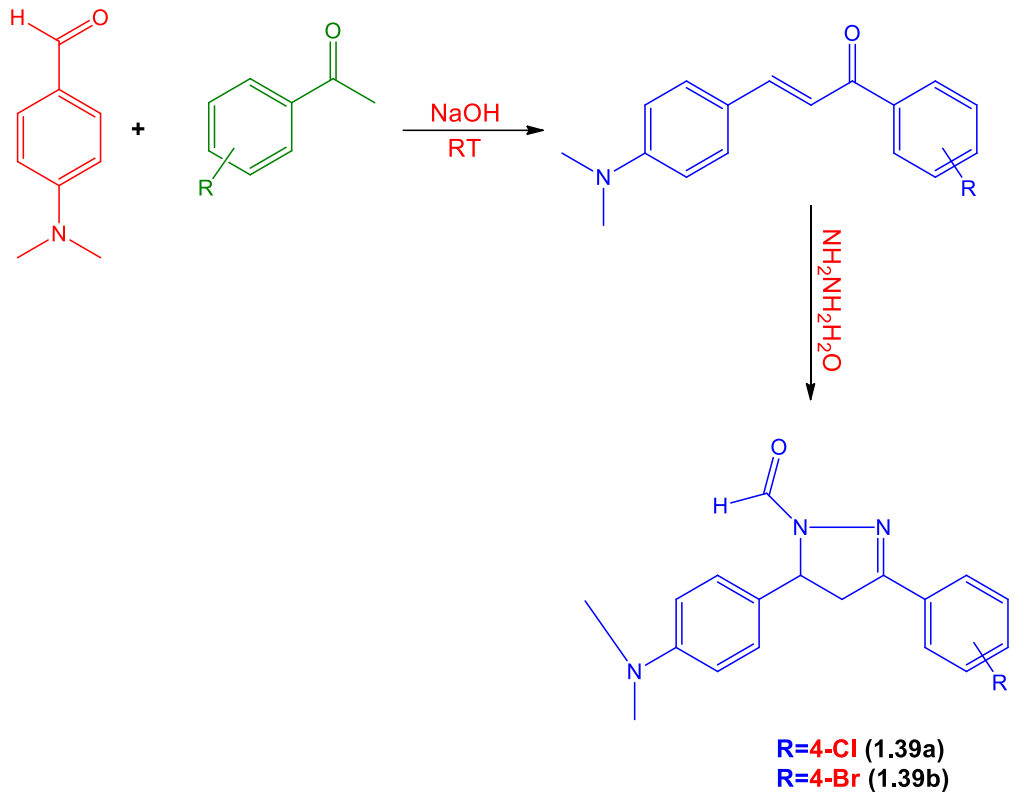
Scheme 1.33. Synthesis of substituted pyrazolone-pyrazole derivatives.

Faith Tok *et al.* in **2020** synthesized and investigated a series of novel 2-pyrazoline derivatives as a potential anticancer agent (Scheme 1.34). From the study conducted it was reported that compounds **1.37** and **1.38** show cytotoxic effect in Hela, MKN-45, MCF-7 cancer cells and did not show cytotoxicity on NIH-3T3 normal cells. Further, compounds **1.37** and **1.38** also upregulated Bax and downregulated Bcl-2 protein expression levels in cells. Henceforth, the study concluded that compounds **1.37** and **1.38** activate apoptosis by inducing mitochondrial apoptotic proteins in all three cancer cell lines.[112]



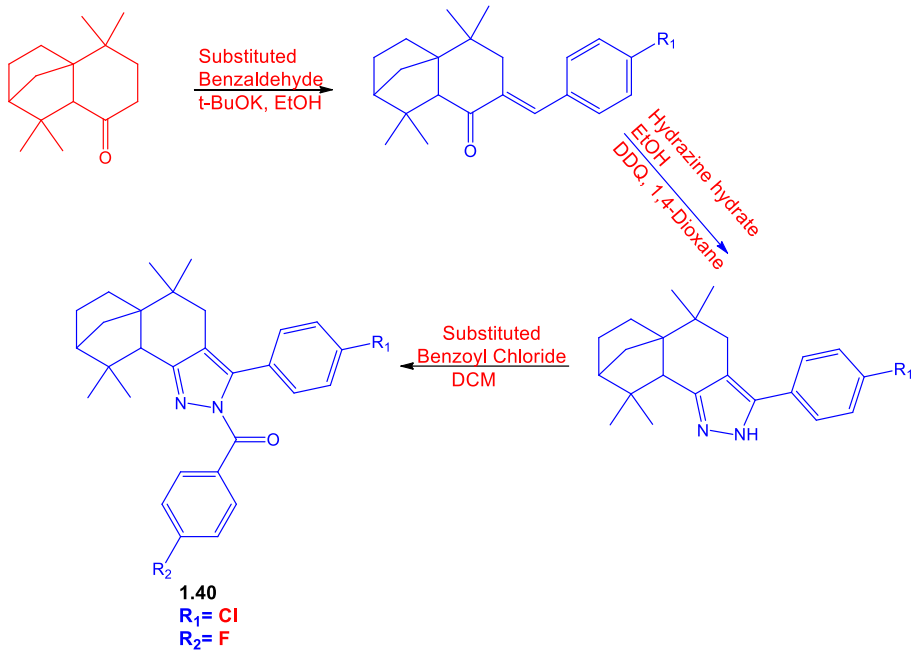
Scheme 1.34. Synthesis of 2-pyrazoline derivatives.

In **2021**, Manish Rama and collaborators design and synthesized pyrazoline containing N-formyl derivatives *via* Michael addition reaction. The novel compounds **1.39a** and **1.39b** shows excellent activity against fibrosarcoma cell lines (HT1080) and human lung cancer (A549) but low toxicity against human primary normal lung cells (HFL-1) (Scheme 1.35).[113]



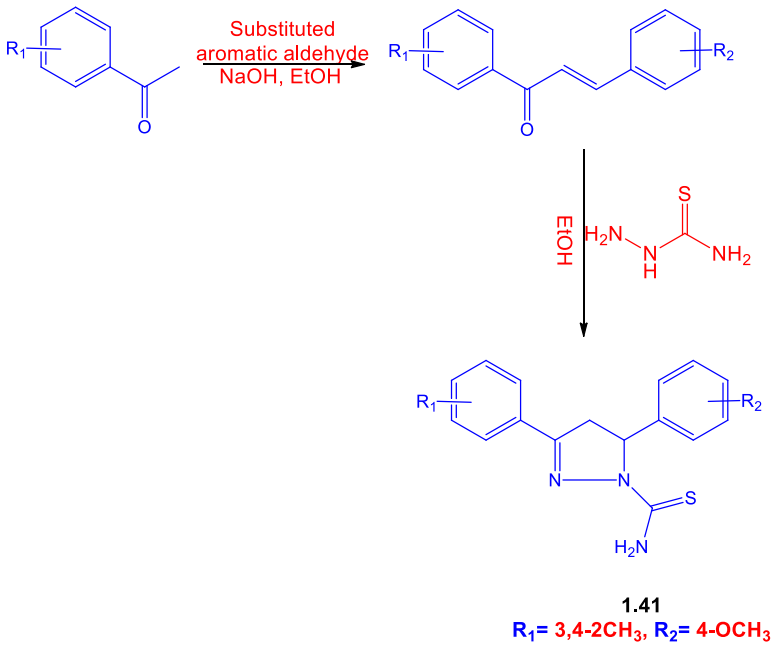
Scheme 1.35. Synthesis of N-formyl pyrazoline derivatives.

Pyrazole ring containing isolongifolanone was designed, synthesized and evaluated for their antiproliferative activity in **2021** by Yunyun Wang *et al.* Among the series of newly synthesized pyrazole, compound **1.40** shows excellent activity towards MCF-7 cancer cells and also induced the generation of intracellular ROS and mitochondrial depolarization. Further, the studies also reveal that compound **1.40** induced apoptosis *via* activation of caspase-3 and PARP through Bcl decreasing and increasing Bax and p53 (Scheme 1.36).[114]



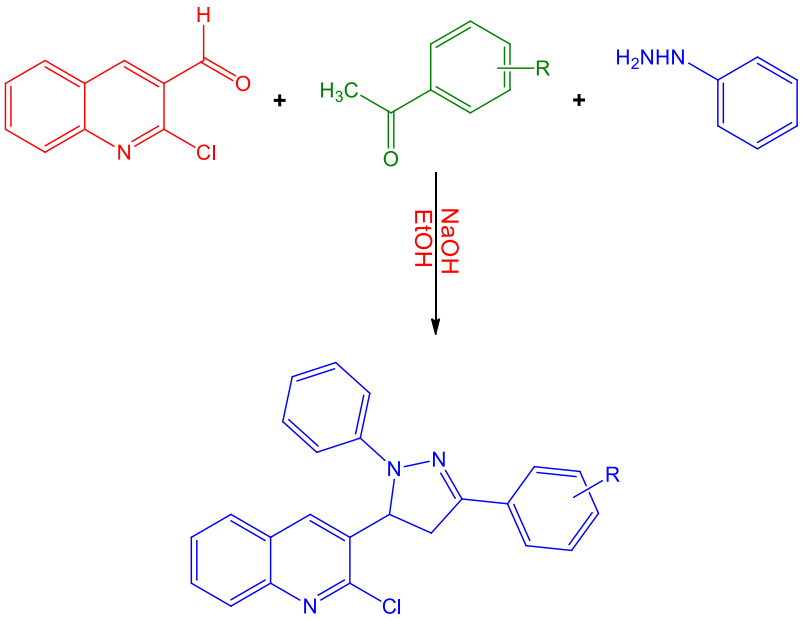
Scheme 1.36. Synthesis of pyrazole ring containing isolongifolanone derivatives.

Peng-cheng Lv and collaborators design and developed a series of pyrazole-thiourea derivatives in **2010** and investigated their EGFR inhibitory activity. The investigation report concluded that compound **1.41** exhibits potent activity against MCF-7 with IC50 value of 0.08 μM (Scheme 1.37).[115]



Scheme 1.37. Synthesis of pyrazole derivatives containing thiourea skeleton.

In **2020**, K. Santhosh Kumar and co-workers synthesized several quinoline pyrazoline derivatives and evaluated their anticancer activity against A375, MCF-7 and HT-29 by MTT assay method. Compounds **1.42a**, **1.42b** and **1.42e** exhibit potential activity against A375 and MCF-7 cells whereas compounds **1.42c**, **1.42d** and **1.42f** showed potent activity against HT-29 cells (Scheme 1.38).[116]



1.42a-f

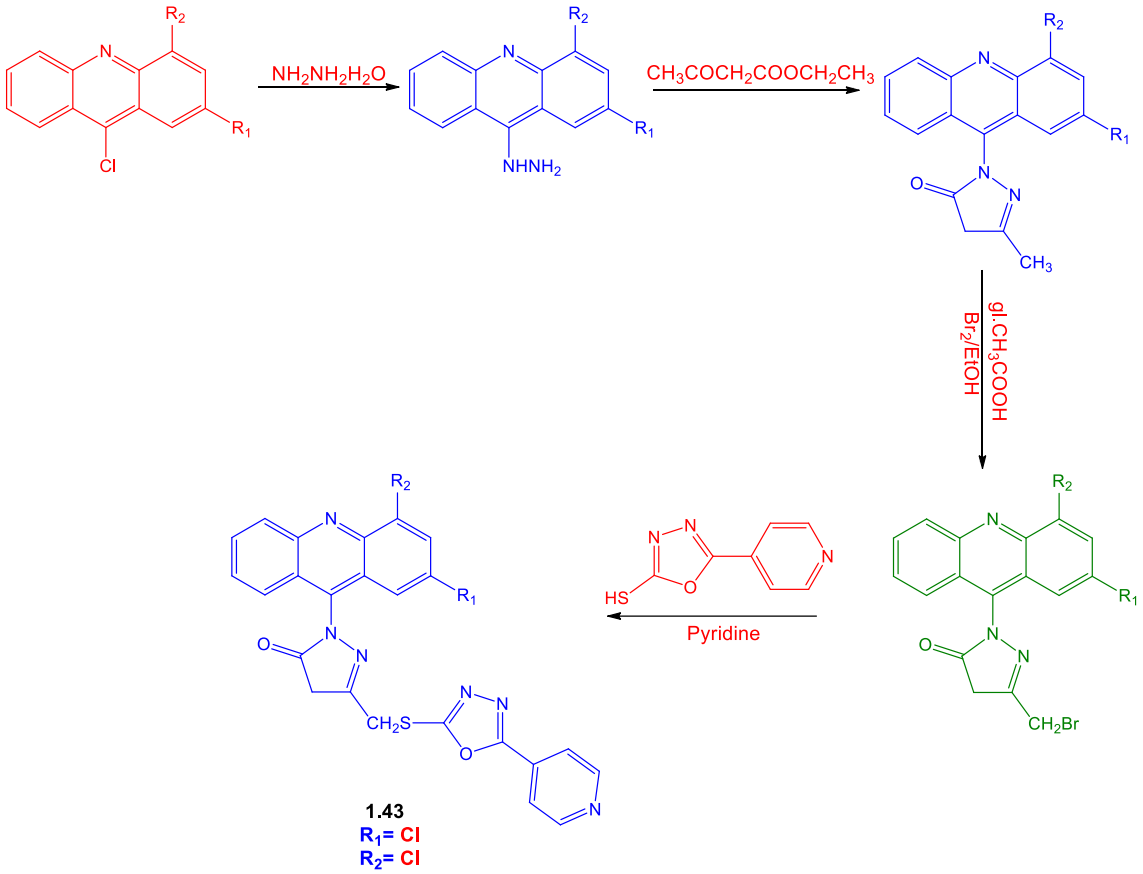
R= 4-F **(1.42a)**
R= 4-Cl **(1.42b)**
R= 4-Br **(1.42c)**
R= 4-NO_2 **(1.42d)**
R= 1-Naphthyl **(1.42e)**
R= 2-Naphthyl **(1.42f)**

Scheme 1.38. Synthesis of quinoline-pyrazoline derivatives.

1.4.2.3 Pyrazole with Anti-inflammatory Activity

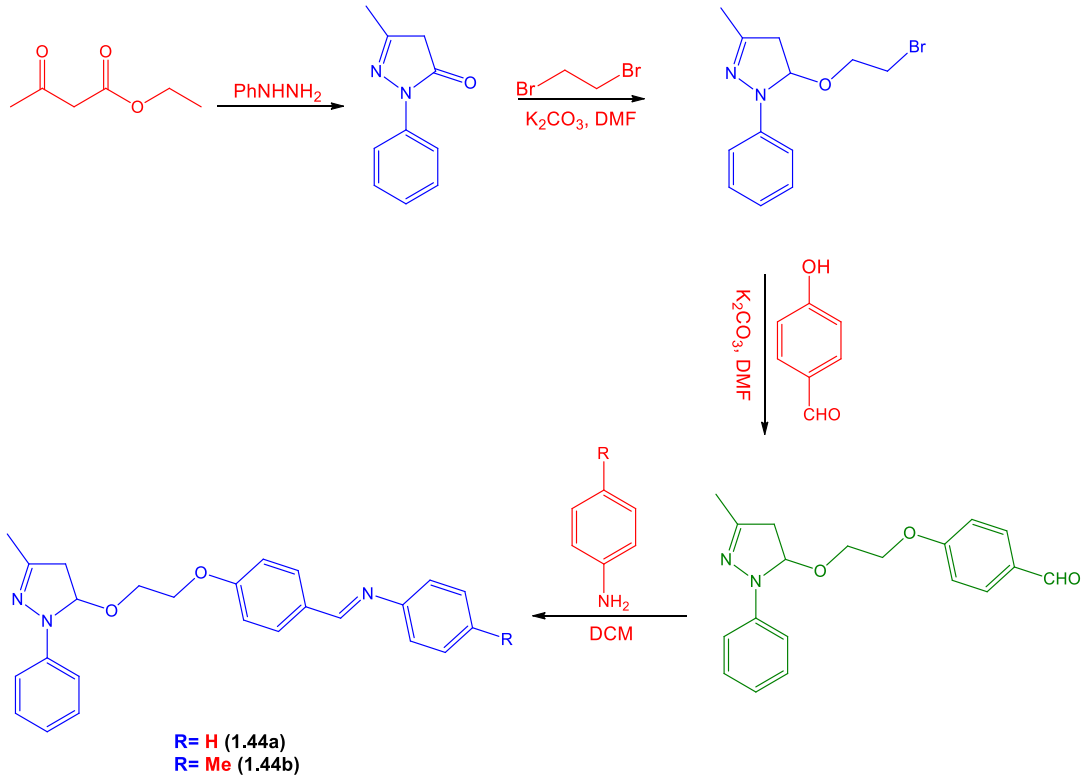
Inflammation is a multifactorial process that reflects the response of the body to various harmful stimuli. Inflammation is caused by an increased movement of leucocytes from the blood into the injured tissue and is reflected by many disorders such as redness of the skin, pain, swelling, and loss of function in the case of acute inflammation while chronic inflammation may lead to arthritis, asthma, and psoriasis.[117] Although Non-steroidal inflammatory drugs (NSAIDs) are among the most widely used drugs for treating inflammatory disorders, they display several side effects, such as gastrointestinal mucosal damage, renal toxicity and bleeding.[118,119] Therefore, the search for novel anti-inflammatory

drugs continues with numerous studies focused on the design and synthesis of newer drugs with minimal adverse side effects and a high safety margin. Several pyrazole compounds have been reported to be effective therapeutic agents for the treatment of inflammation. Substituted acridinyl pyrazoline derivatives and their anti-inflammation activity was synthesized and evaluated by Trilok Chandra *et al.* in **2010**. The compound with the strongest anti-inflammation activity was shown by **1.43** and overall, the compounds bearing electronegative atoms play a key role in enhancing the activity of the compound (Scheme 1.39).[120]



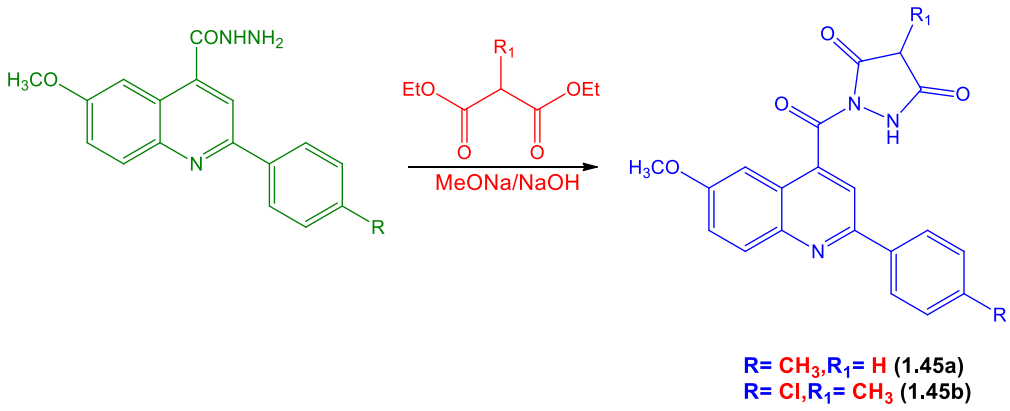
Scheme 1.39. Synthesis of acridinyl pyrazoline derivatives.

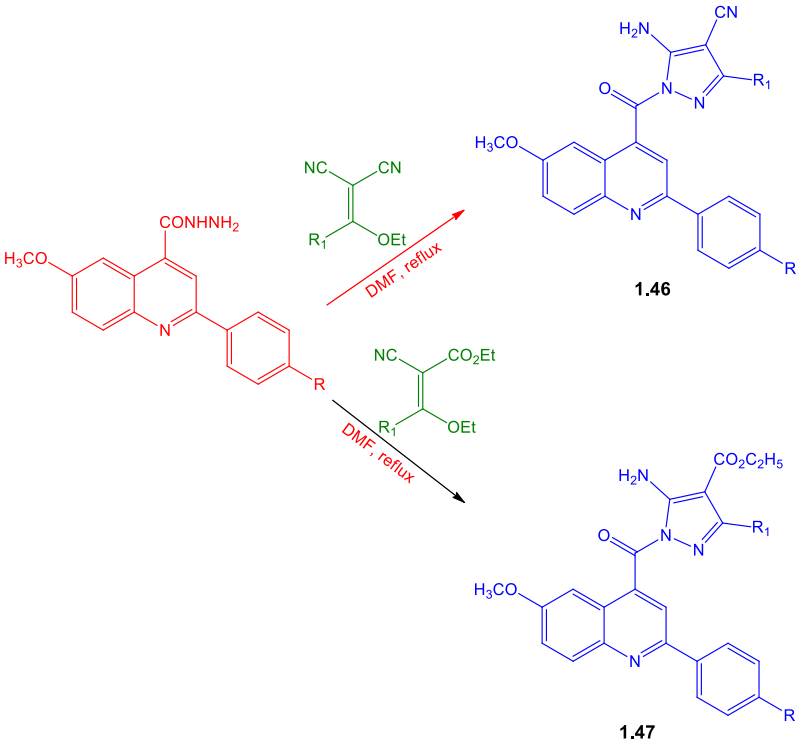
Ashish Kumar Tewari *et al.* in **2014** synthesized several pyrazoline derivatives and tested their anti-inflammatory activity which was performed using carrageenan mouse paw edema bioassay. The novel compounds **1.44a** and **1.44b** manifest the best among the series with comparable anti-inflammatory activity to the reference drug Nimesulide (Scheme 1.40).[121]



Scheme 1.40. Synthesis of pyrazoline derivatives.

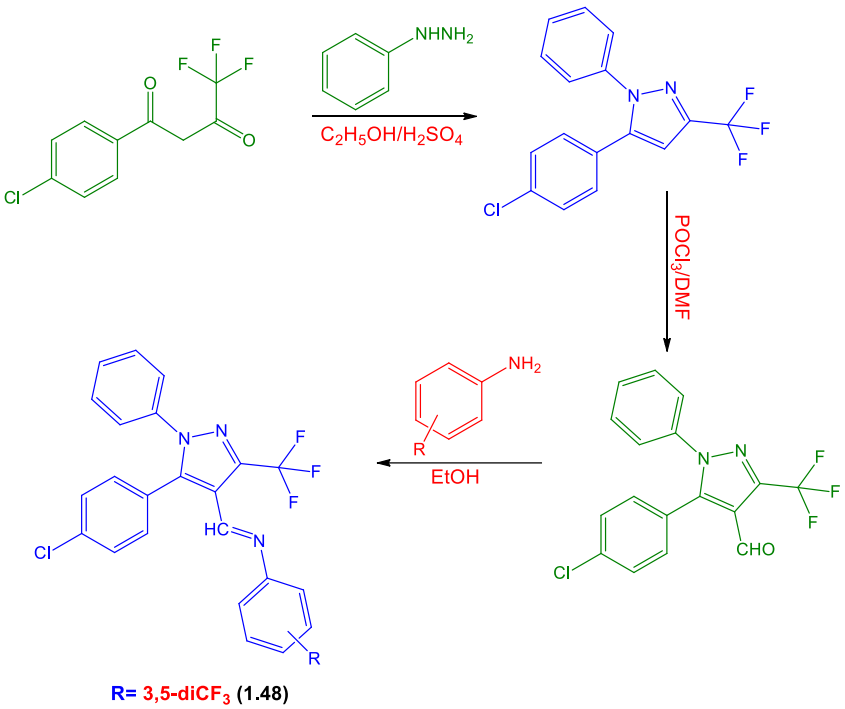
A series of quinolines-pyrazole derivatives were developed by using Pfitzinger reaction and their anti-inflammatory activity and ulcerogenic effect were tested in the year **2015** by Said A.H. El-Feky and co-workers (Scheme 1.40). Molecular docking was also studied to rationalize the possible selectivity against COX-2 enzyme. They have reported that compound **1.47** exhibits the highest anti-inflammatory activity and also shows the best binding pose in the receptor protein COX-2. Furthermore, from the results, it was also found out that compounds **1.45a**, **1.45b**, **1.46** and **1.47** were devoid of ulcerogenic activity.[122]





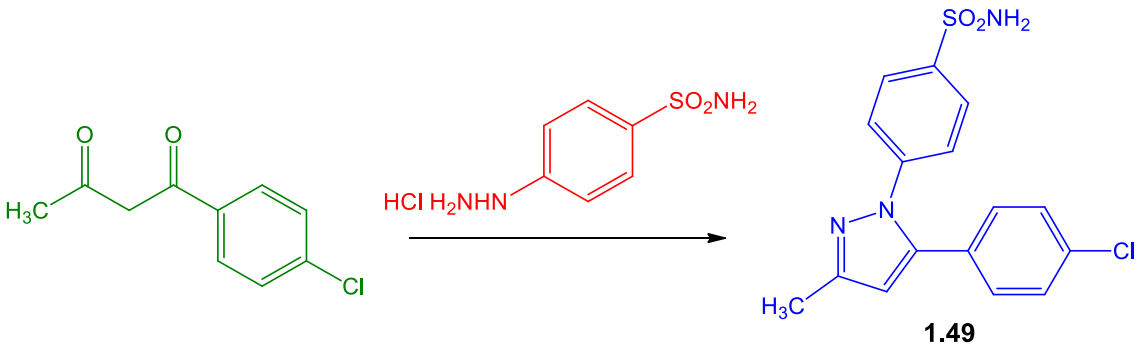
Scheme 1.41. Synthesis of quinoline incorporated pyrazole derivatives.

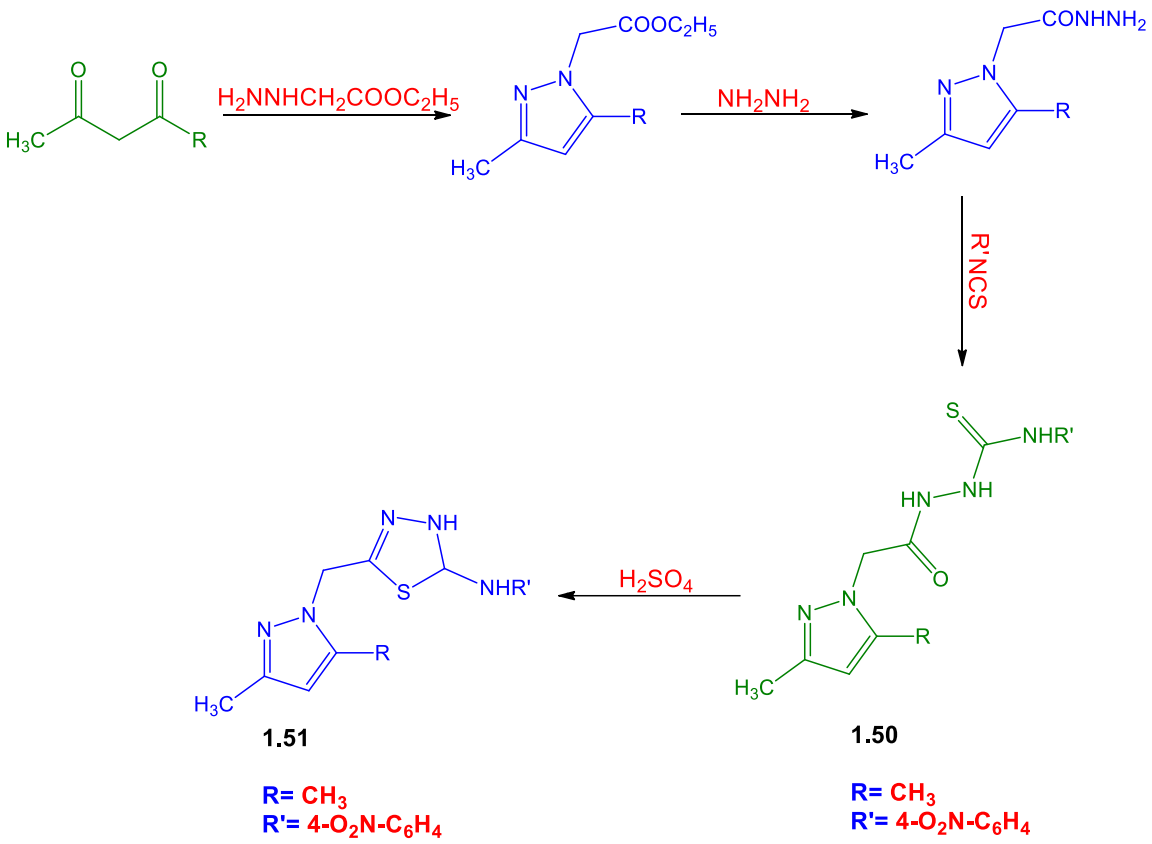
In **2012**, Magda A.-A. El-Sayed *et al.* were able to synthesize some pyrazole derivatives. Among all investigated series, the synthesized compound **1.48** exhibits anti-inflammatory activitywhich was performed in *vivo* using the carrageenan-induced rat paw edema model (Scheme 1.42).[123]



Scheme 1.42. Synthesis of triarylpyrazole derivatives.

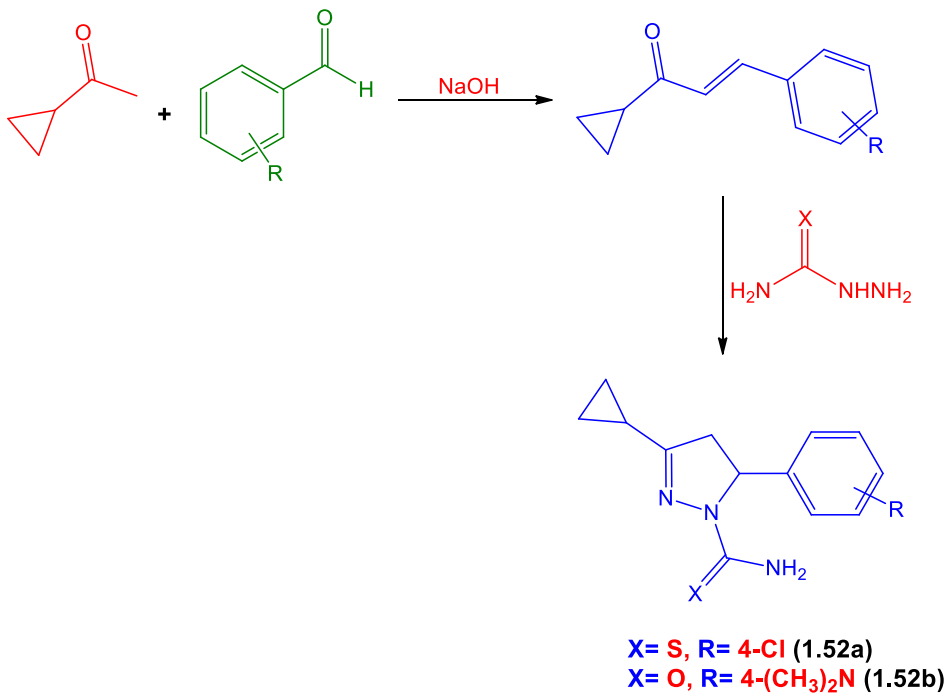
Samir M. El-Moghazy *et al.* in **2012** synthesized and developed a series of pyrazole derivatives and *in vivo* anti-inflammatory activity was investigated by employing the standard acute carrageenan-induced paw edema method. The most active compounds **1.49**, **1.50** and **1.51** were also tested for ulcerogenic liability in rats comparing with the reference drugs indomethacin and celecoxib (Scheme 1.43).[124]





Scheme 1.43. Synthesis of pyrazole derivatives with anti-inflammatory activity.

Nadia A. Khalil *et al.* in **2012** developed a series of pyrazoline derivatives and evaluated the series for anti-inflammatory and antioxidant activity. The results obtained from the studies reveals that compound **1.52a** and **1.52b** shows the highest anti-inflammatory and free-radical scavenging activities (Scheme 1.44).[125]

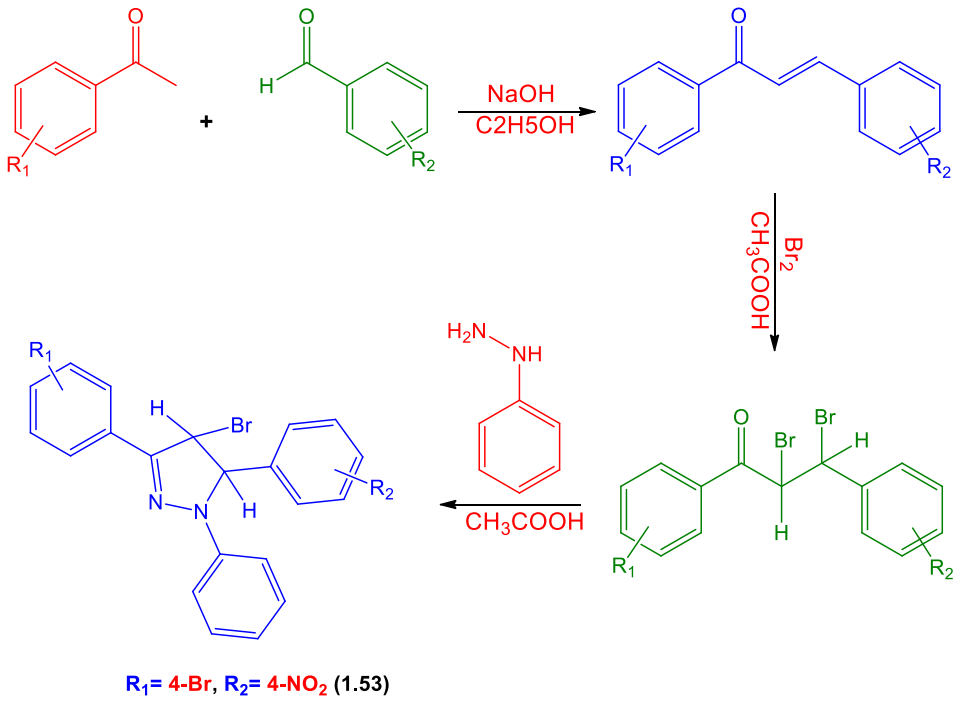


Scheme 1.44. General synthetic pathway of pyrazoline derivatives.

1.4.2.4 Pyrazole with Anti-oxidant Activity

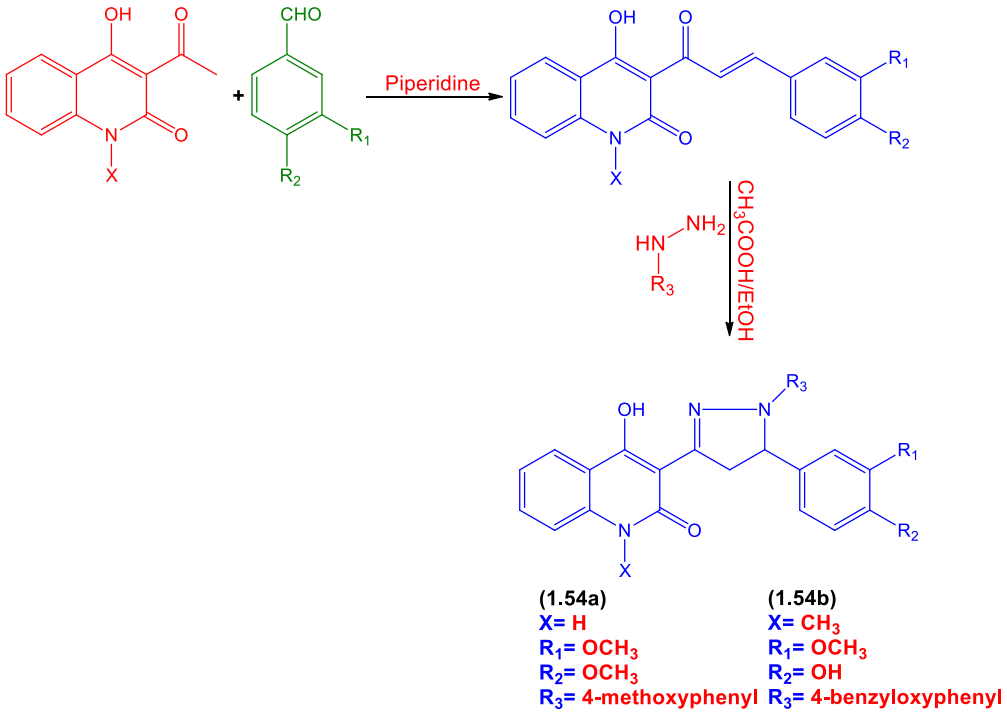
Free radicals are highly reactive molecular species containing an unpaired electron in an atomic orbital. They are capable of attacking the healthy cells of the body and damage all major components of cells, including DNA, proteins, carbohydrates and lipids[126] Cells with damaged DNA stagnate and are prone to developing cancer and growths. They are responsible for accelerating the aging process causing wrinkles age spots and diabetes, Alzheimer's disease, cardiovascular disease, alcohol-induced liver disease, neural disorders.[64] Antioxidants, also known as free radicals scavengers, are chemicals that prevent damages by interacting and neutralizing the free radicals radical[127] Although, the body itself produces antioxidants known as endogenous antioxidants, the capacities of endogenous antioxidants are affected by age, diet, and health status of the individuals. Therefore, external (exogenous) dietary antioxidant sources are required to diminish the increasing effects of such.[128] As such, the searches for new antioxidants have garnered immense attention**.** Anjan Kumar *et al.* in **2013** synthesized a series of 4-bromo pyrazolines derivatives employing Claisen-Schmidt condensation. The antioxidant activity was studied using the DPPH method and was found out that the compound **1.53** having 4-bromo and 4-

nitro groups present in the phenyl rings of pyrazoline nucleus exhibit the highest activity (Scheme 1.45).[129]



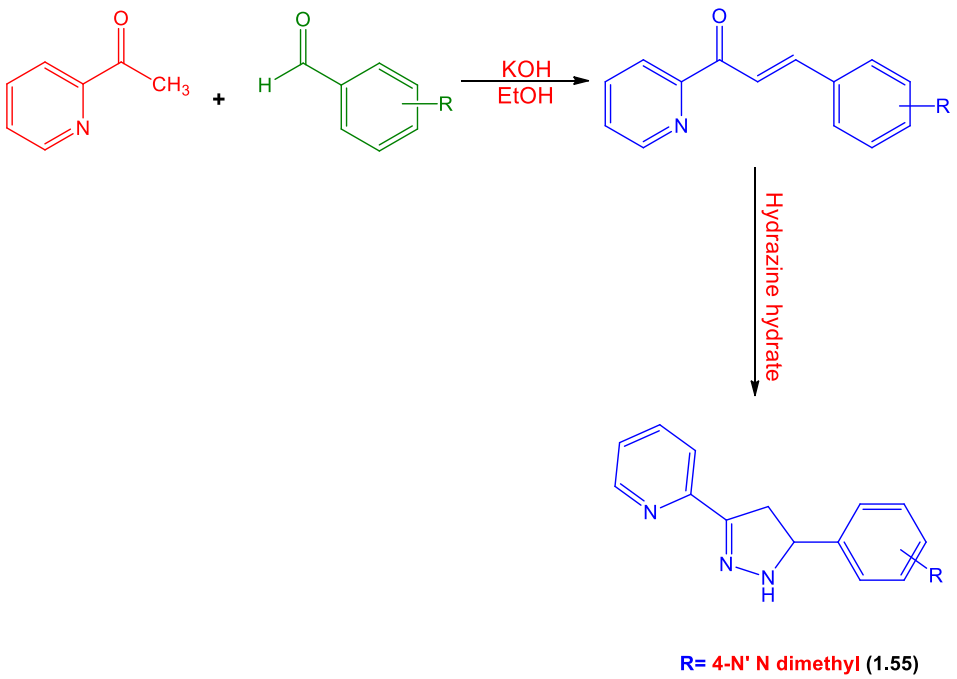
Scheme 1.45. Synthesis of pyrazoline derivatives with antioxidant activity.

Ioanna Kostopoulou and co-workers synthesized a series of quinolinone-pyrazoline hybrids in **2020**. Antioxidant activity was performed using the DPPH method which reveals that compounds **1.54a** and **1.54b** shows the best DPPH scavenging ability.[130]



Scheme 1.46. Synthesis of quinolinone-pyrazoline derivatives.

Pyrazoline derivatives bearing pyridyl moiety were synthesized and further evaluated for their antioxidant activity by Imtiyaz Hussain lone and co-workers in **2014**. The synthesized compounds exhibited good antioxidant activity with slight variation due to the presence of different substituents on the phenyl ring (Scheme 1.47). Compound **1.55** exhibited the maximum antioxidant activity which may be due to the availability of more electron clouds on the core molecule.[131]



Scheme 1.47. Synthesis of pyrazoline derivatives bearing pyridyl moiety.

1.5 Rationale and Aim of the Research Work

In view of the wide applications and biological activities of various chalcones, pyrazoles and pyrazolines we thought it will be worthwhile to design and synthesize organic octadecanamide chalcone derivatives and their further synthetic extension towards the synthesis of octadecanamide pyrazoline scaffolds to explore their anti-microbial activity. The present research investigation describes the synthesis of natural products-based organic octadecanamide chalcones and octadecanamide pyrazolines, spectral characterization, results and discussion and their anti-microbial activity and their molecular docking studies is detailed in chapters 2-4.

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Chapter 2

Synthesis of Octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl] amide Chalcones by Conventional Method and their Antimicrobial Activity

Abstract

In this chapter, a new series of octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives under base-catalyzed Claisen-Schmidt condensation weredesigned, synthesized and characterized by spectral analysis (FT-IR, 1H-NMR, 13C-NMR and MS). All the synthesized compounds were evaluated for their in vitro antimicrobial activity against two bacterial strains, *Escherichia coli* and *Staphylococcus aureus* and two fungal strains, *Penicillium italicum* and *Fusarium oxysporum* using the well-diffusion method. The antimicrobial studies revealed that compounds **2.6a**, **2.6b**, **2.6c** and **2.6d** showed promising antibacterial activity against both the bacterial strains, while compounds **2.6c** and **2.6d** exhibited good antifungal activity against both the fungal strains.

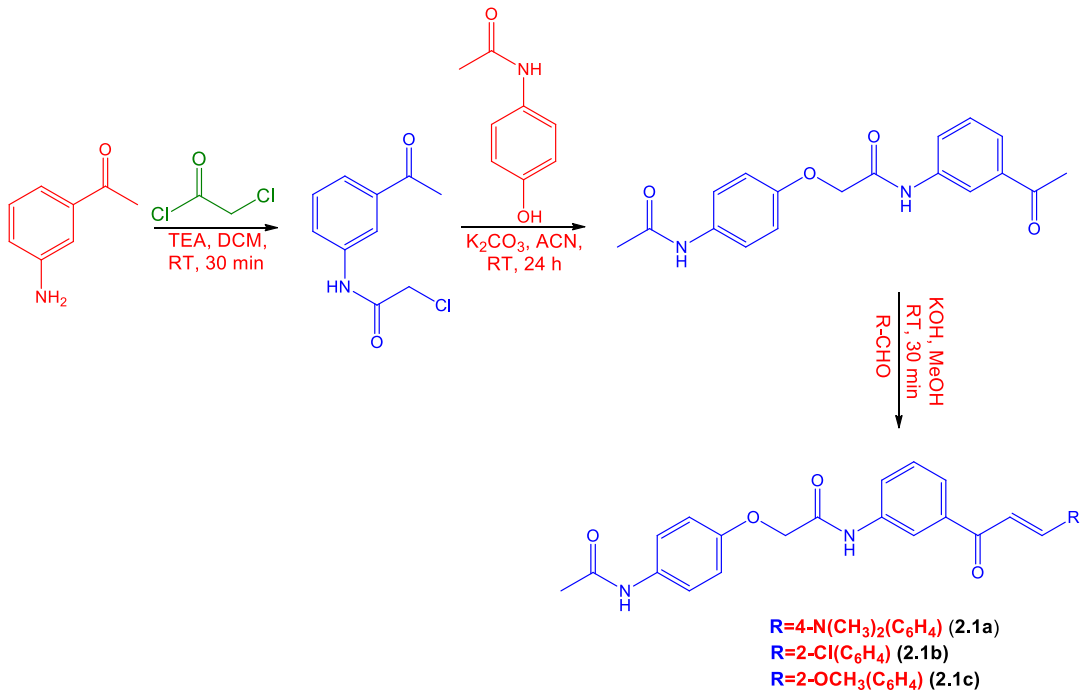
2.1 INTRODUCTION

Chalcones are considered as one of the most versatile compounds due to their diverse distribution in nature with a vast array of biological activity. Naturally, chalcones are found plenty in plants[1] and are secondary plant metabolite.[2] They fall under the flavonoid family and serves as key precursors in the synthesis of many biologically active compounds such as pyrazolines, pyrimidines, aurones, benzalcoumaranones, aurones, flavones, flavanones etc.[3-4] Chalcones manifest a bright yellow-colored compounds[5] and they can also be synthetically prepared in the laboratory from commercially available chemicals.[6] Two aromatic rings attached on the opposite end with α, β-unsaturated carbonyl system constitute the general structure of chalcone. The aromatic ring in chalcone structure is a completely delocalized system.[7] The conventional or most common reaction for preparing chalcone is Claisen-Schmidt condensation which is generally catalyzed by base but acid-catalyzed are also employed sometimes.[8] Claisen-Schimdt condensation of chalcone is achieved by fusing acetophenone with benzaldehyde catalyzed by base like NaOH, KOH, $Ba(OH)_2$, MgO and acid catalysts like HCl, BF_3, B_2O_3, $AlCl_3$ etc.[9] Chalcone possesses a wide spectrum of biological activity with different compounds showing good activity against antifungal, anti-oxidant,[10] antimalarial,[11] anti-bacterial,[12] anti-inflammatory,[13] anti-invasive,[13] antimicrobial activity,[14] anti-diabetic activity,[15] analgesic,[16] anti-ulcerative, antiviral,[17] Antileishmanial,

antituberculosis,[18] antipyretic, anti-hepatotoxic, antiallergic,[19] antiparasitic, antileishmanial, antitubercular,[20] antiplatelet, antitubercular, antihyperglycemic,[21] immunosuppressive, antinociceptive properties[22] anti-HIV, tyrosine kinase inhibitors[23] antiangiogenic[24] anti-spasmolytic activity, anti-invasive activity[25] effects on cancer cell growth[26] cytotoxic and anticancer activity.[27] The diverse nature and vast spectrum of biological importance have prompted many researchers around the world to synthesized chalcone.

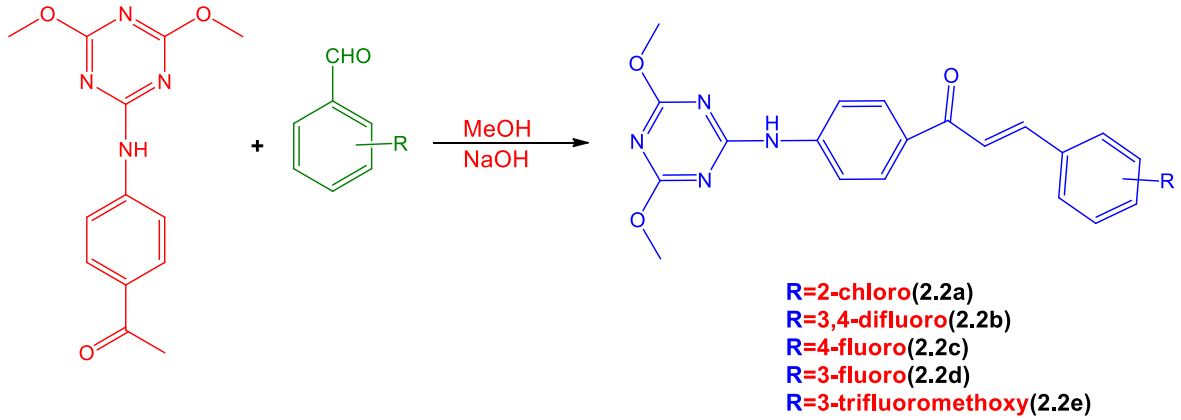
In recent years, the resistance of microbes to various antimicrobial drugs has raised major concerns over public health. Therefore, there is an urgent need to design and develop newer drugs with a wide spectrum of antimicrobial activity. Considering the versatile nature and broad spectrum of biological activity of chalcones, some recent investigations of chalcone derivatives and their role in biological activity are discussed below.

Sivalingam Lakshmanan *et al.* in **2021** synthesized some chalcone derivatives and studied their cytotoxicity as well as cell morphology analysis. The results reveal that the newly prepared chalcone derivatives exhibited potential cytotoxic action against A549 cells (human lung cancer) and no action was observed for HEL-299(human normal lung fibroblasts). The compounds **2.1a**, **2.1b** and **2.1c** in comparison with standard drug fluorouracil was observed to be more potent towards cytotoxic activity at 10 mM/mL against A549 as well as inducing cell death by apoptosis (Scheme 2.1).[28]



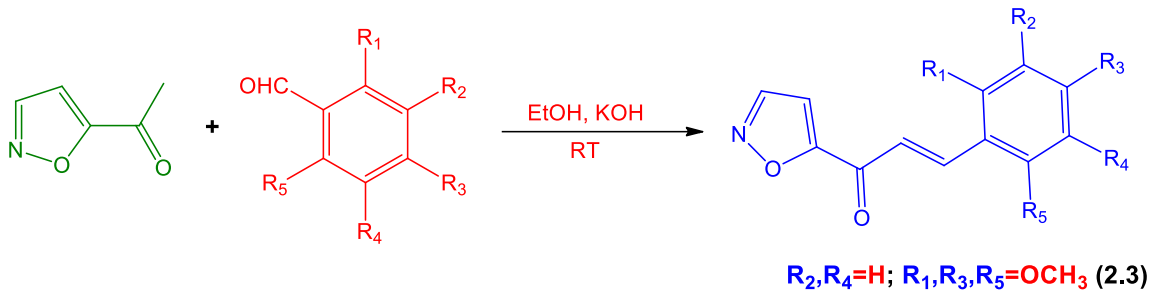
Scheme 2.1. Synthesis of chalcone derivatives as potent anaplastic lymphoma kinase inhibitors.

In the year **2020**, R.S. Shinde and collaborators design and developed a series of triazine chalcone derivatives and evaluated them for their antimicrobial activity. The synthesized derivatives were found to possess potential antimicrobial activity against the bacterial strains as well as the fungal strains. The compounds **2.2a**, **2.2b**, **2.2c**, **2.2d** and **2.2e** triazine chalcones showed immense potential antimicrobial activity with 1 to 1.5-fold times more active in comparison to that of standard reference drugs Ciprofloxacin and Miconazole. They also reported that the presence of substitution on the benzene ring of triazine chalcones like methoxy, benzeloxy, fluorine and acetal group was found to have more effect as antimicrobial agents (Scheme 2.2).[29]



Scheme 2.2. Synthesis of aryl substituted triazine chalcones.

Afzal Shaik and co-workers in **2020** was able to design and synthesized a series of chalcone containing Isoxazole ring derivatives. They tested the compounds for their potential anticancer, antimicrobial and antioxidant activity (Scheme 2.3). It was reported that compound **2.3** exhibited the best activity against bacterial strains with MIC at 1 µg/mL as well as antioxidant activity with IC50 at 5 ±1 µg/mL. All the synthesized compounds were also found to be non-toxic on normal human cell lines (LO2). Compound **2.3** substituted with 2,4,6-trimethoxy on the phenyl ring showed the most potent against bacterial strains and antioxidant activity which also suggested the importance of electronic property of the substituent on the phenyl ring in deciding the potency of a compound.[30]



Scheme 2.3. Synthesis of isoxazolylchalcones.

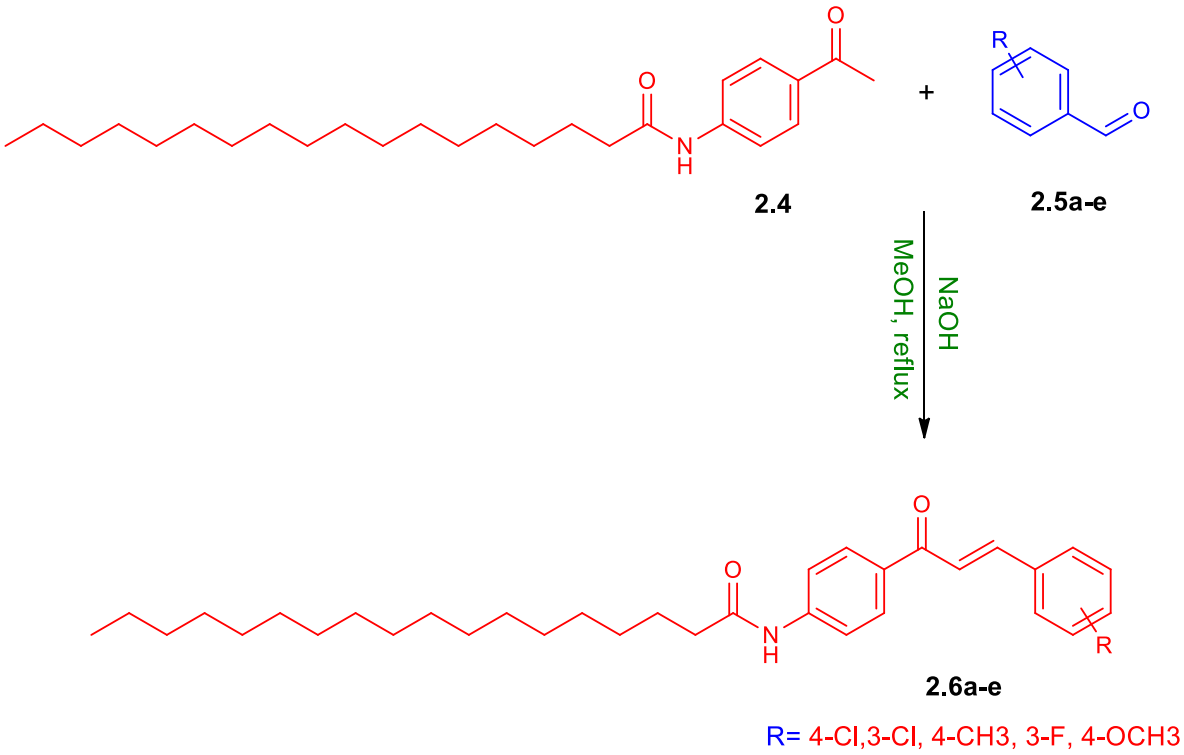
2.2 EXPERIMENTAL SECTION

2.2.1 Materials and Methods

All the reagents and solvents were purchased from commercially available sources and used without further purification. Melting points were recorded in open capillaries using IKON melting point apparatus and are uncorrected. FT-IR spectra of the compounds were recorded on Perkin-Elmer spectrophotometer (Spectrum-Two) using KBr disk and values are expressed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra for the compounds were recorded using Bruker 300 MHz spectrophotometer in $CDCl_3$ as a solvent and TMS as an internal standard, values are given in parts per million (ppm). Mass spectra for the compounds were recorded on Advion Expression (S)CMS system. Progress of the reactions was monitored by Thin Layer Chromatography (TLC) with silica gel plates (Merck) using ethylacetate and *n*-hexane (3:7) as a solvent system and visualized under UV-light/iodine vapors.

2.2.2 General procedure for the synthesis of octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (2.6a-e)

To a mixture of octadecanoic acid (4-acetyl-phenyl)-amide (**2.4**) (0.401g, 1 mmol), 4-chlorobenzaldehyde (**2.5a**) (0.106 g, 1 mmol) in methanol (15 mL) was added NaOH (40%) and the reaction mixture was stirred under reflux conditions for 24 h. The progress of the reaction was monitored by TLC. After completion of the reaction, crushed ice was added and filtered. The solid product was washed with water (3-4 times) to obtain the pure octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone (**2.6a**). The same method was followed for the synthesis of other compounds (**2.6b-e**).



Scheme 2.4. Synthesis of octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (**2.6a-e**).

2.3 RESULTS AND DISCUSSION

2.3.1 Chemistry

In the present study, we report the synthesis of octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives under base-catalyzed Claisen-Schmidt condensation. The synthetic route begins with the reaction of octadecanoic acid (4-acetyl-phenyl)-amide (**2.4**) and substituted benzaldehydes (**2.5a-e**) in the presence of NaOH in methanol under reflux to yield the corresponding octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (**2.6a-e**) (Scheme 2.4). The chalcone derivatives synthesized were reflux with constant stirring with most of the reaction completing within 24 hours depending upon the types of aldehyde used. The preliminary confirmation of the final product obtained was confirmed by thin layered chromatography with the formation of a single spot on the TLC plate. Figure 1 indicates that the halogen group in chalcone derivatives was highly reactive giving a good amount of solid product. This may be due to the electron-withdrawing group which made it more electropositive which is likely bound to be attacked by the nucleophile. The base-catalyst NaOH used also enhances the reactivity of the reaction.

The Variations in the reaction mixture color and physical properties was observed with reaction initially after mixing the reactant shows pale yellow insoluble solid which upon further heating and stirring turns to a dark yellow soluble solution. Generally, upon careful observation, almost all the reactions started to form their product within 20-30 minutes of continuous refluxing. It was observed that in many reactions, the formation of foam type on the top of the reaction mixture indicates the initial formation of the product which was confirmed by thin layer chromatography or simply TLC. The compounds bearing substituents of chloro and fluoro shows good yields. The workup procedure employed was quite simple with ice cold water wash for 3-4 times. The color of the final solid product obtained after filtration and drying gives a varied range with most of the compound exhibiting pale yellow to bright yellow. The melting points of the synthesized compounds ranges from 116-161^{o}C. The structures of octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (**2.6a-e**) (Figure 2.1) were elucidated by FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy. The yield percentage and melting points of octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (**2.6a-e**) are presented in Table 2.1.

Table 2.1. Synthesis of octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (**2.6a-e**).

Entry	R	Product	Yield(%)	M.P.(^{o}C)
1	4-Cl	**2.6a**	78	158-161
2	3-Cl	**2.6b**	82	118-120
3	4-CH_3	**2.6c**	70	132-135
4	3-F	**2.6d**	80	116-117
5	4-OCH_3	**2.6e**	68	125-128

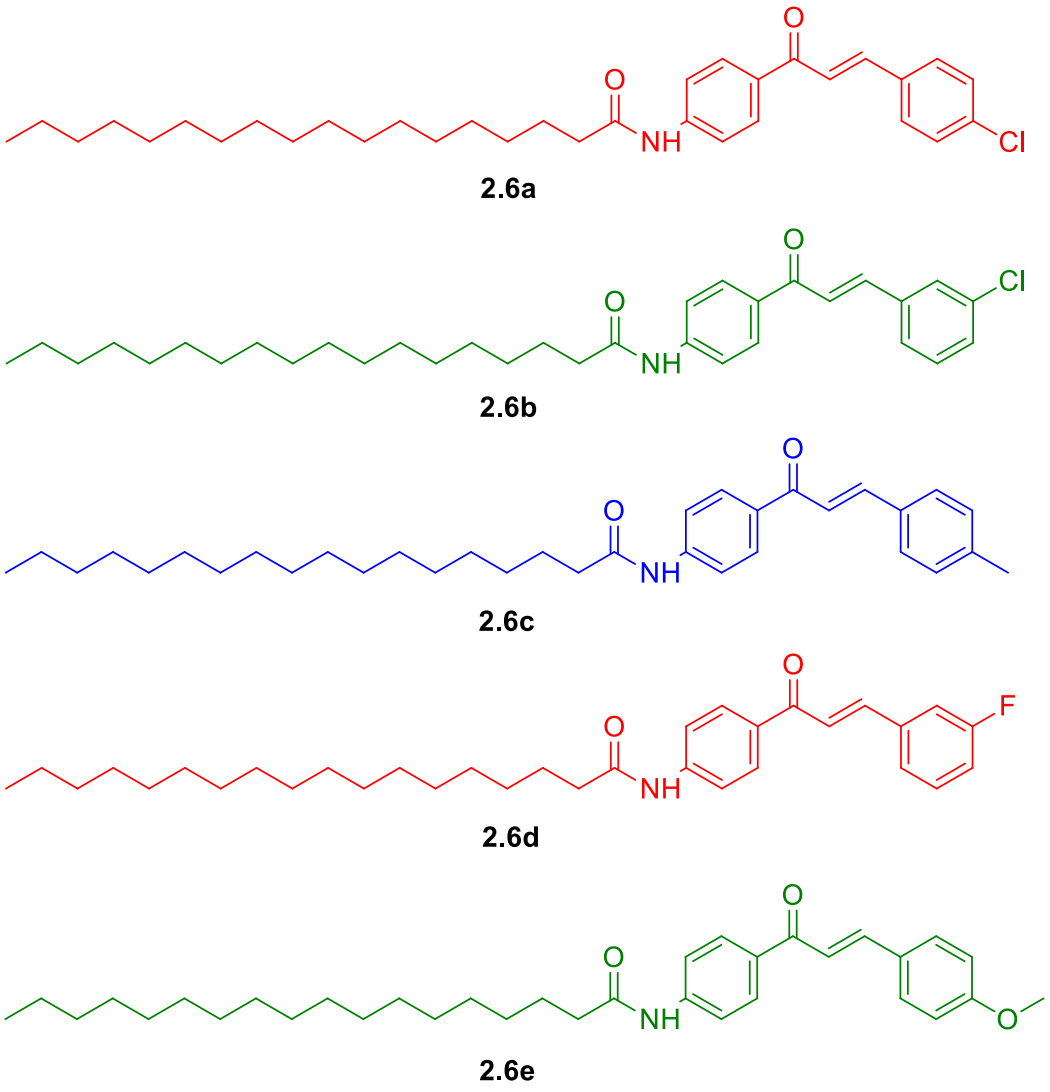


Figure 2.1. Structures of newly synthesized octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcones (2.6a-e).

2.3.2 Biological Activity

2.3.2.1 Materials and Methods

The antimicrobial activity of the synthesized chalcone derivatives was evaluated against bacteria and fungi by agar well diffusion method.[31,32] *Escherichia coli* and *Staphylococcus aureus* were used as test organisms for antibacterial study whereas, *Penicillium italicum* and *Fusarium oxysporum* were used as test organisms for antifungal study.

The bacterial strains were grown in nutrient broth media at 37°C±2°C for 24 hours. An aliquot of 200 µL of the freshly grown bacterial culture containing approximately 10^5-10^6 CFU/mL was spread over nutrient-agar plates. Wells were made on the agar plates using a

sterilized cork borer and the test compounds were added into their respective marked wells. The synthesized compounds (**2.6a-e**) were tested in concentrations of 10µg/uL using dimethyl sulfoxide (DMSO) as solvent. Streptomycin was taken as the positive standard. The plates were incubated at 37℃ for 24 hours. The antibacterial activity of the compounds was determined by measuring the diameter of the inhibition zone.

All those compounds (2.6a-e) screened for antibacterial activity were also tested for their antifungal activity in Sabouraud dextrose agar medium by following the same well diffusion method against *Penicillium italicum* and *Fusarium oxysporum*. Fresh fungal cultures were grown in Sabouraud dextrose broth at 27℃±2℃ for around 48 hours. Around 200µL of the freshly grown cultures were seeded on Sabouraud agar plates. Wells were made on the agar plates and the test compounds (2.6a-e) at 10µg/µL concentration were added into the wells. The plates were incubated at 27℃ for 48 hours. The antifungal activity of the synthesized compounds was evaluated by measuring the diameter of the inhibition zone. The antifungal activity of the test compounds was compared with the standard drug Fluconazole at the same concentration. All experiments were performed in triplicate to confirm reproducibility.

2.3.2.2 Antimicrobial Activity

The compounds (2.6a-e) were screened for their *in vitro* antibacterial activity against gram-negative bacteria (*Escherichia coli*) and gram-positive bacteria (*Staphylococcus aureus*), and antifungal activity against *Penicillium italicum* and *Fusarium oxysporum*. The activity was determined using well-diffusion method by measuring the diameter of the zone of inhibition in mm at a concentration of 10µg/uL in DMSO. The results of the antibacterial and antifungal activity of the tested compounds are presented in Table 2.2. All the synthesized compounds showed appreciable antibacterial activity for both gram-positive and gram-negative bacteria except compound 2.6e, which did not show any activity against *Escherichia coli*. The maximum inhibition against both *Escherichia coli* and *Staphylococcus aureus* was shown by compound 2.6a containing Cl substituents in para position in which the zone of inhibition was almost comparable to the standard drug.

The compounds also displayed antifungal activity against *Fusarium oxysporum* and *Penicillium italicum*. depicting its antifungal activity. All the tested compounds showed antifungal activity against *Fusarium oxysporum*. The maximum inhibition towards *Fusarium oxysporum* was exhibited by compound **2.6c** containing methyl group and **2.6d** containing Fluoro group. Whereas, the antifungal activity against *Penicillium italicum* was shown only

by compound **2.6c**, **2.6d** and **2.6e**, while **2.6a** and **2.6b** showed no potency.

Table 2.2. Antimicrobial activity of the tested compounds (**2.6a-e**).

	Zone of inhibition(mm)			
Compound	***Escherichia coli***	***Staphylococcus aureus***	***Fusarium oxysporum***	***Penicillium italicum***
2.6a	28	29	11	-
2.6b	12	11	15	-
2.6c	16	16	19	7
2.6d	14	14	19	7
2.6e	-	15	4	9
Streptomycin	30	32	-	-
Flucanazole	-	-	28	27

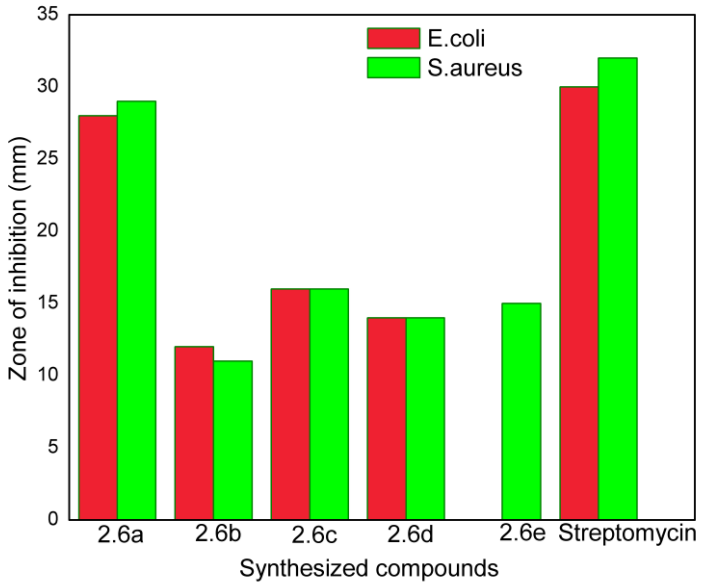


Figure 2.2. Bar graph of compounds **2.6a-e** tested against bacterial strain.

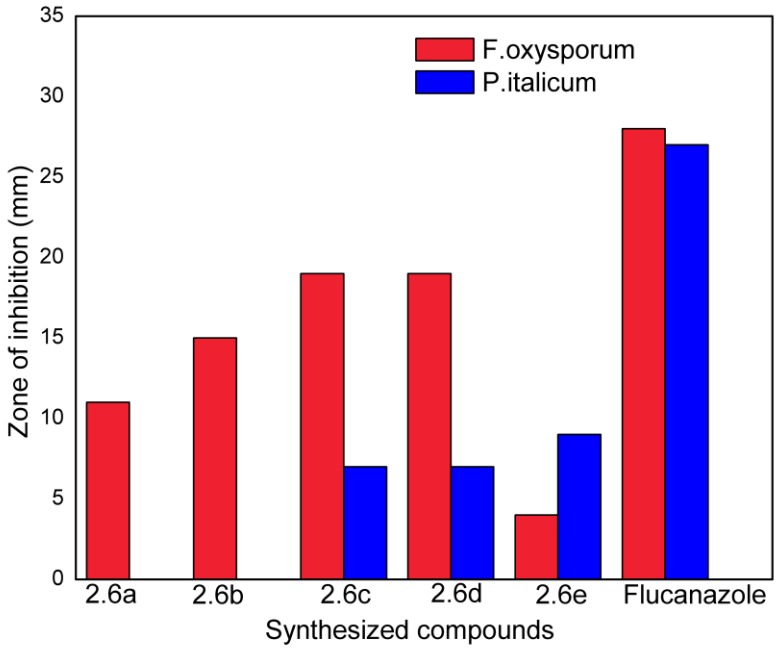


Figure 2.3. Bar graph of compounds **2.6a-e** tested against fungal strain.

2.4 CONCLUSION

In conclusion, a series of novel octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (2.6a-e) were synthesized and characterized by various spectroscopic methods such as IR, 1H-NMR, 13C-NMR and MS. The newly synthesized octadecanamide chalcone compounds (2.6a-e) displayed appreciable antibacterial activity against gram-positive *Escherichia coli* and gram-negative *Staphylococcus aureus*. All the compounds except 2.6e showed good antibacterial activity against the tested strains, among which compound 2.6a exhibited the most promising antibacterial activity which was almost comparable to that of standard drug Streptomycin. The results for antifungal studies indicated that all the compounds 2.6a, 2.6b, 2.6c, 2.6d and 2.6e exhibited antifungal activity against *Fusarium oxysporum*, however only compound 2.6c, 2.6d and 2.6e inhibited the growth of *Penicillium italicum*. Overall, the results revealed that the newly synthesized compounds can be of potential value in using as antifungal and antibacterial agents.

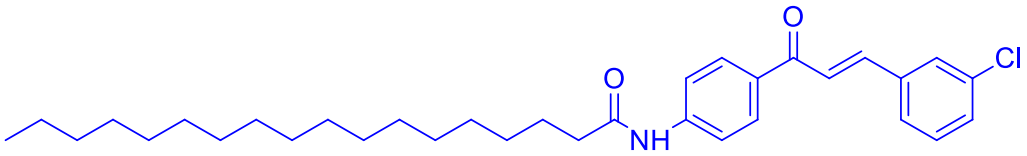
2.5 SPECTRAL CHARACTERIZATION DATA

2.5.1 N-(4-((E)-3-(4-chlorophenyl)acryloyl)phenyl)stearamide (2.6a)

2.6a

Pale yellow solid; yield 78%; M.P. =158-161°C; FT-IR (KBr, cm^{-1}): 3,285 (NH), 3,041 (Ar-CH), 1,658 (C=O), 1,556 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$), δ, ppm: 8.04-8.01(d, J = 8.72 Hz, 1H, Ar-H), 7.78-7.66 (m, 3H, Ar-H), 7.59-7.48 (m, 4H, Ar-H), 7.41-7.38 (m, 2H, HC=CH), 2.42-2.37 (t, 2H, CH_2), 1.79-1.70 (q, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3); ^{13}C NMR (300 MHz, DMSO-d_6), δ_C, ppm: 194.17, 184.04, 174.68, 167.02, 164.16, 161.84, 132.69, 131.30, 130.60, 130.14, 128.21, 128.01, 123.72, 118.39, 31.30, 29.03, 28.81, 28.70, 22.09; MS: m/z 524.2 (M^+).

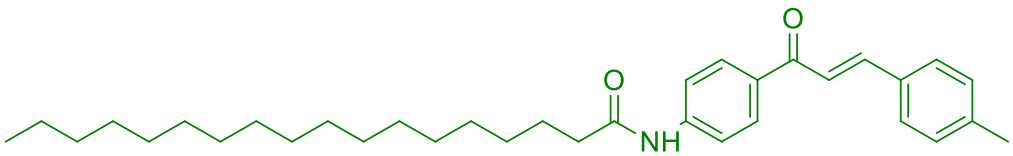
2.5.2 N-(4-((E)-3-(3-chlorophenyl)acryloyl)phenyl)stearamide (2.6b)



2.6b

Bright yellow solid; yield 82%; M.P. =118-120°C; FT-IR (KBr, cm^{-1}): 3,324 (NH), 3,046 (Ar-CH), 1,662 (C=O), 1,563 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$), δ, ppm: 8.18-8.15(d, J = 8.70 Hz, 1H, Ar-H), 8.08-7.92 (m, 4H, Ar-H), 7.80-7.77 (m, 3H, Ar-H), 7.49-7.45 (m, 2H, HC=CH), 6.63-6.60 (d, J = 8.81Hz, 1H, Ar-H), 2.37-2.33 (t, 2H, CH_2), 1.59-1.46 (q, 2H, CH_2), 1.22 (s, 28H, $(CH_2)_{14}$), 0.85-0.82 (t, 3H, CH_3); ^{13}C NMR(300 MHz, DMSO-d_6), δ_C, ppm: 185.61, 171.95, 154.01, 139.63, 133.72, 131.25, 130.56, 129.99, 129.46, 127.81, 127.55, 127.47, 124.03, 118.28, 112.68, 31.25, 28.99, 28.67, 22.05, 13.91; MS: m/z 524.2 (M^+).

2.5.3 N-(4-((E)-3-p-tolylacryloyl)phenyl)stearamide (2.6c)



2.6c

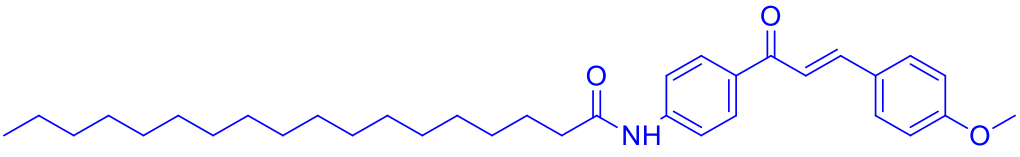
Yellow solid; yield 70%; M.P. =132-135 ^{o}C; FT-IR (KBr, cm^{-1}): 3,309 (NH), 3,040 (Ar-CH), 1,672 (C=O), 1,556 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$), δ, ppm: 8.04-8.01 (d, J = 8.16 Hz,1H, Ar-H), 7.95-7.92 (d, J = 8.46 Hz, 1H, Ar-H), 7.82-7.66 (m, 3H, Ar-H), 7.56-7.47 (m, 3H, Ar-H), 7.24-7.21 (m, 2H, HC=CH), 2.42-2.39 (m, 4H, $(CH_2)_2$), 1.77-1.72 (t, 3H, CH_3), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3), ^{13}C NMR(300 MHz, $CDCl_3$), δ_C, ppm: 187.96, 162.20, 143.74, 133.19, 129.96, 129.77, 129.49, 129.01, 128.90, 128.79, 121.17, 120.96, 118.29, 112.68, 31.25, 28.99, 22.05, 21.06, 13.91; MS: m/z 502.2 (M-1).

2.5.4 N-(4-((E)-3-(3-fluorophenyl)acryloyl)phenyl)stearamide (2.6d)

2.6d

Bright yellow solid; yield 80%; M.P. =116-117^{o}C; FT-IR (KBr, cm^{-1}): 3,323 (NH), 3,040 (Ar-CH), 1,683 (C=O), 1,556 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$), δ, ppm: 8.04-8.01(d, J = 8.86 Hz, 1H, Ar-H), 7.94-7.92 (d, J = 8.74 Hz, 1H, Ar-H), 7.78-7.67 (m, 2H, HC=CH), 7.55-7.49 (m, 1H, Ar-H), 7.41-7.32 (m, 4H, Ar-H), 7.14-7.06 (m, 1H, Ar-H), 2.42-2.32 (q, 2H, CH_2), 1.76-1.72 (m, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$), δ_C, ppm: 188.76, 172.07, 164.82, 161.55, 142.69, 141.86, 133.44, 131.33, 130.14, 124.63, 123.06, 119.16, 117.29, 114.09, 38.01, 32.06, 29.83, 25.60, 22.82, 14.25; MS: m/z 507.0 (M^+).

2.5.5 N-(4-((E)-3-(4-methoxyphenyl)acryloyl)phenyl)stearamide (2.6e)



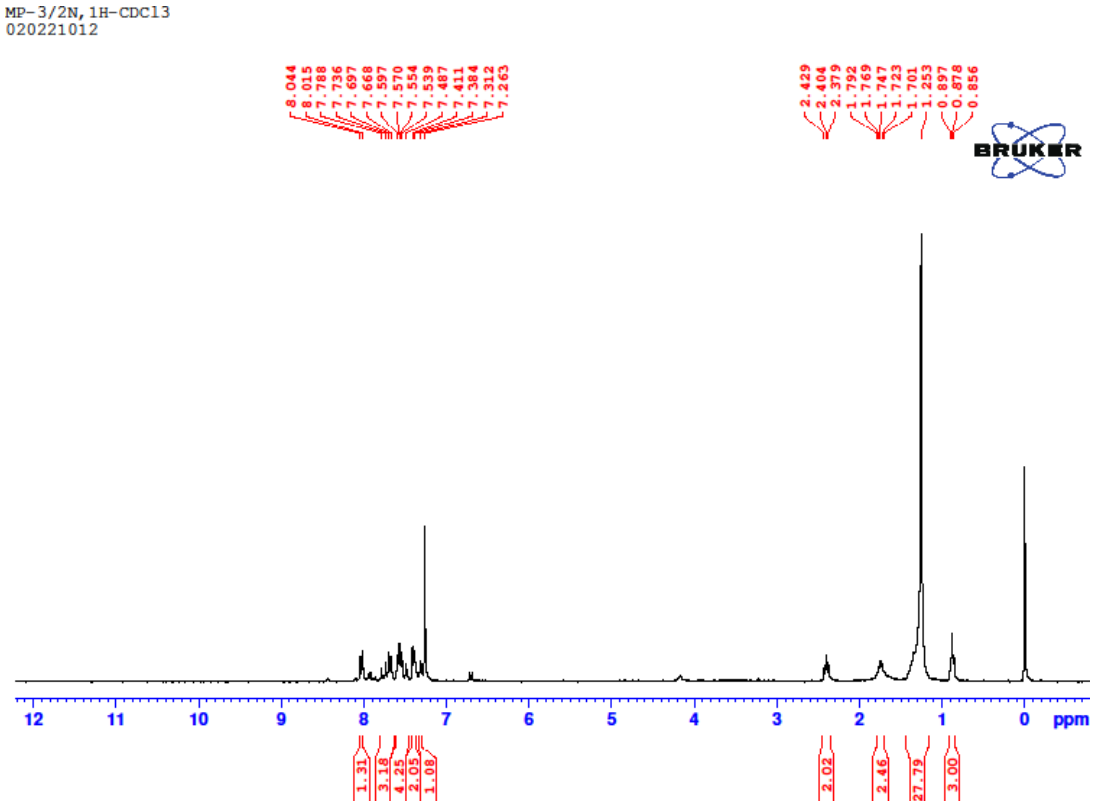
2.6e

Yellow solid; yield 68%; M.P. =125-128^{o}C; FT-IR (KBr, cm^{-1}): 3,306 (NH), 3,034 (Ar-CH), 1,674 (C=O), 1,557 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$), δ, ppm: 8.03-8.00(d, J = 8.42 Hz, 2H, Ar-H), 7.68-7.59 (m, 4H, Ar-H), 7.44-7.39 (m, 2H, HC=CH), 6.95-6.92 (d, J = 8.07 Hz, 2H, Ar-H), 3.86-3.85 (t, 3H, CH_3), 2.42-2.37 (t, 2H, CH_2), 1.79-1.69 (q, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$), δ_C, ppm: 189.21, 172.00,

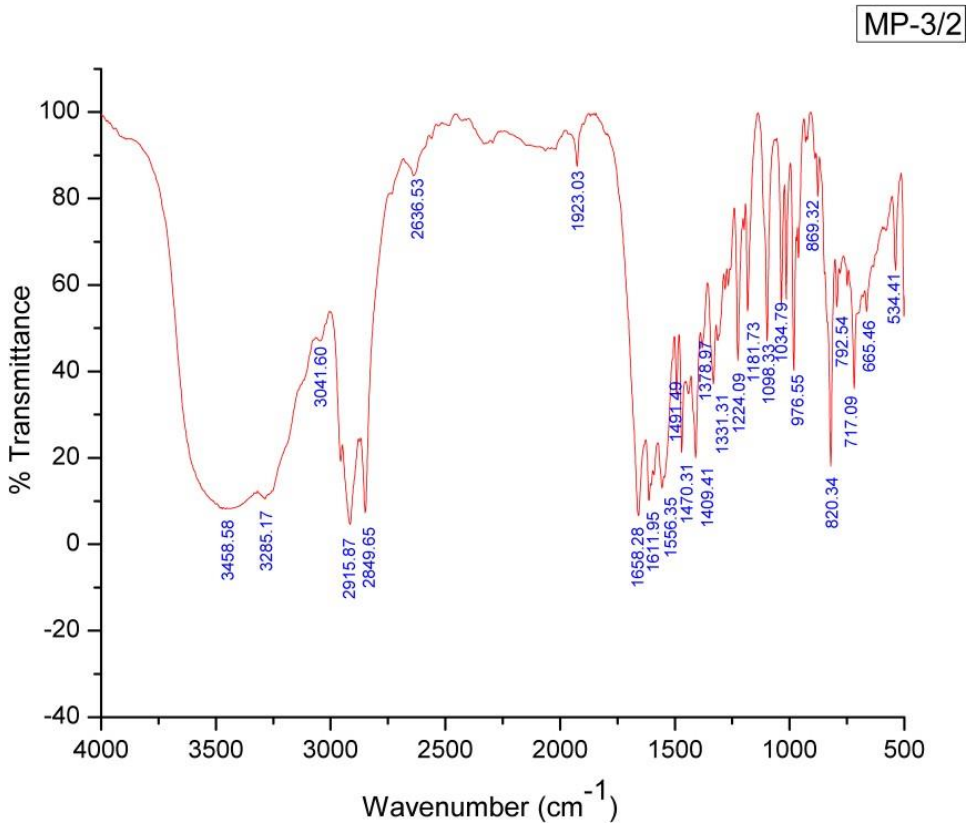
161.80, 144.56, 142.30, 134.02, 131.13, 130.36, 129.98, 127.79, 119.57, 119.09, 114.55, 114.06, 55.53, 32.06, 29.83, 25.63, 22.82, 14.25; MS: m/z 519.0 (M^+).

2.6 REPRESENTATIVE SPECTRA OF OCTADECANAMIDE CHALCONES (2.6a-e)

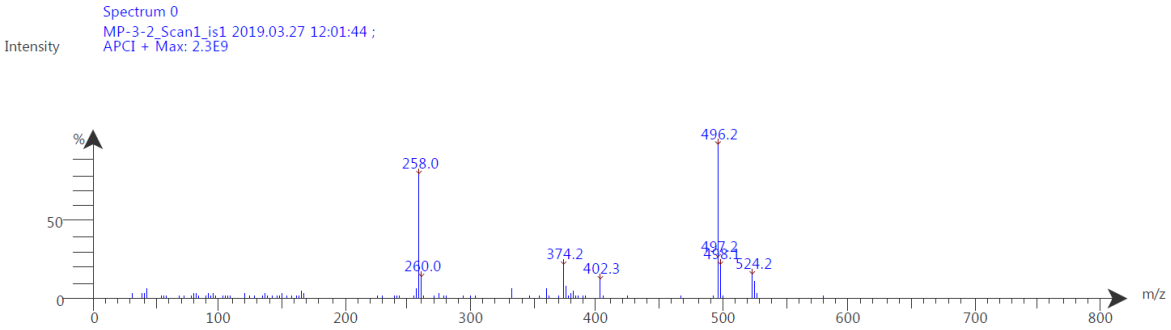
2.6.1 1H NMR Spectrum of N-(4-((E)-3-(4-chlorophenyl)acryloyl)phenyl)stearamide (2.6a)



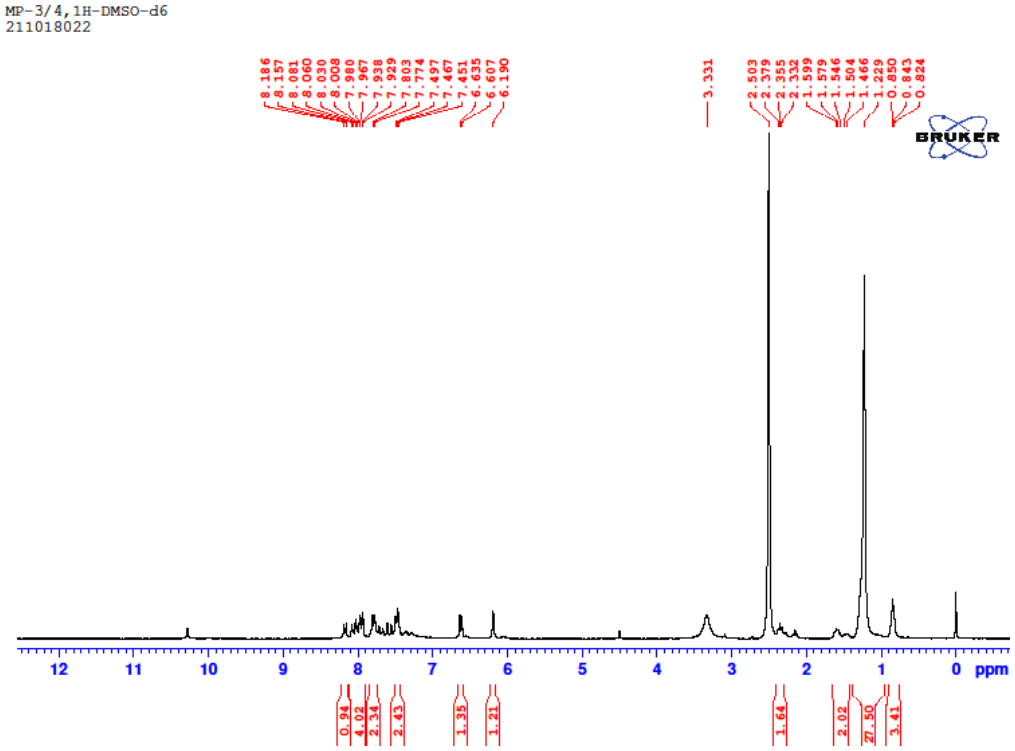
2.6.2 FT-IR Spectrum of N-(4-((E)-3-(4-chlorophenyl)acryloyl)phenyl)stearamide (2.6a)



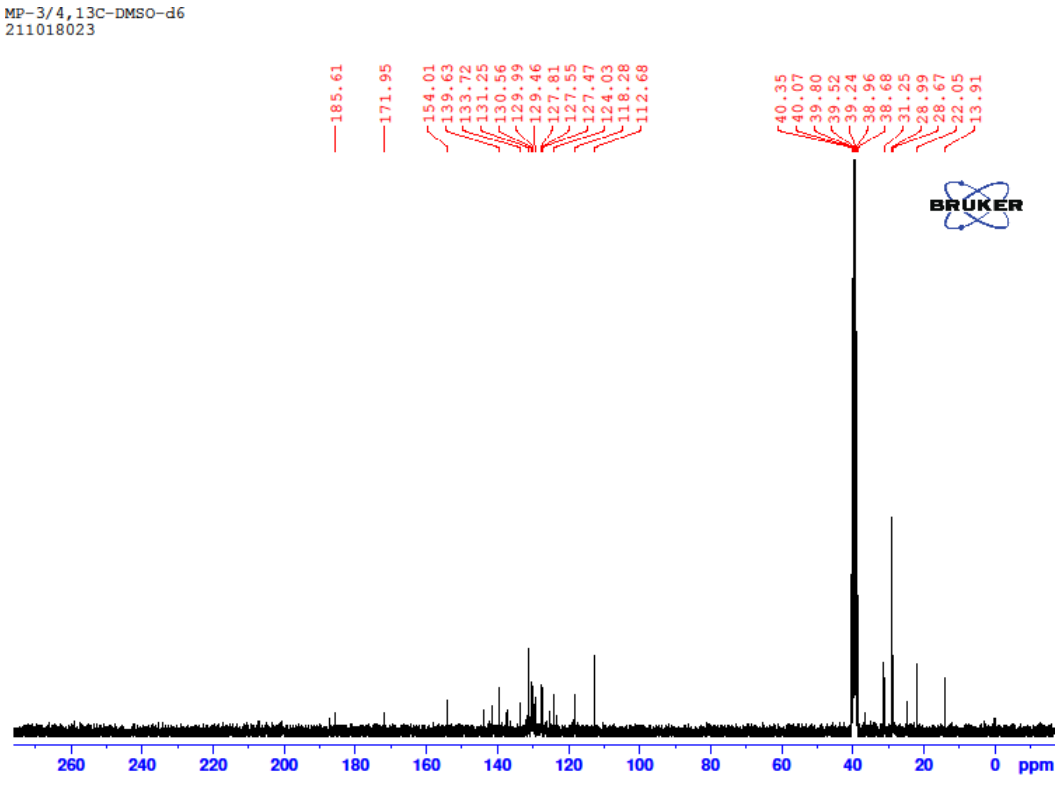
2.6.3 Mass Spectrum of N-(4-((E)-3-(4-chlorophenyl)acryloyl)phenyl)stearamide (2.6a)



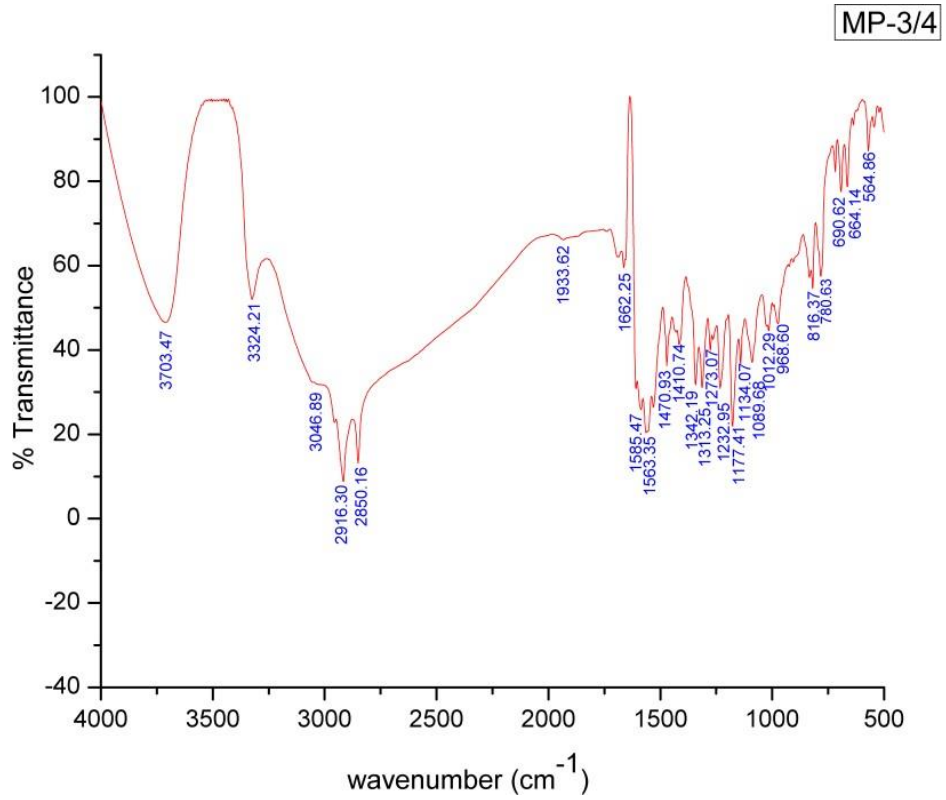
2.6.4 1H NMR Spectrum of N-(4-((E)-3-(3-chlorophenyl)acryloyl)phenyl)stearamide (2.6b)



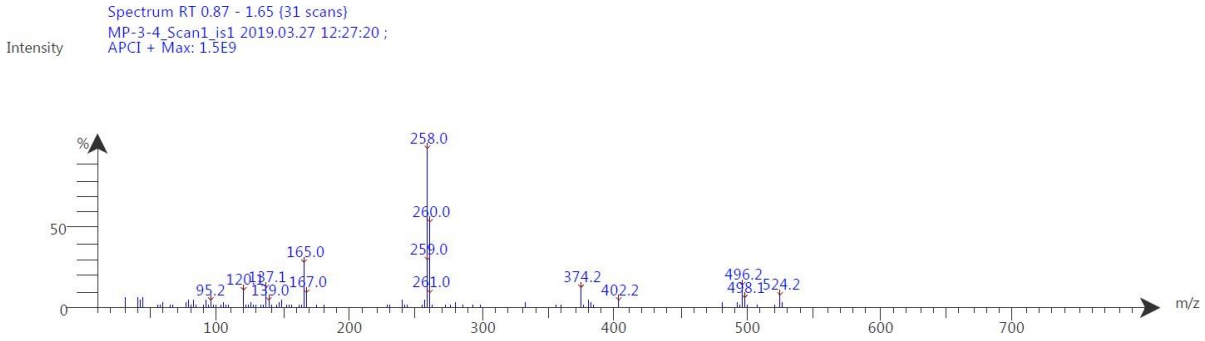
2.6.5 13C NMR Spectrum of N-(4-((E)-3-(3-chlorophenyl)acryloyl)phenyl)stearamide (2.6b)



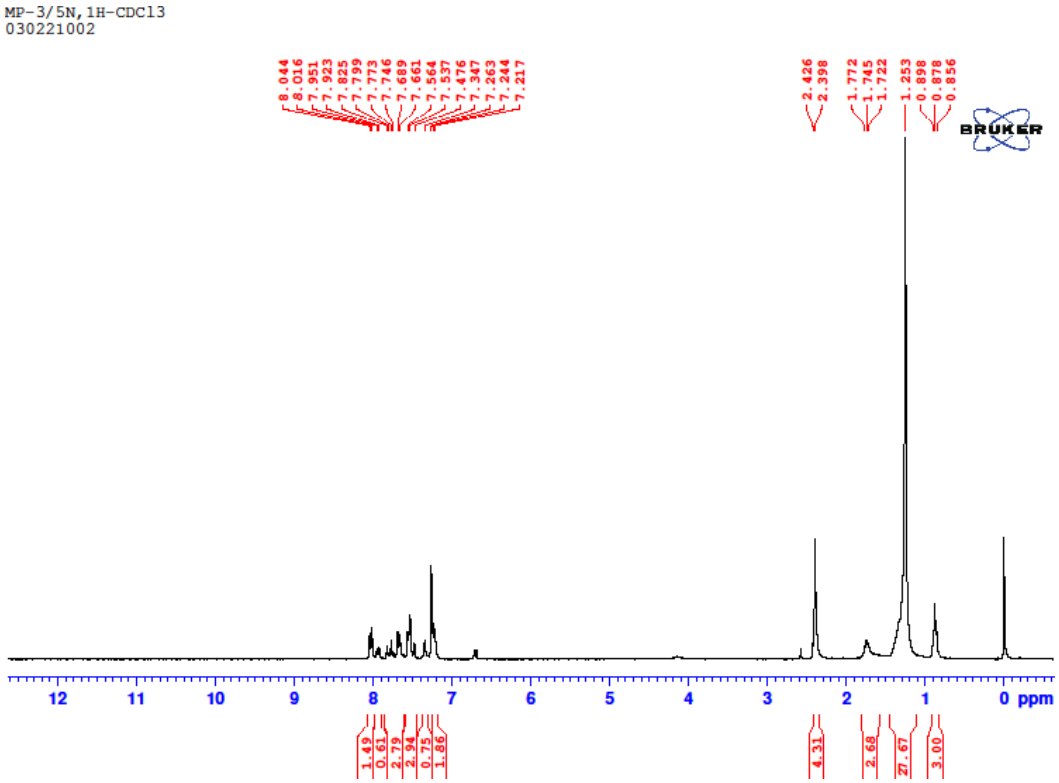
2.6.6 FT-IR Spectrum of N-(4-((E)-3-(3-chlorophenyl)acryloyl)phenyl)stearamide (2.6b)



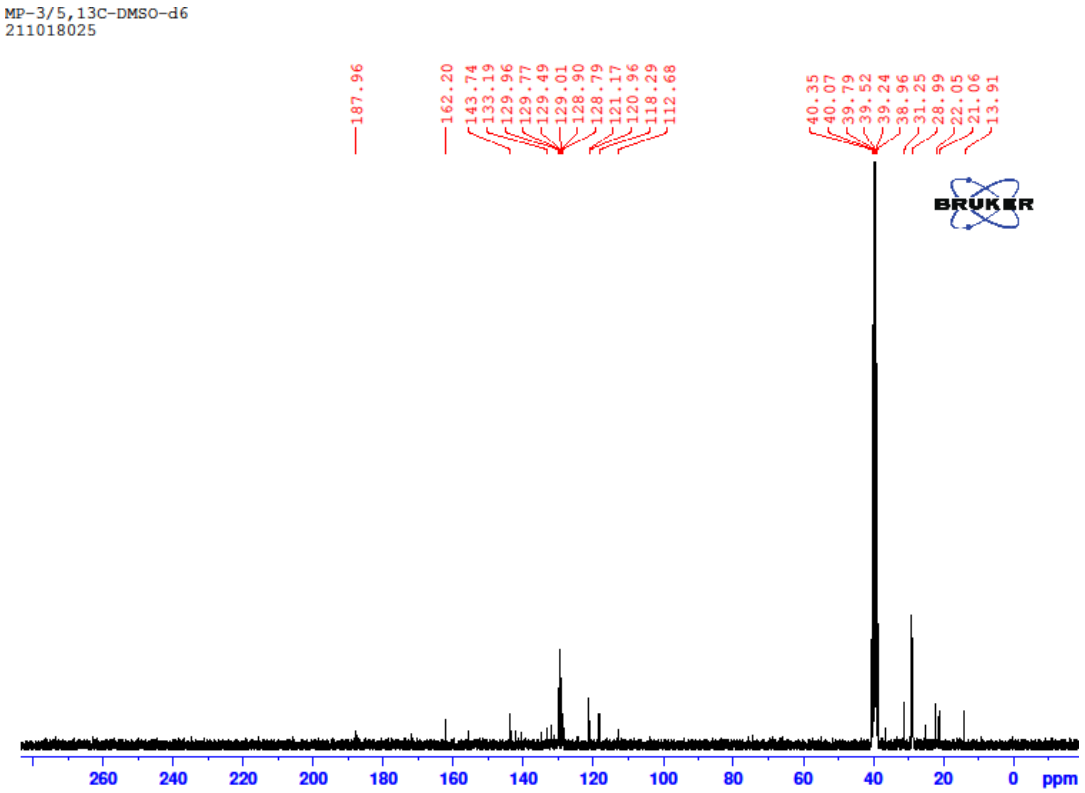
2.6.7 Mass Spectrum of N-(4-((E)-3-(3-chlorophenyl)acryloyl)phenyl)stearamide (2.6b)



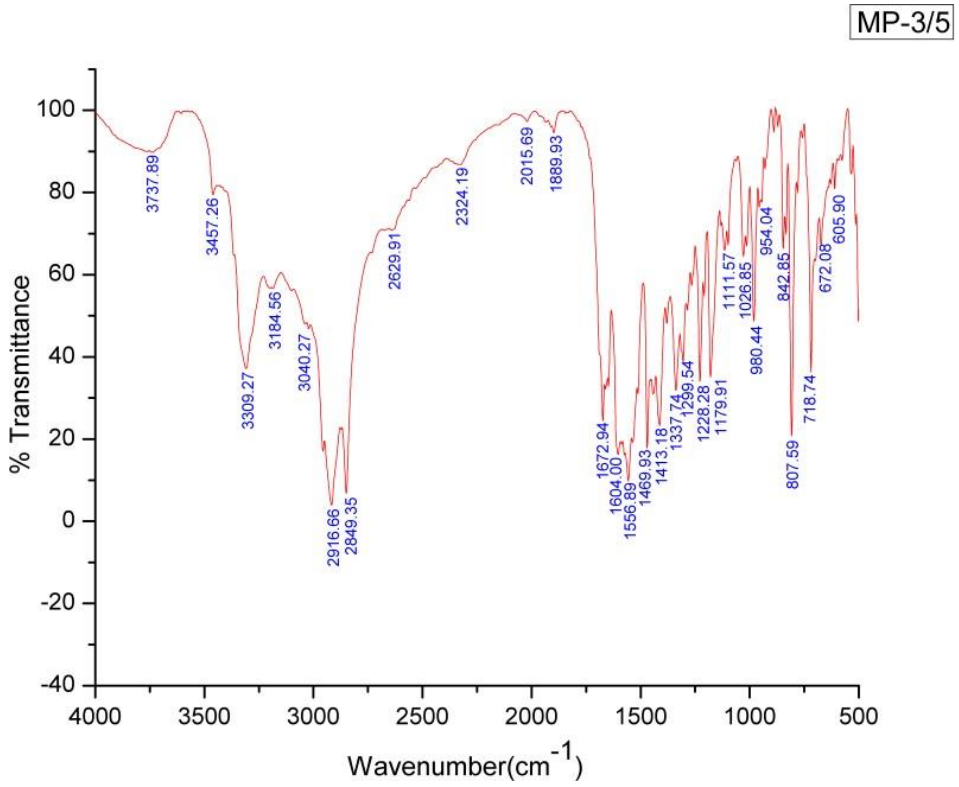
2.6.8 1H NMR Spectrum of N-(4-((E)-3-p-tolylacryloyl)phenyl)stearamide (2.6c)



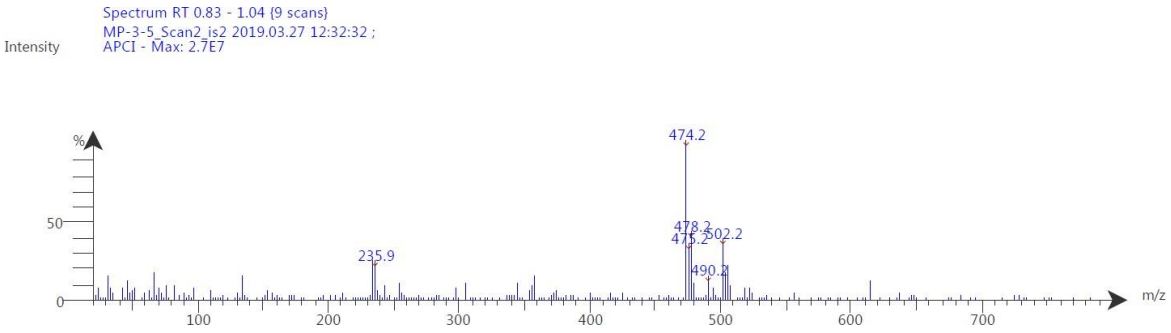
2.6.9 13C NMR Spectrum of N-(4-((E)-3-p-tolylacryloyl)phenyl)stearamide (2.6c)



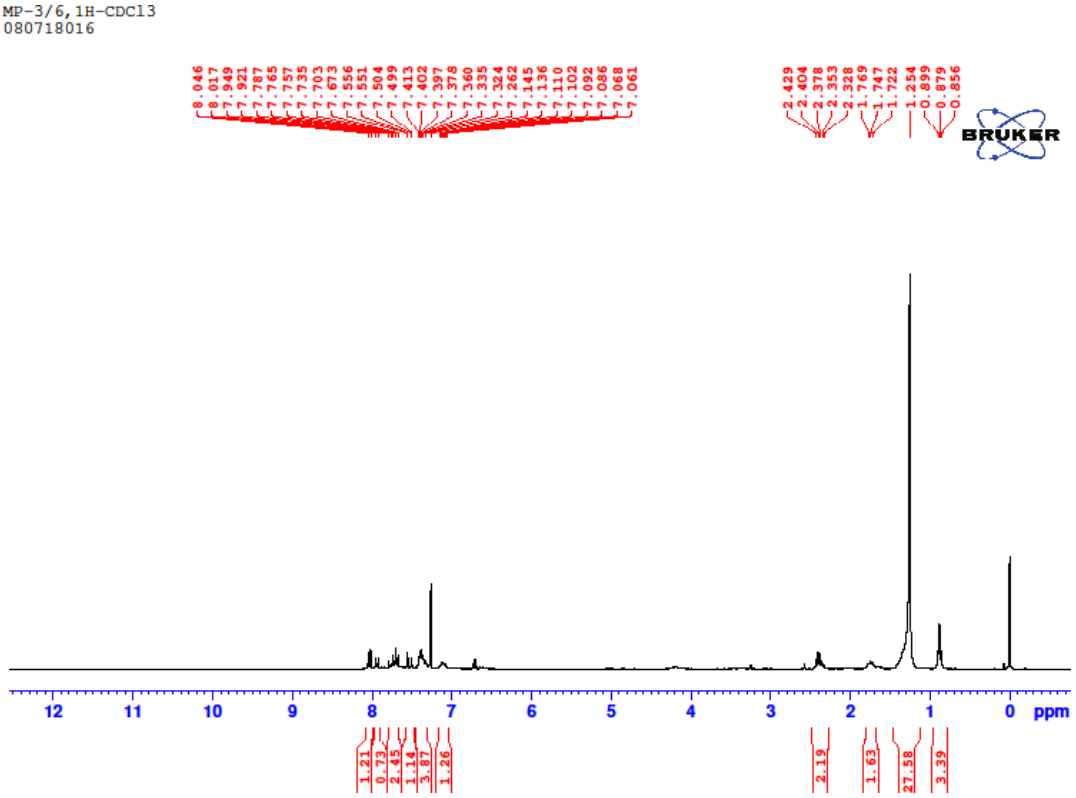
2.6.10 FT-IR Spectrum of N-(4-((E)-3-p-tolylacryloyl)phenyl)stearamide (2.6c)



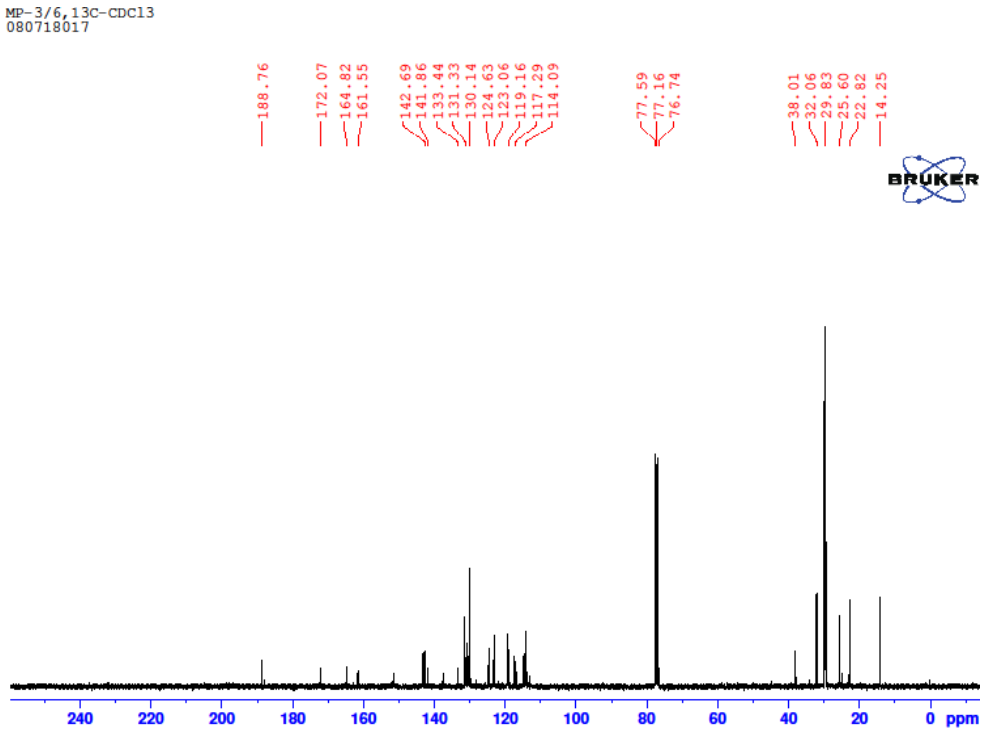
2.6.11. Mass Spectrum of N-(4-((E)-3-p-tolylacryloyl)phenyl)stearamide (2.6c)



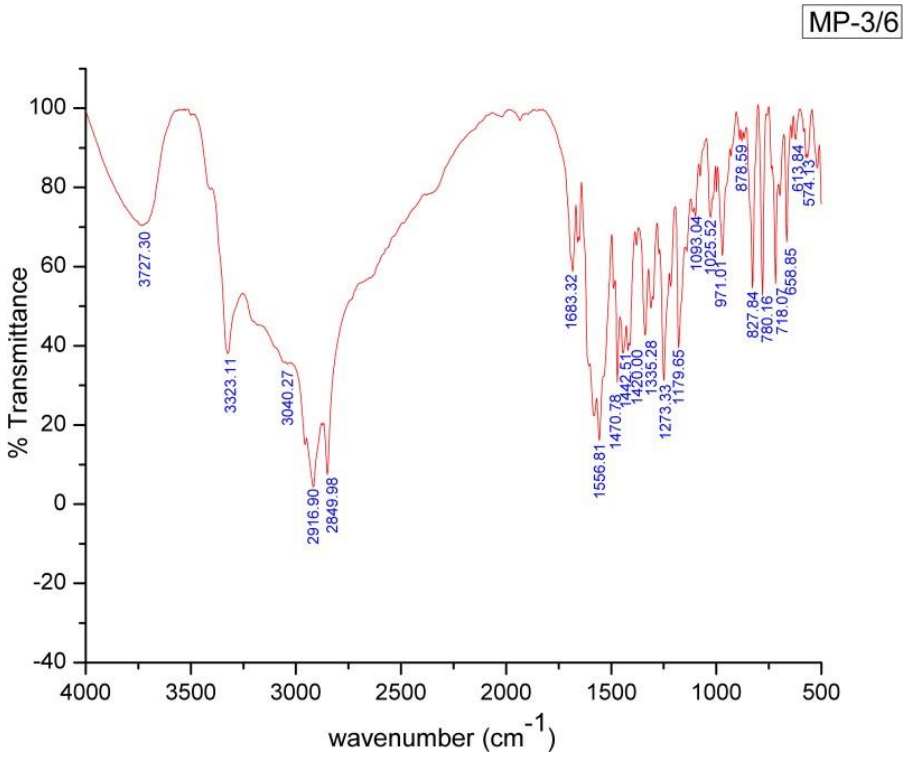
2.6.12 1H NMR Spectrum of N-(4-((E)-3-(3-fluorophenyl)acryloyl)phenyl)stearamide (2.6d)



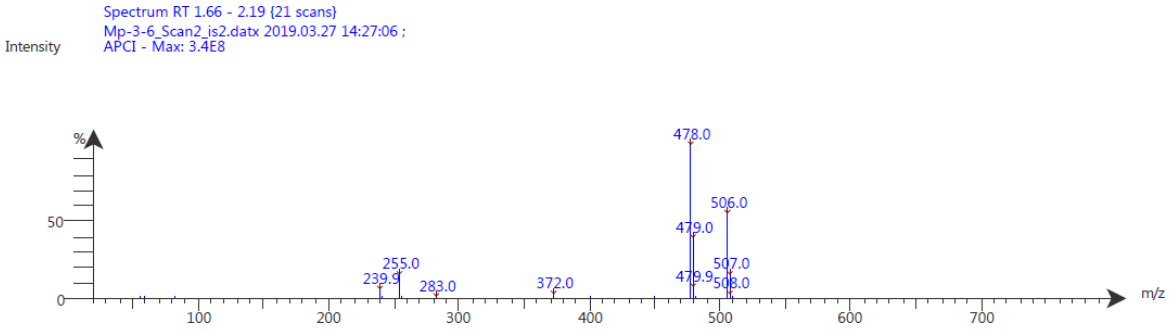
2.6.13 13C NMR Spectrum of N-(4-((E)-3-(3-fluorophenyl)acryloyl)phenyl) stearamide (2.6d)



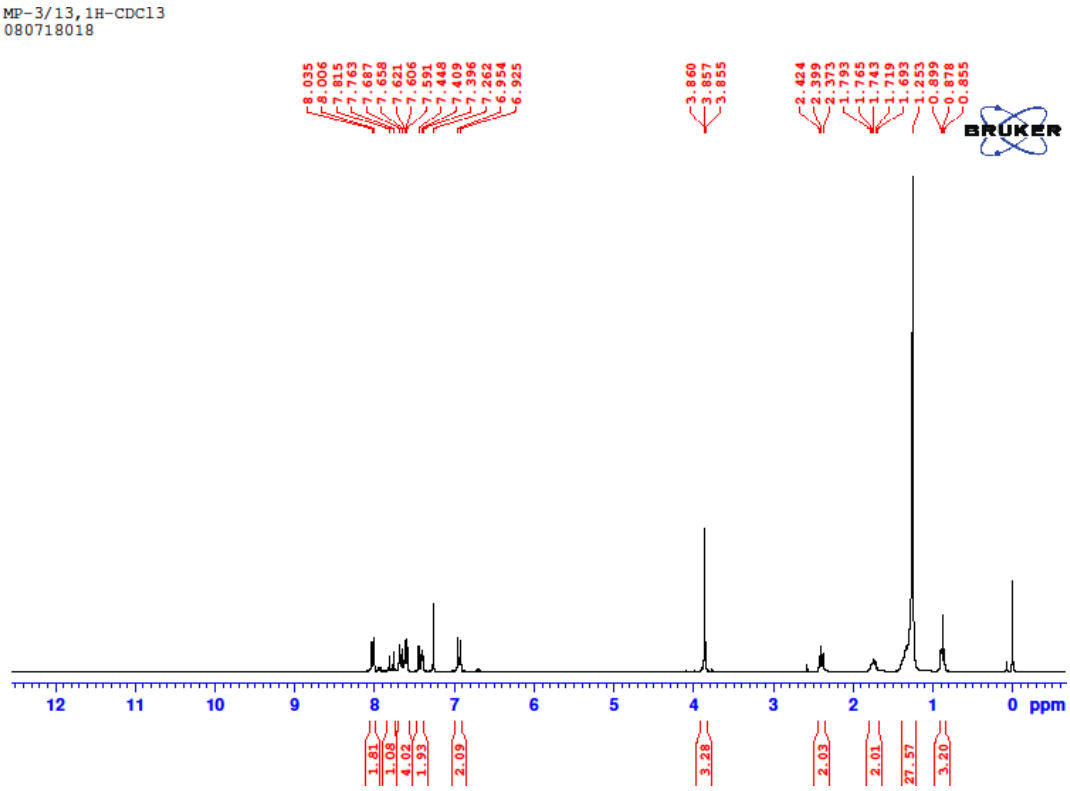
2.6.14 FT-IR Spectrum of N-(4-((E)-3-(3-fluorophenyl)acryloyl)phenyl)stearamide (2.6d)



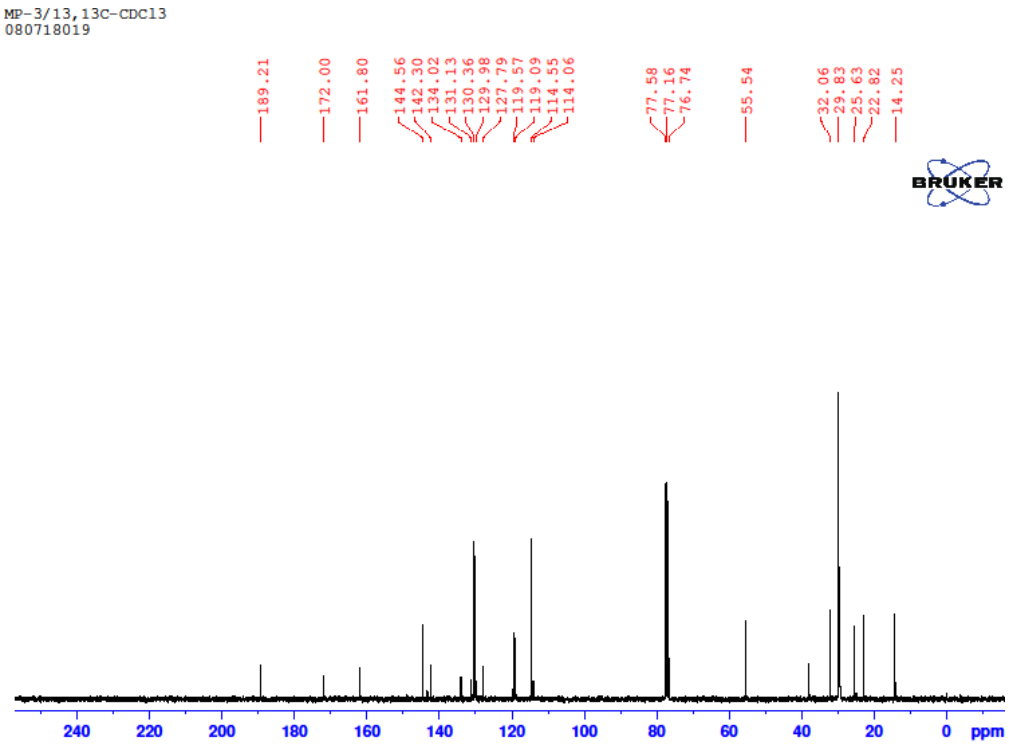
2.6.15 Mass Spectrum of N-(4-((E)-3-(3-fluorophenyl)acryloyl)phenyl)stearamide (2.6d)



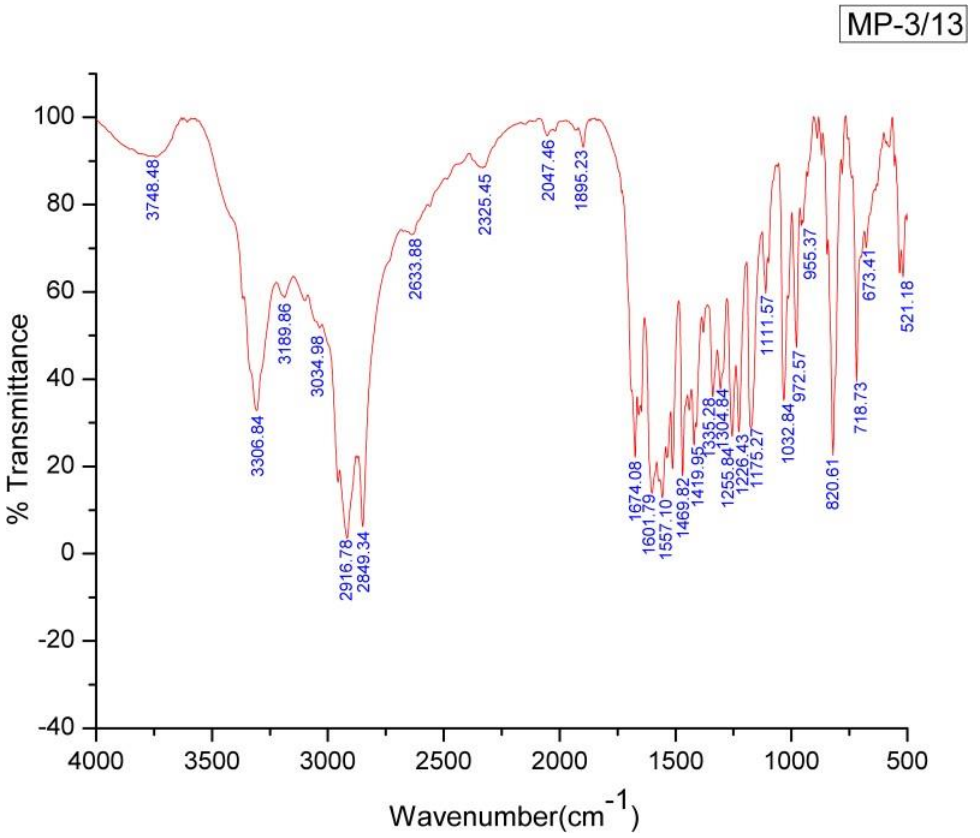
2.6.16 1H NMR Spectrum of N-(4-((E)-3-(4-methoxyphenyl)acryloyl)phenyl) stearamide (2.6e)



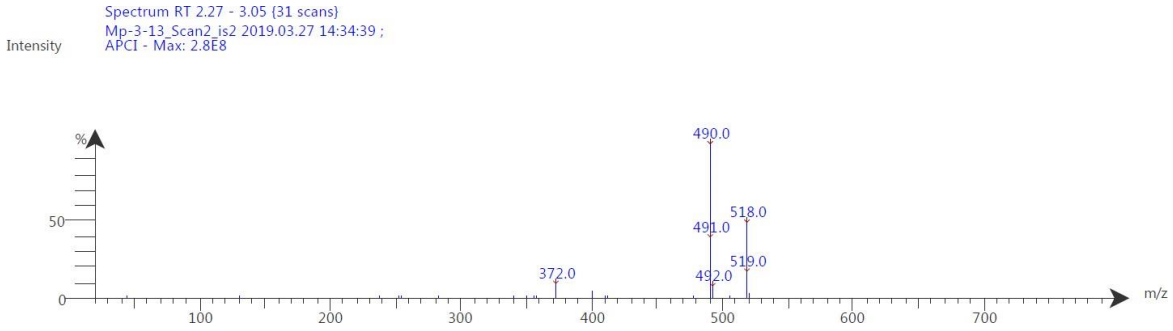
2.6.17 13C NMR Spectrum of N-(4-((E)-3-(4-methoxyphenyl)acryloyl)phenyl) stearamide (2.6e)



2.6.18 FT-IR Spectrum of N-(4-((E)-3-(4-methoxyphenyl)acryloyl)phenyl)stearamide (2.6e)



2.6.19 Mass Spectrum of N-(4-((E)-3-(4-methoxyphenyl)acryloyl)phenyl)stearamide (2.6e)



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Chapter 3

Synthesis of Octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide Pyrazoline derivatives and their Antimicrobial Activity

Abstract

In this chapter, a new series of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives were designed, synthesized and characterized by spectral analysis (IR, 1H-NMR, 13C-NMR and Mass). All the compounds were screened for *in vitro* antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. All the compounds exhibited significant antibacterial activity with minimum inhibitory concentrations ranging from 1.25mg/mL to 2.5mg/mL. The newly synthesized compounds were also found to have good antifungal activity against *Fusarium oxysporum* and *Penicillium italicum* with all the compounds showing MIC of 2.5mg/mL for both the fungal strains.

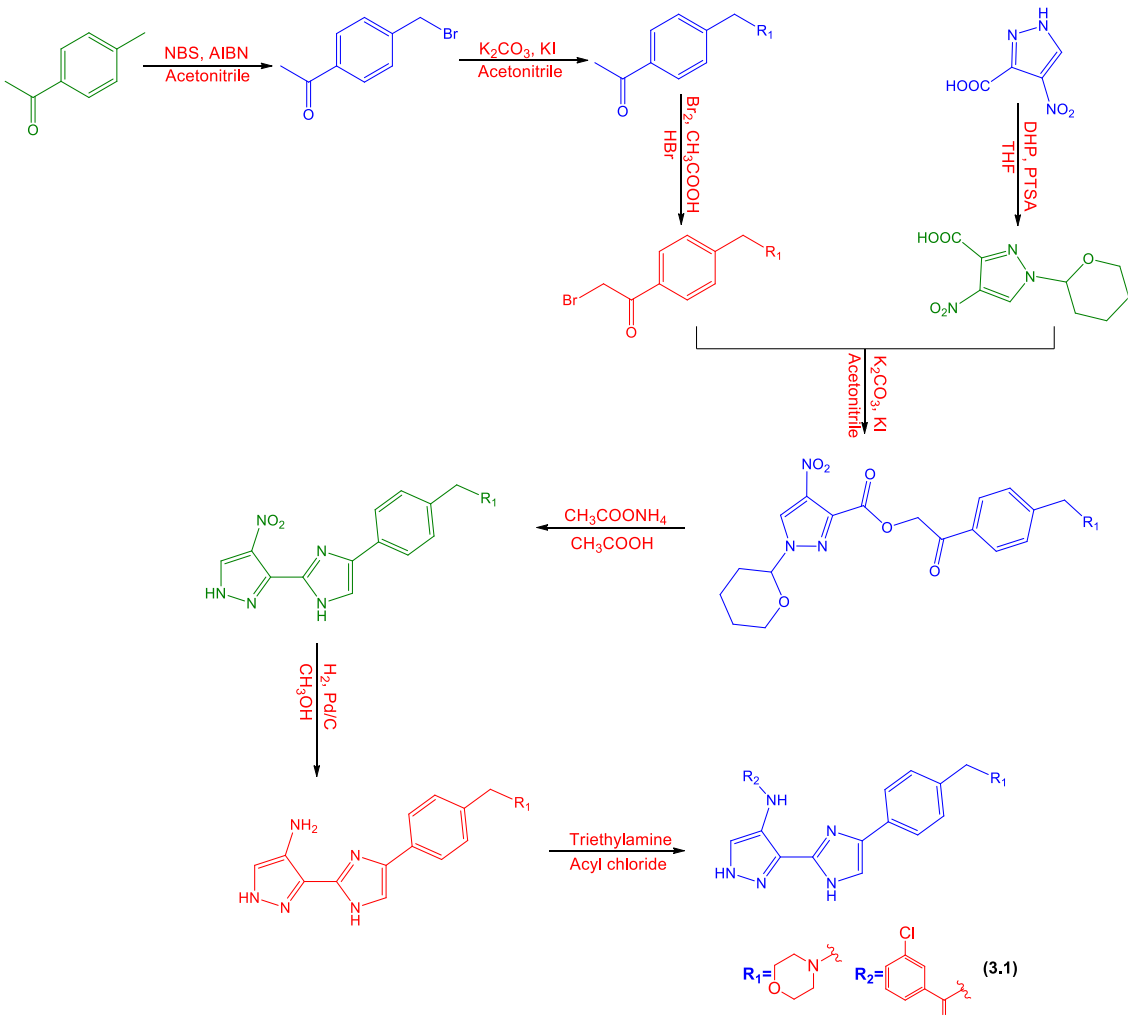
3.1 INTRODUCTION

Heterocycles are a unique class of compounds that plays an important role in medicinal chemistry. They can be acidic as well as basic which can interact with nucleophilic and electrophilic reactants.[1] Pyrazoles are an important class of heterocycles characterized by a 5-membered ring with three carbon atoms and two nitrogen atoms. Ludwig Knorr first coin the term Pyrazole in 1883. They are also known as alkaloids owing to their composition and unique pharmacological effects on humans. The first pyrazole present in nature was isolated from seeds of watermelons in 1959.[2] They are very rare in nature due to their difficulty in the formation of N-N bond by the living organisms.[3] Naturally, pyrazoles are extracted from Houttuynia cordata, which is a common plant from tropical Asia.[4] Pyrazoles are also found in natural products such as vitamins, hormones and alkaloids.[5] Pyrazolines are the partially reduced forms of pyrazole and the completely reduced form is pyrazolidine.[6] The substituted pyrazoles are synthesized by two classical methods which involve approaches on intermolecular [3+2] cycloadditions of 1,3-dipoles to alkynes or by condensations of hydrazines with 1,3-dicarbonyl compounds or their 1,3-dielectrophile equivalents.[7] Pyrazoles are the backbones for many numbers of compounds for widespread applications as building

blocks of a large number of compounds, agrochemicals, catalysis as well as in medicine.[8] Pyrazole and its derivatives play an important role in the field of medicinal chemistry with a long history of applications in pharmaceutical, agrochemical[2] as well as chemical industry[9] and made up the core part of many compounds and have been reported to show a wide range of activities such as antimicrobial, antiviral, antitumor, anti-depressant, insecticides, fungicides,[10] anti-histaminic,[11] anti-fungal, anti-convulsant, anti-viral,[8] antitubercular, anti-inflammatory,[12] anticancer, analgesic, antihypertensive and CNS activity like antiepileptic, antidepressant, etc.[13]

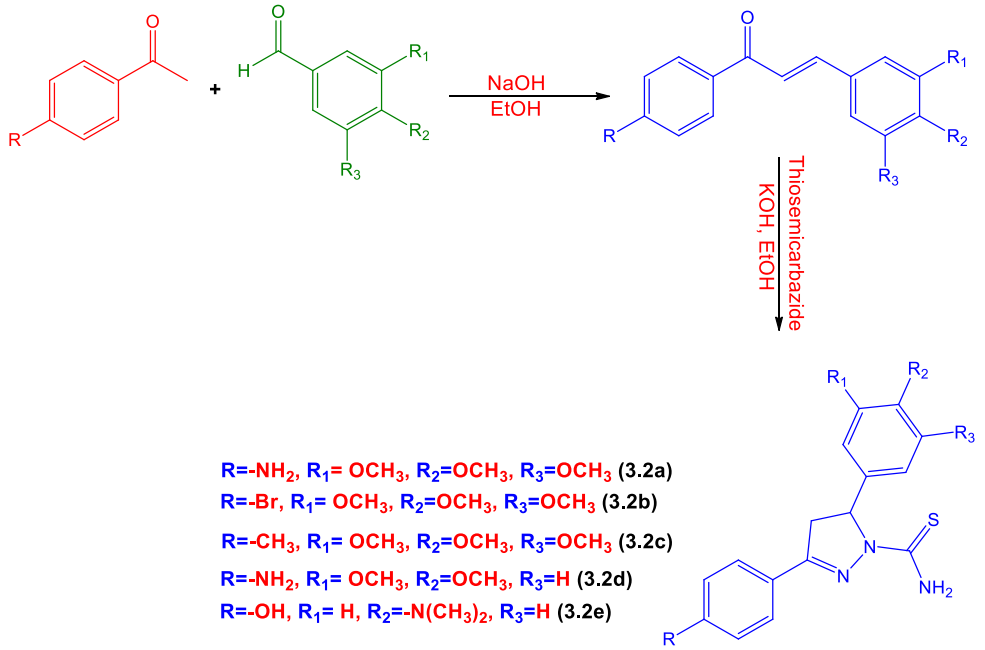
At present, antimicrobial resistance is a vital concern that poses a major threat to public health and therefore, it is necessary to develop new antibiotic analogues to combat the drug resistance. Pyrazole ring fused in chalcone structure have drawn considerable attention as antimicrobial agents to fight against the resistance towards antifungal and antibacterial strains.[14] Henceforth, in the present study, we report the design and synthesis of a series of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl) phenyl]-amide pyrazoline derivatives (**3.6a-f**). Further considering the potential antibacterial properties of pyrazole derivatives, the synthesized compounds were tested for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against *Fusarium oxysporum* and *Penicillium italicum*. Some recently investigated pyrazoles and pyrazolines and their biological activities are discussed below.

In **2021**, You-Guang Zheng and co-workers performed kinase assay of the newly synthesized pyrazole derivatives. Among the series, compound **3.1** expressed inhibiting activities against four kinases JAK2, JAK3, Aurora A and Aurora B with IC50 values at 0.166 μM, 0.057 μM, 0.939 μM and 0.583 μM respectively. Compound **3.1** also exhibited antiproliferative activities against K562 (human chronic myeloid leukemia cells) and also induced cell cycle arrest in G2 phase by inhibiting the proliferation of cells (Scheme 3.1).[15]



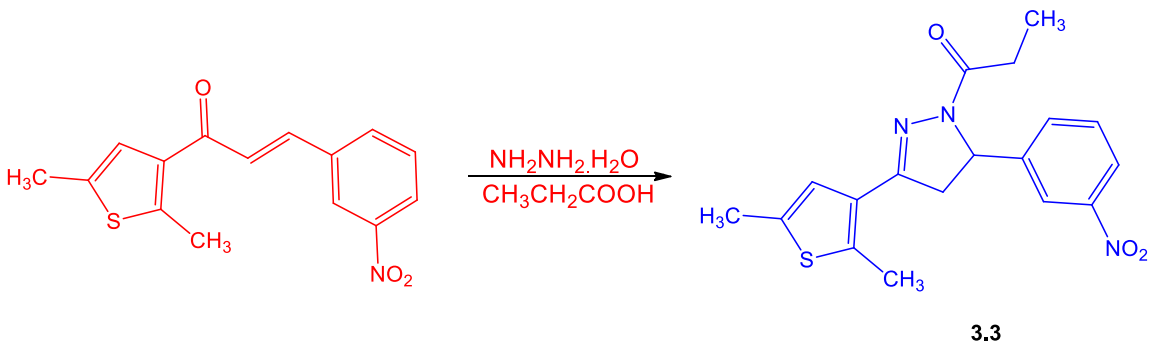
Scheme 3.1. Synthesis of pyrazole derivatives as potent JAK 2/3 and aurora A/B kinases multi-targeted inhibitors.

Yazgi Dizdaroglu *et al.*in **2020** design and synthesized a series of pyrazoline derivatives and evaluated the human isoforms hCA I and hCA II inhibitory properties. All the newly synthesized pyrazoline derivatives (**3.2a-e**) expressed potential inhibitory action at very low nanomolar concentrations with values ranging between 21.98 nM and 25.14 nM. Further, they also reported that molecular docking studies also supported the observed inhibitory actions against hCA I and hCA II (Scheme 3.2).[16]



Scheme 3.2. Synthesis of pyrazole derivatives as carbonic anhydrase inhibitors.

Mohammad Asad *et al.* design and synthesized a series of pyrazoline derivatives in **2021** from chalcones and hydrazine hydrate. The novel derivatives synthesized were tested for their efficacy towards gram-positive and gram-negative bacterial strains. It was observed that compound **3.3** manifests the best result among the derivatives with MIC (minimum inhibitory concentration) at 32 µg/mL for *Escherichia coli* and *Staphylococcus aureus* whereas for *Streptococcus pyogenes* and *S. typhimurium* the MIC was observed at 64 µg/mL (Scheme 3.3).[17]



Scheme 3.3. Synthesis of N -acyl-2-pyrazolines from chalcones in the presence of aliphatic acids.

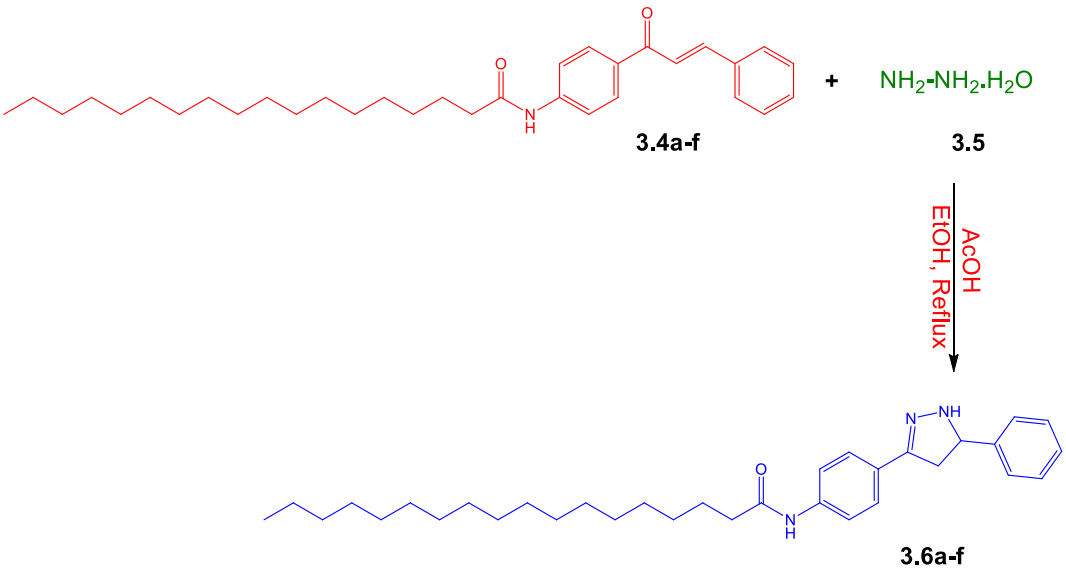
3.2 EXPERIMENTAL SECTION

3.2.1 Materials and Methods

All the reagents and solvents were purchased from commercially available sources and used without further purification. Melting points were recorded in open capillaries using IKON melting point apparatus and are uncorrected. FTIR spectra of the compounds were recorded on Perkin-Elmer spectrophotometer (Spectrum-Two) using KBr disk and values are expressed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra for the compounds were recorded using Bruker 300 MHz spectrophotometer in $CDCl_3$ as a solvent and TMS as an internal standard, values are given in parts per million (ppm). Mass spectra for the compounds were recorded on Advion Expression (S)CMS system. Progress of the reactions was monitored by Thin Layer Chromatography (TLC) with silica gel plates (Merck) using ethylacetate and *n*-hexane (3:7) as a solvent system and visualized under UV-light/iodine vapors.

3.2.2 General procedure for the synthesis of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives (3a-f)

To a mixture octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide **3.4a** (1 mmol), hydrazine hydrate **3.5** (10 mmol) in ethanol was added AcOH and the reaction mixture was stirred under reflux conditions for 24 h. The progress of the reaction was monitored by TLC. After completion of the reaction, crushed ice was added and filtered. The solid product was washed with water (3-4 times) to obtain the pure octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline (**3.6a**). The same method was followed for the synthesis of other compounds (**3.6b-f**) (Scheme 3.4 and Figure 3.1).



Scheme 3.4. Synthesis of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl) phenyl]-amide pyrazoline derivatives (**3.6a-f**).

3.3 RESULTS AND DISCUSSION

3.3.1 Chemistry

The present study reports the synthesis of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives (Scheme 3.4). The reaction was carried out starting from octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (**3.4a-f**) with hydrazine hydrate (**3.5**) in the presence of acetic acid (AcOH) refluxing for 24h. The newly synthesized pyrazoline derivatives were obtained in good yield and characterized by spectroscopic techniques such as 1H-NMR, 13C-NMR, IR and Mass.

The Infrared spectrum of the newly synthesized pyrazoline derivatives (**3.6a-f**) shows the appearance of a new absorption band at 3275 and 1593 cm^{-1} for NH and C=N groups respectively. The N-N group also appears at 1026 cm^{-1}. The 1H-NMR showed the $-CH_3$ group as a triplet at δ 0.89-0.85 ppm and the $-CH_2$ protons of the pyrazoline ring resonated as a pair of doublet of doublets at δ 3.17-3.10 ppm, 3.77-3.67 ppm and 5.61-5.55 ppm. In 13C-NMR, it was also observed that the characteristic chemical shift values appear at δ 42.55-41.59 ppm, 60.10-55.48 ppm, 154.35-153.77 ppm for pyrazoline rings carbons CH_2, CH and C=N respectively. The yield percentage and melting point of

octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives (**3.6a-f**) are presented in Table 3.1

Table 3.1. Synthesis of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives (**3.6a-f**).

Entry	R	Product	Yield (%)	M.P. (^{o}C)
1	Benz (3.4a)	**3.6a**	85	102-105
2	3-Cl (3.4a)	**3.6b**	75	70-74
3	4-CH_3 (3.4a)	**3.6c**	71	80-82
4	3-Br (3.4a)	**3.6d**	81	75-79
5	4-OCH_3 (3.4a)	**3.6e**	72	86-89
6	2-Cl (3.4a)	**3.6f**	78	60-63

3.3.2 Biological Activity

3.3.2.1 Materials and Methods

3.3.2.1.1 Antibacterial

The antibacterial activity of the newly synthesized compounds (3.6a-f) was tested against gram-negative bacteria *Escherichia coli* (*E.coli*) and gram-positive bacteria *Staphylococcus aureus* (*S.aureus*). The antibacterial activity was studied by determining the minimum inhibitory concentration (MIC) of the compounds. MIC is the lowest concentration of the test compounds which prevents the growth of bacteria.18 The bacterial strains were grown in 250 ml of nutrient broth overnight at 37ºC and the freshly grown culture broth was further centrifuged at 3000 rpm for 15 minutes. The bacterial cells were further resuspended in sterile Phosphate buffered solution (PBS) to obtain a cell count of approximately 108 CFU/mL. MIC was determined by taking 10mg/mL initial concentration of the compounds prepared using DMSO as the solvent. Each compound was further serially diluted two-fold with nutrient broth and inoculated with 200µL of ~108 CFU/mL of *E.coli* cells and incubated at 37ºC for 24 hours. The antibiotic Streptomycin (1 mg/ml) was taken as the standard reference. The lowest concentration of the compounds at which no visible growth was observed was recorded as the MIC. All experiments were performed in triplicates.

3.3.2.1.2 Antifungal

The compounds (**3.6a-f**) were also tested for antifungal activity against *Fusarium oxysporum* and *Penicillium italicum* by following the same two-fold dilution method to determine the MIC. Fresh fungal cultures were grown in potato dextrose broth at 27℃±2℃ for around 48 hours. Initially, 10mg/mL of each compound was taken and the test compounds (**3.6a-f**) were further serially diluted two-fold using potato dextrose broth. Around 200µL of the freshly grown fungal cultures were inoculated on each tube and were incubated at 27℃ for 48 hours. The lowest concentration, which showed no growth was considered as the MIC for each drug. Antibiotic Fluconazole was used as a standard drug for antifungal activity. All experiments were performed in triplicate to confirm reproducibility.

3.3.2.2 Antimicrobial Activity

The antimicrobial study of the newly synthesized compounds (**3.6a-f**) was performed against gram-negative bacteria *Escherichia coli (E.coli)* and gram-positive bacteria *Staphylococcus aureus (S.aureus)* and two different fungi *Fusarium oxysporum* and *Penicillium italicum*. The standard antibiotics namely Streptomycin and Fluconazole were used as a positive control for bacteria and fungi, respectively. The antimicrobial activity was recorded for each tested compound as the minimum inhibition concentration (MIC). The results of the antimicrobial activity of the newly synthesized compounds are presented in Table 3.2. From the results, it is observed that all the compounds exhibited considerable antibacterial activity against both the tested strains. The MIC of compounds, **3.6d**, **3.6e** and **3.6f** was 1.25mg/mL for both the tested bacterial strains. Compounds **3.6a** and **3.6c** showed MIC at 2.5 mg/mL against both *E.coli* and *S.aureus*, while compound **3.6b** showed activity at 1.25mg/mL and 2.5mg/mL for *E.coli* and *S.aureus* respectively. For antifungal activity, it was found that all the compounds showed the same MIC at 2.5mg/mL for both the fungal strains, *Fusarium oxysporum* and *Penicillium italicum*. The compounds also displayed antifungal activity against *Fusarium oxysporum* and *Penicillium italicum*. depicting its antifungal activity. All the tested compounds showed antifungal activity against *Fusarium oxysporum*. The maximum inhibition towards *Fusarium oxysporum* was exhibited by compound **3.6c** containing methyl group and **3.6d** containing bromo group. Whereas, the antifungal activity against *Penicillium italicum* was shown only by compound **3.6c**, **3.6d** and **3.6e**,

while **3.6a** and **3.6b** showed no potency.

Table 3.2. Antimicrobial activity of the tested compounds (**3.6a-f**).

	Minimum inhibitory concentration (mg/mL)			
Compound	***Escherichia coli***	***Staphylococcus aureus***	***Fusarium oxysporum***	***Penicillium italicum***
3.6a	2.5	2.5	2.5	2.5
3.6b	1.25	2.5	2.5	2.5
3.6c	2.5	2.5	2.5	2.5
3.6d	1.25	1.25	2.5	2.5
3.6e	1.25	1.25	2.5	2.5
3.6f	1.25	1.25	2.5	2.5
Streptomycin	0.07	0.15	-	-
Flucanazole	-	-	0.06	0.03

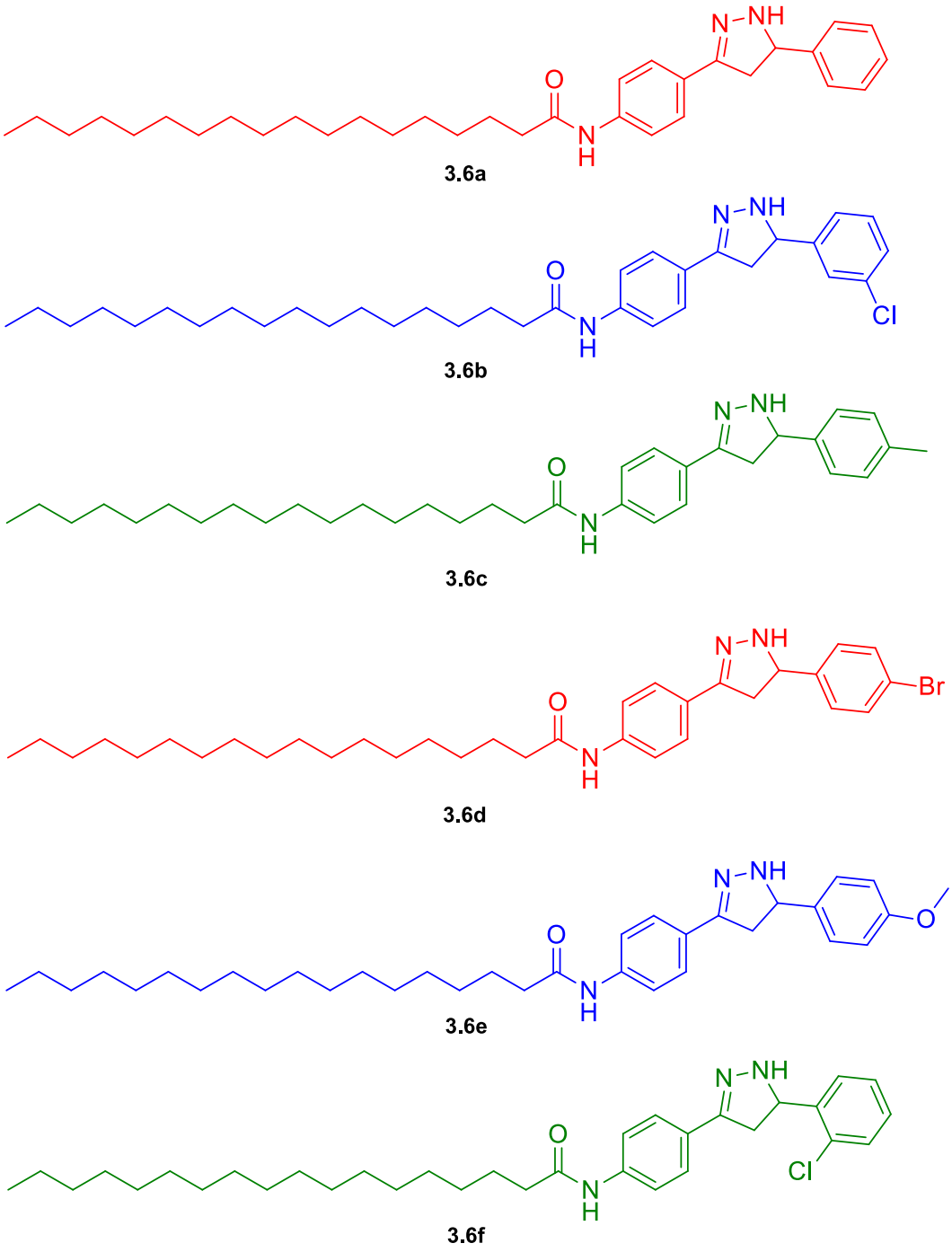


Figure 3.1. Structures of newly synthesized octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives(**3.6a-f**).

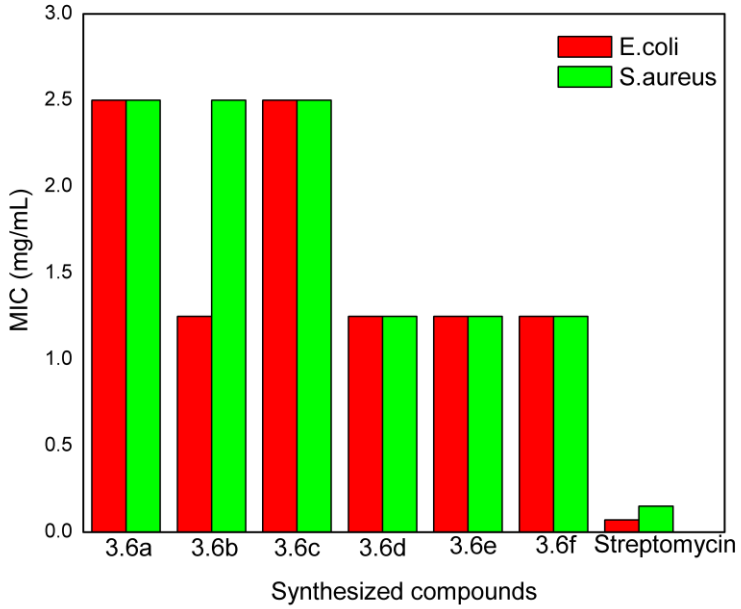


Figure 3.2. Bar graph of compounds **3.6a-f** against bacterial strains.

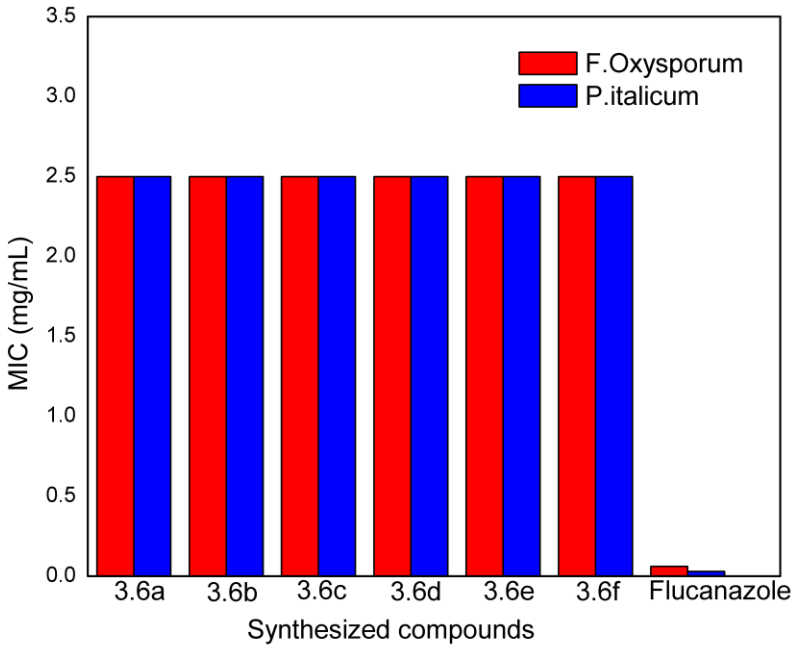


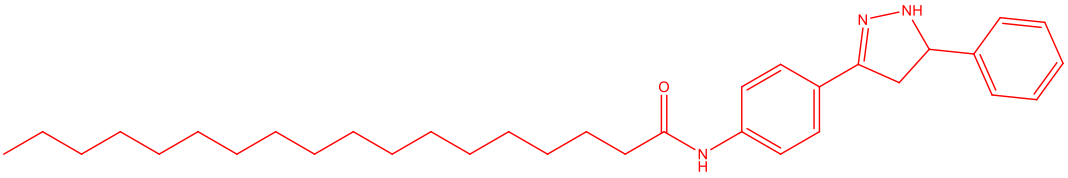
Figure 3.3. Bar graph of compounds **3.6a-f** against fungal strains.

3.4 CONCLUSION

All the newly synthesized compounds (**3.6a-f**) exhibited appreciable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Besides, the compounds also showed activity against two fungal strains, *Fusarium oxysporum* and *Penicillium italicum.* Molecular docking studies (Chapter-4) revealed a common interaction for both the synthesized compounds and standard drugs with the target protein. Thereby, future studies on these compounds might increase their potency and thus, may be used as a potential lead for the development of novel antimicrobial agents.

3.5 SPECTRAL CHARACTERIZATION DATA

3.5.1 N-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3.6a)



3.6a

Brown solid, yield: 85%, m.p.: 102-105 ^{o}C. FT-IR (KBr, cm^{-1}): 3275.27(N-H), 3027.99(Ar-CH), 1695.17(C=O), 1593.31(C=N), 1249.61(C-N), 1026.43(N-N). 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.72-7.67 (m, 2H, Ar-H), 7.60-7.57 (m, 2H, Ar-H), 7.46-7.42 (m, 1H, Ar-H), 7.36-7.28 (m, 2H, Ar-H), 7.22-7.20 (m, 2H, Ar-H), 5.61-5.55 (dd, 1H, -CH), 3.77-3.67 (dd, 1H, -CH_2), 3.17-3.10 (dd, 1H, -CH_2), 2.36-2.32 (t, 2H, CH_2), 1.72-1.61 (*q*, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3). 13C NMR (300 MHz, $CDCl_3$), δ (ppm): 178.90, 171.89, 169.10, 153.82, 141.91, 140.17, 129.03, 127.80, 127.63, 126.98, 126.39, 125.64, 120.23, 119.60, 60.10, 42.53, 34.19, 32.06, 29.81, 29.51, 29.49, 29.40, 29.25, 24.93, 22.82, 22.06, 14.24. MS (m/z): 504.8 (M+1) was observed for $C_{33}H_{49}N_3O$, Calc. (m/z): 503.76 for $C_{33}H_{49}N_3O$.

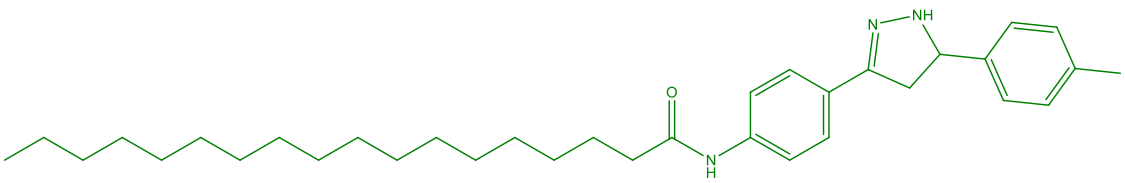
3.5.2 N-(4-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3.6b)

3.6b

Pale yellow solid, yield: 75%, m.p.: 70-74 ^{o}C. FT-IR (KBr, cm^{-1}): 3311.04(N-H), 3050.54(Ar-CH), 1685.84(C=O), 1598.75(C=N), 1255.05(C-N), 1007.77(N-N). 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.78-7.58 (m, 3H, Ar-H), 7.44-7.34 (m, 1H, Ar-H), 7.26-7.18 (m, 3H, Ar-H), 7.12-7.10 (m, 1H, Ar-H), 5.57-5.51 (dd, 1H, -CH), 3.78-3.68 (dd, 1H, -CH_2), 3.14-3.07 (dd, 1H, -CH_2), 2.37-2.32 (t, 2H, CH_2), 1.74-1.61 (*q*, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3). 13C NMR (300 MHz, $CDCl_3$), δ (ppm): 178.79, 172.03, 169.26, 167.81, 153.83, 143.88, 140.37, 134.93, 130.36, 128.05, 127.67, 125.81, 123.96, 119.67, 59.62, 42.42, 34.29, 32.05, 29.80, 29.60, 29.48, 29.41, 29.27,

24.99, 22.81, 22.00, 20.68, 14.23. MS (m/z): 539.0 (M+1) was observed for $C_{33}H_{48}ClN_3O$, Calc. (m/z): 538.21 for $C_{33}H_{48}ClN_3O$.

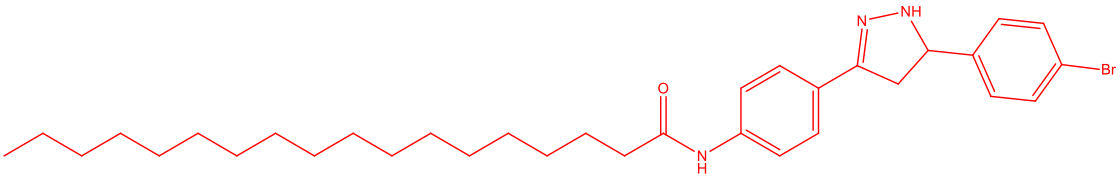
3.5.3 N-(4-(5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3.6c)



3.6c

Pale yellow solid, yield: 71%, m.p.: 80-82 ºC. FT-IR (KBr, cm^{-1}): 3270.60(N-H), 3038.10(Ar-CH), 1694.40(C=O), 1601.86(C=N), 1258.94(C-N), 1012.44(N-N). 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 9.11 (s, 1H, NH, D_2O exchangeable), 7.69-7.54 (m, 3H, Ar-H), 7.44-7.34 (m, 1H, Ar-H), 7.25-7.19 (m, 1H, Ar-H), 7.12-7.10 (m, 3H, Ar-H), 5.57-5.51 (dd, 1H, -CH), 3.75-3.65 (dd, 1H, $-CH_2$), 3.15-3.08 (dd, 1H, $-CH_2$), 2.37-2.33 (t, 2H, CH_2), 2.0 (s, 3H, Ar-CH_3), 1.74-1.63 (*q*, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3).13C NMR(300 MHz, $CDCl_3$), δ (ppm): 178.39, 172.12, 170.95, 169.11, 168.03, 154.04, 144.79, 140.34, 138.96, 137.47, 129.65, 127.56, 125.55, 119.62, 59.90, 42.55, 37.82, 32.03, 29.79, 29.59, 29.46, 29.40, 29.27, 25.63, 22.79, 22.04, 14.22. MS (m/z): 518.7 (M+1) was observed for $C_{34}H_{51}N_3O$, Calc. (m/z): 517.79 for $C_{34}H_{51}N_3O$.

3.5.4 N-(4-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide(3.6d)

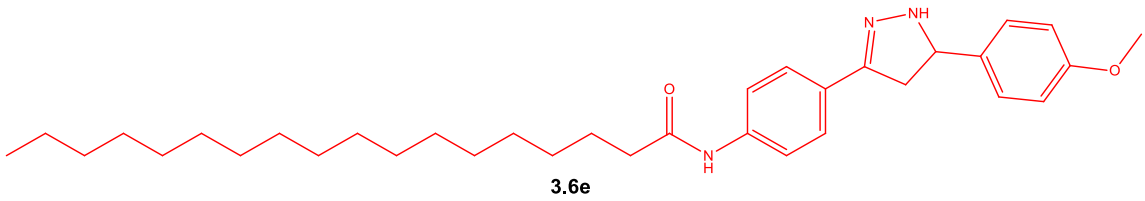


3.6d

Pale yellow solid, yield: 81%, m.p.: 75-79 ºC. FT-IR (KBr, cm^{-1}): 3256.60(N-H), 3038.10(Ar-CH), 1688.95(C=O), 1587.86(C=N), 1259.72(C-N), 1003.11(N-N). 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 9.20 (s, 1H, NH, D_2O exchangeable), 7.77-7.65 (m, 2H, Ar-H), 7.60-7.53 (m, 2H, Ar-H), 7.44-7.41 (m, 2H, Ar-H), 7.26 (m, 1H, Ar-H), 7.11-7.08 (m, 1H, Ar-H), 5.54-5.49 (dd, 1H, -CH), 3.77-3.67 (dd, 1H, $-CH_2$), 3.10-3.05 (dd, 1H, $-CH_2$),

2.36-2.31 (t, 2H, CH_2), 1.71-1.60 (*q*, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3). 13C NMR (300 MHz, $CDCl_3$), δ (ppm): 178.15, 172.25, 171.05, 169.21, 168.15, 167.86, 154.00, 140.85, 140.53, 132.10, 128.61, 127.58, 127.45, 126.49, 121.64, 119.66, 59.57, 42.34, 34.20, 32.01, 29.78, 29.57, 29.49, 29.44, 29.38, 29.24, 22.77, 21.97, 14.19. MS (m/z): 582.4 (M^+) was observed for $C_{33}H_{48}BrN_3O$, Calc. (m/z): 582.66 for $C_{33}H_{48}BrN_3O$.

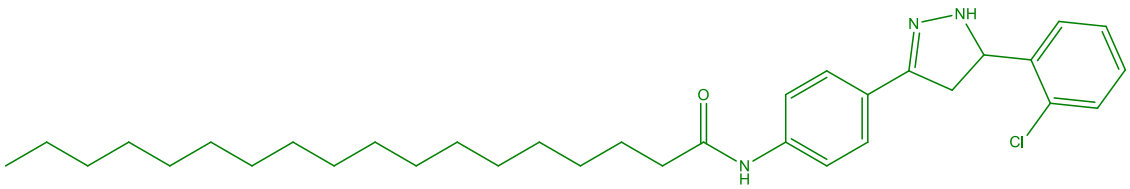
3.5.5 N-(4-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3.6e)



3.6e

Yellow solid, yield: 72%, m.p.: 86-89 °C. FT-IR (KBr, cm^{-1}): 3332.82(N-H), 3036.30(Ar-CH), 1696.67(C=O), 1601.36(C=N), 1249.24(C-N), 1029.50(N-N). 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.66-7.60 (m, 3H, Ar-H), 7.26-7.13 (m, 1H, Ar-H), 6.96-6.82 (m, 2H, Ar-H), 6.29-6.24 (m, 2H, Ar-H), 5.54-5.51 (dd, 1H, -CH), 3.84-3.68 (t, 3H, -CH_3), 3.15-3.09 (dd, 1H, -CH_2), 2.40-2.35 (t, 2H, CH_2), 2.16-2.05 (dd, 1H, -CH_2), 1.71-1.64 (*q*, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3).13C NMR (300 MHz, $CDCl_3$), δ (ppm): 178.72, 171.82, 169.02, 159.16, 153.77, 140.08, 134.18, 128.55, 127.62, 127.02, 126.37, 119.59, 114.50, 114.36, 59.58, 55.48, 42.44, 37.97, 34.13, 32.06, 29.81, 29.60, 29.50, 29.41, 29.24, 25.63, 24.92, 22.82, 14.25. MS (m/z): 532.3 (M-1) was observed for $C_{34}H_{51}N_3O_2$, Calc. (m/z): 533.79 for $C_{34}H_{51}N_3O_2$.

3.5.6 N-(4-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3.6f)

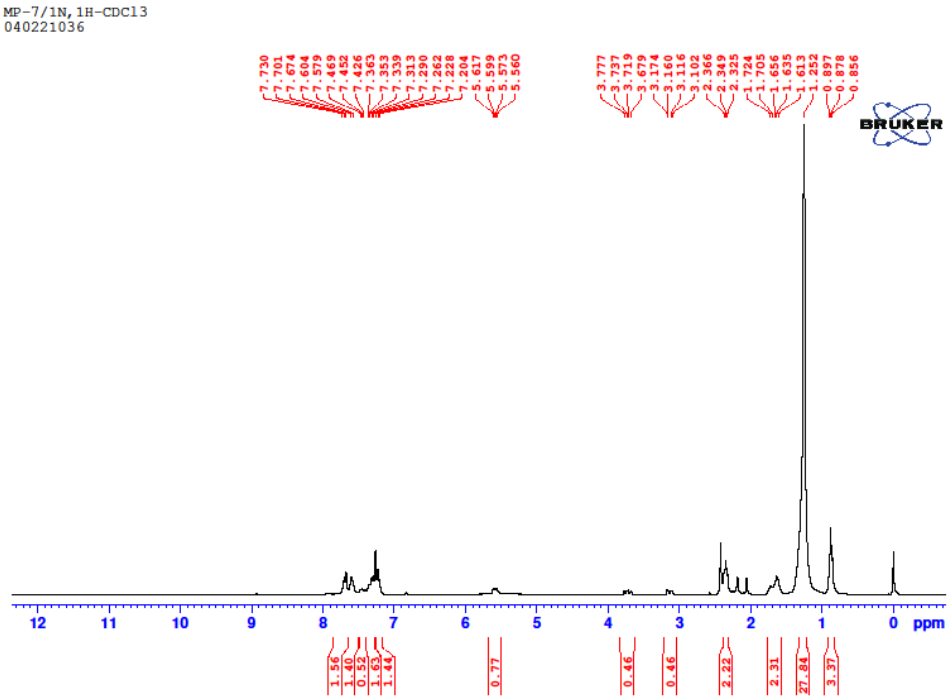


3.6f

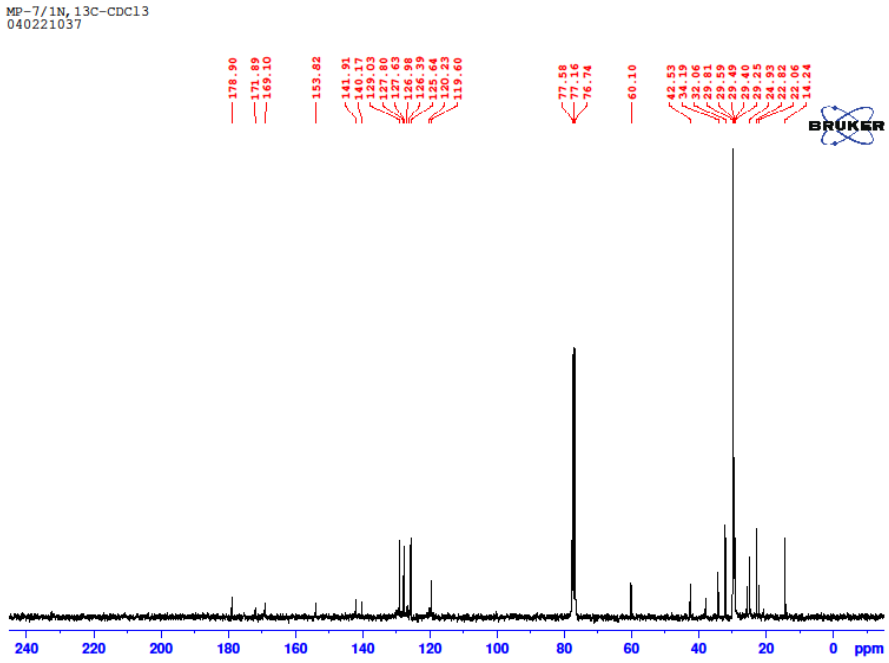
Pale yellow solid, yield: 78%, m.p.: 60-63 ^{o}C. FT-IR (KBr, cm^{-1}): 3216.33(NH), 3044.25(Ar-CH), 1699.31(C=O), 1593.41(C=N), 1265.12(C-N), 1034.79(N-N). 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.69-7.66 (m, 1H, Ar-H), 7.62-7.48 (m, 2H, Ar-H), 7.40-7.31 (m, 2H, Ar-H), 7.21-7.18 (m, 1H, Ar-H), 7.06-7.03 (m, 1H, Ar-H), 6.94-6.91 (m, 1H, Ar-H), 5.93-5.87 (dd, 1H, -CH), 3.87-3.77 (dd, 1H, $-CH_2$), 3.07-2.99 (dd, 1H, $-CH_2$), 2.37-2.32 (t, 2H, CH_2), 1.68-1.58 (*q*, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3). 13C NMR (300 MHz, $CDCl_3$), δ (ppm): 179.38, 169.25, 168.99, 154.35, 140.31, 138.50, 131.84, 131.17, 130.14, 128.94, 127.62, 127.40, 125.93, 119.62, 57.87, 41.59, 32.05, 29.80, 29.57, 29.48, 29.38, 29.22, 24.88, 22.81, 14.23. MS (m/z): 550.5 (M+12) was observed for $C_{33}H_{48}ClN_3O$, Calc. (m/z): 538.21 for $C_{33}H_{48}ClN_3O$.

3.6 REPRESENTATIVE SPECTRA OF OCTADECANAMIDE PYRAZOLINES (3.6a-f)

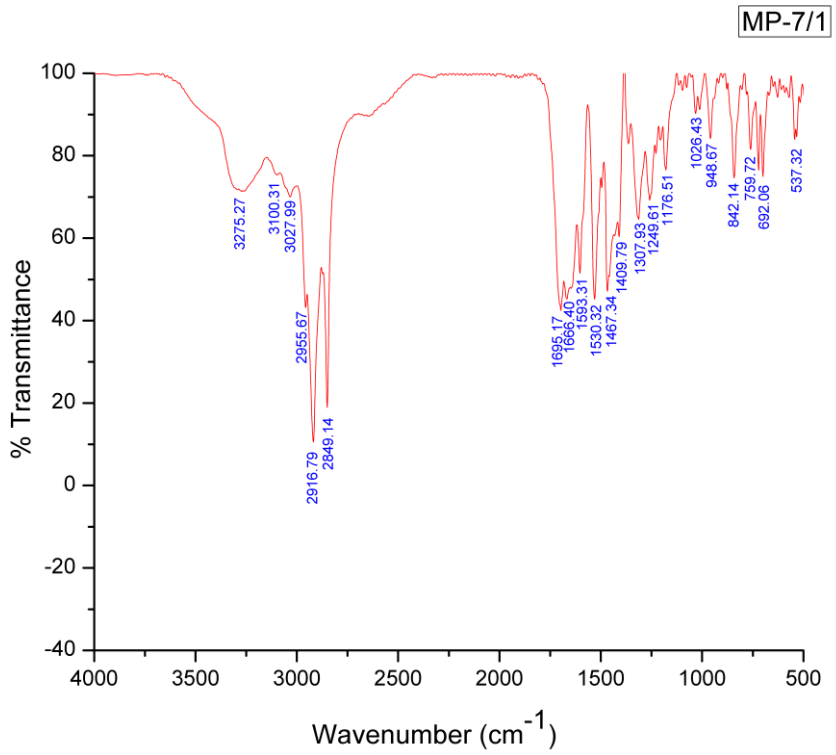
3.6.1 1H NMR Spectrum of N-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6a)



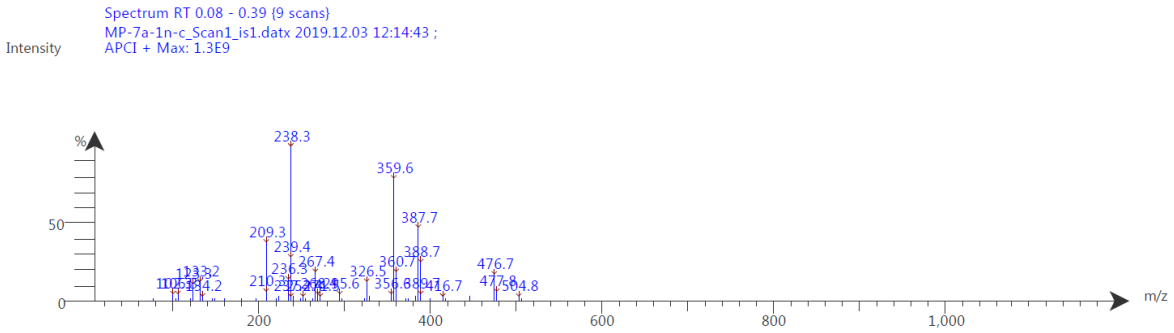
3.6.2 13C NMR Spectrum of N-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3yl)phenyl) stearamide (3.6a)



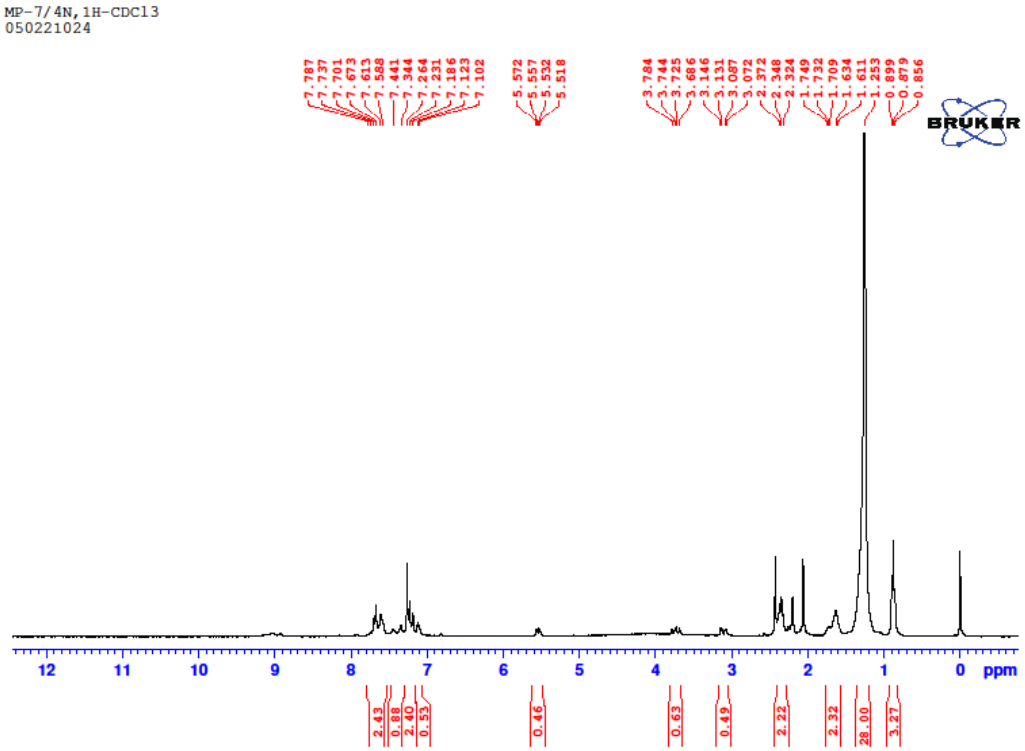
3.6.3 FT-IR Spectrum of N-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6a)



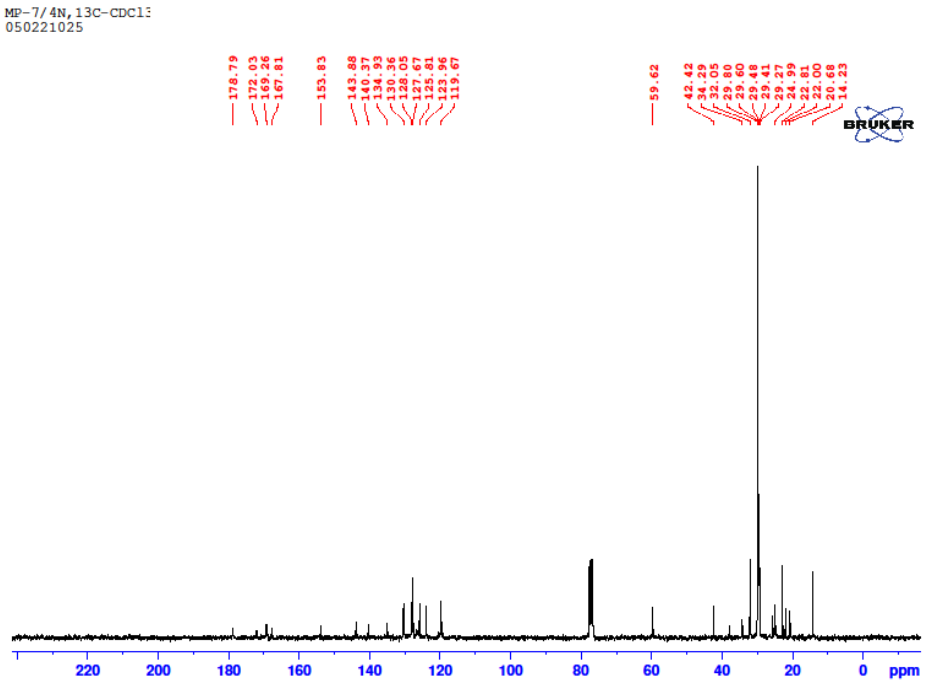
3.6.4 Mass Spectrum of N-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6a)



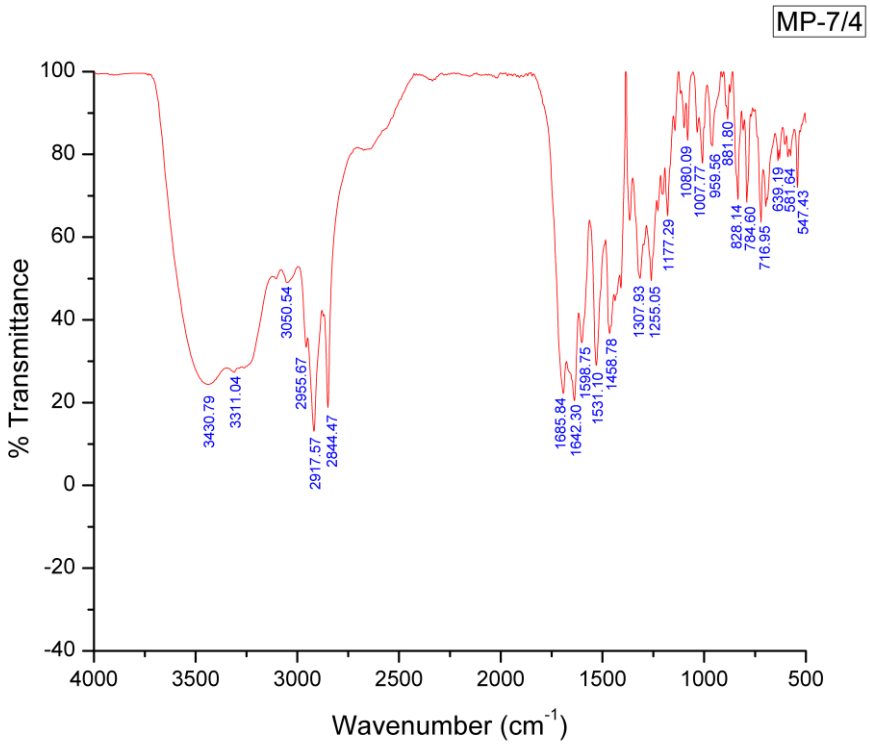
2.6.5 1H NMR Spectrum of N-(4-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3.6b)



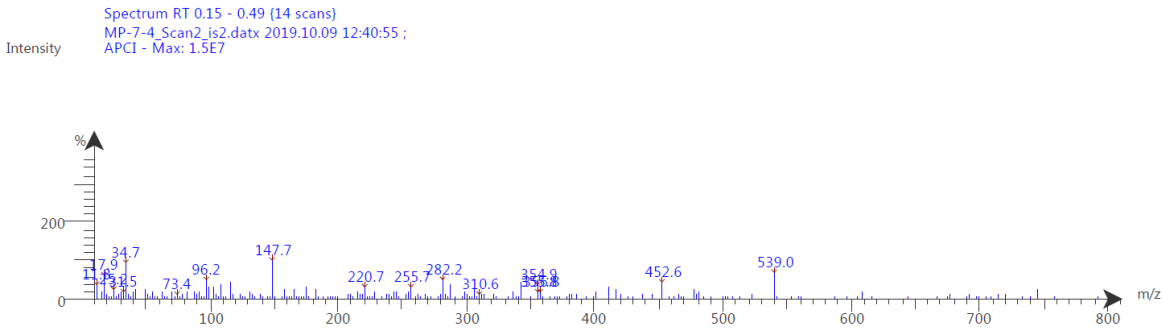
3.6.6 13C NMR Spectrum of N-(4-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3.6b)



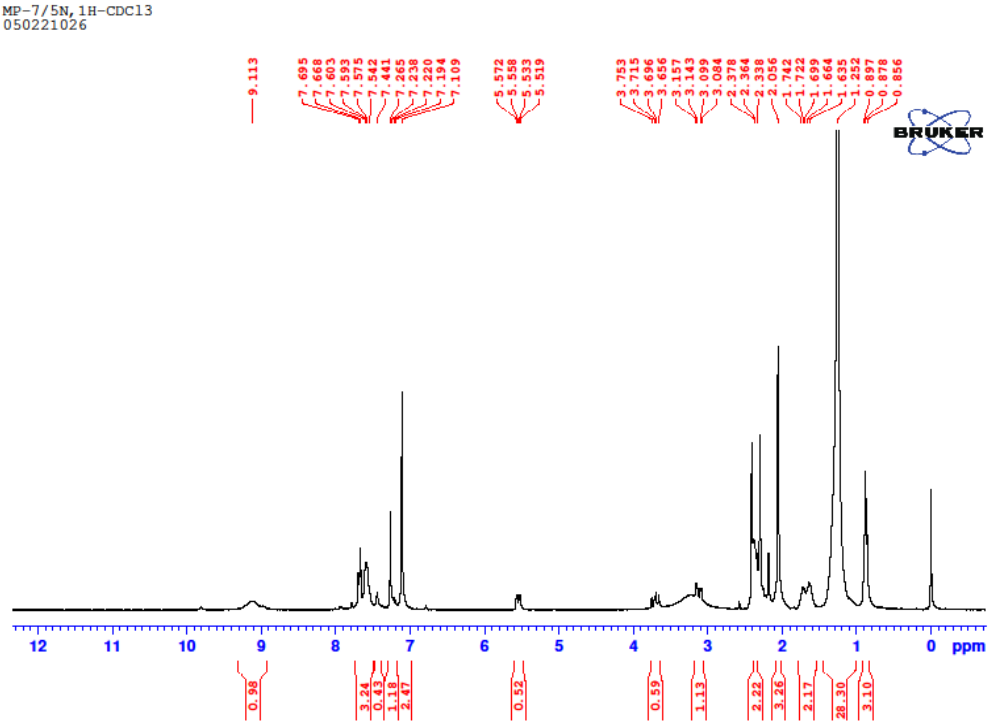
3.6.7 FT-IR Spectrum of N-(4-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6b)



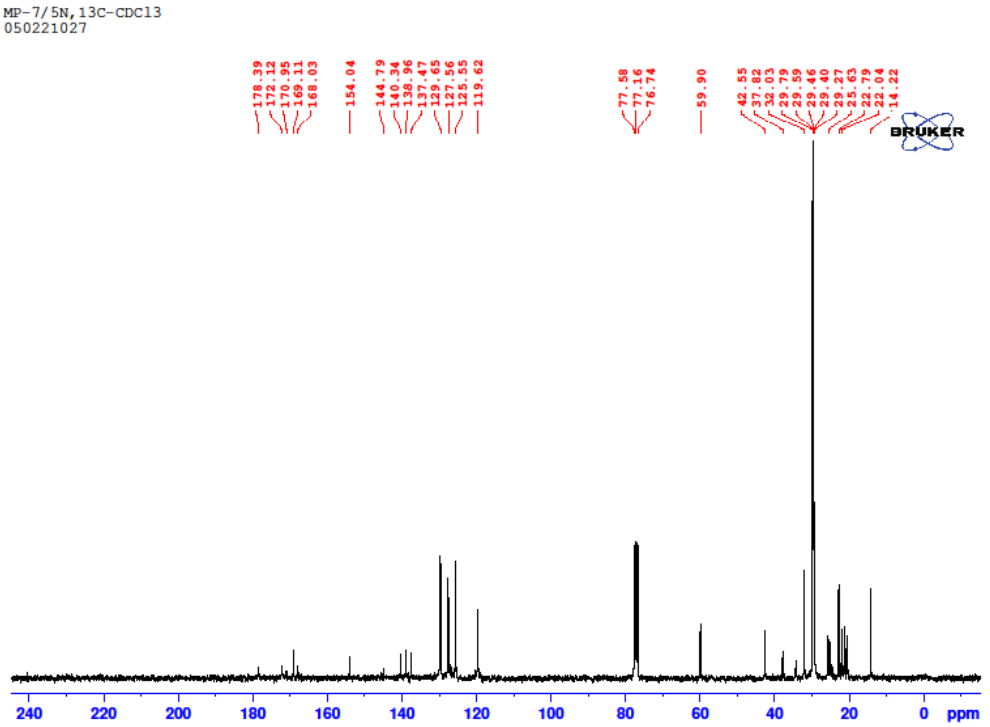
3.6.8 Mass Spectrum of N-(4-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6b)



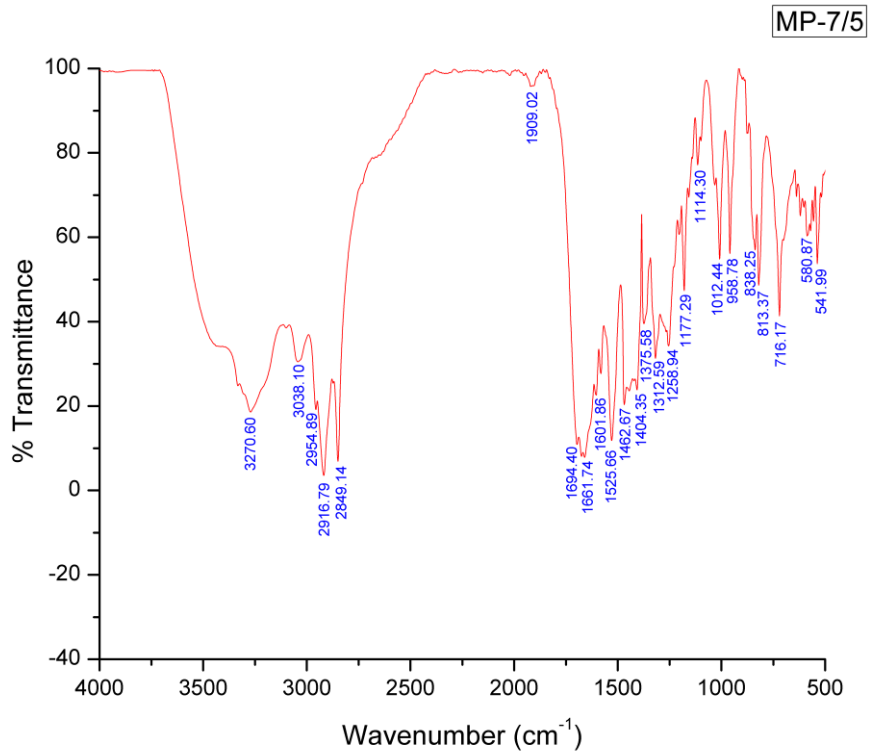
3.6.9 1H NMR Spectrum of N-(4-(5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6c)



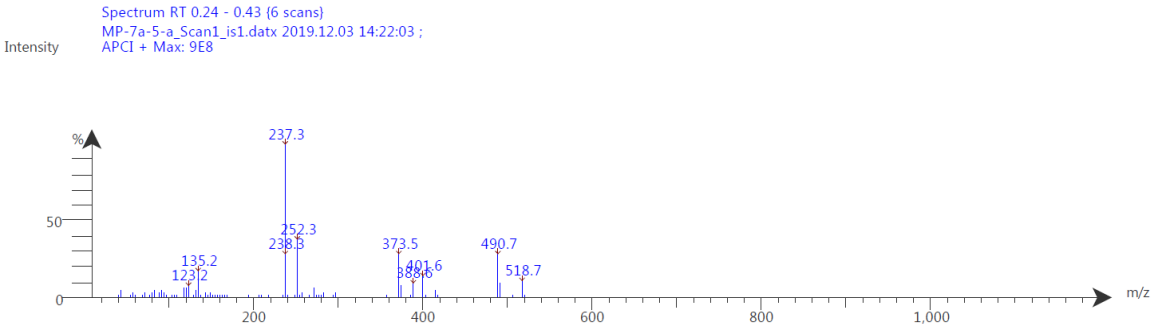
3.6.10 13C NMR Spectrum of N-(4-(5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6c)



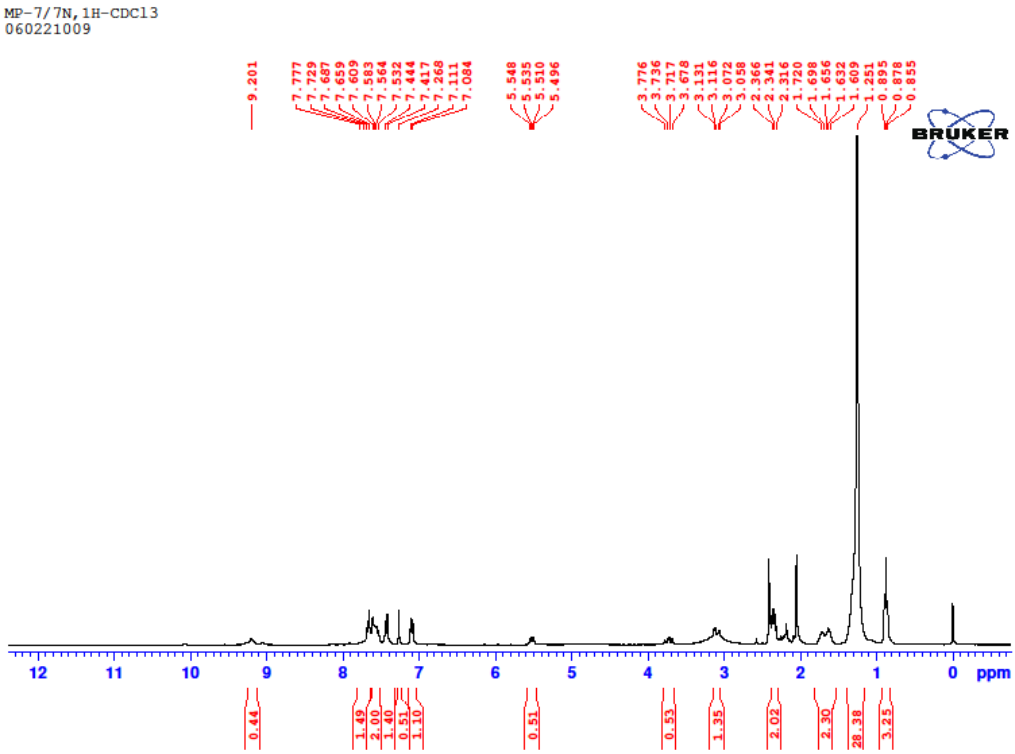
3.6.11 FT-IR Spectrum of N-(4-(5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6c)



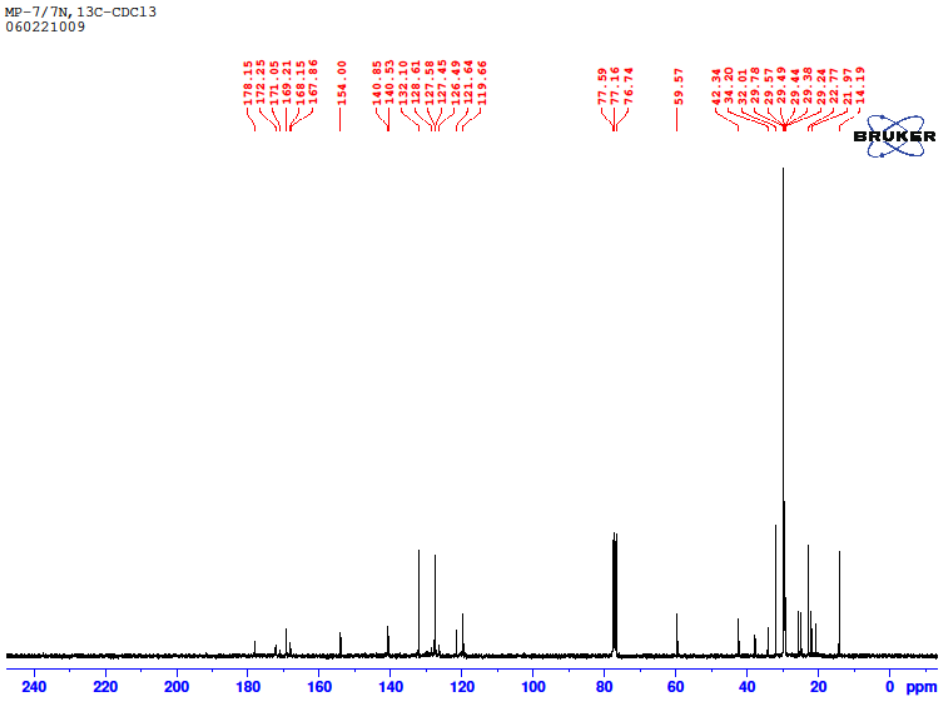
3.6.12 Mass Spectrum of N-(4-(5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl) stearamide (3.6c)



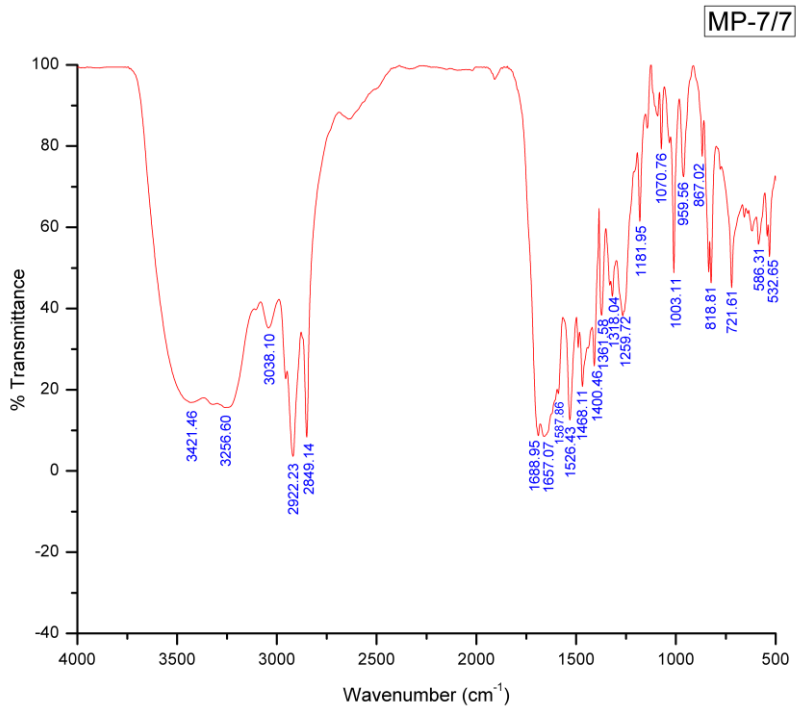
3.6.13 1H NMR Spectrum of N-(4-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3yl) phenyl)stearamide (3.6d)



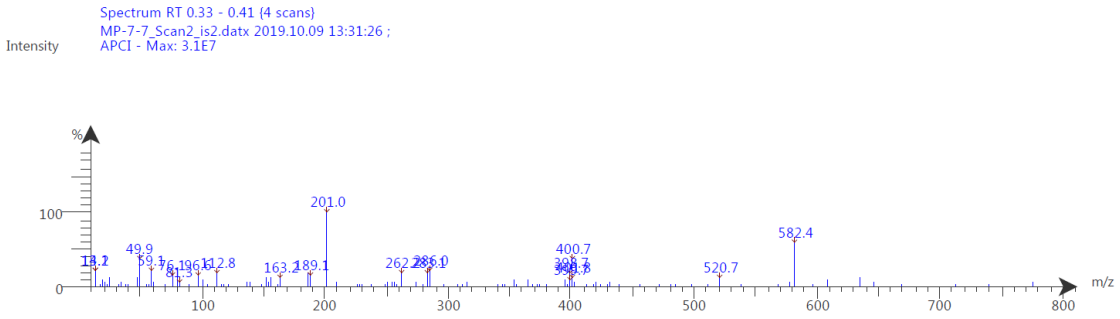
3.6.14 13C NMR Spectrum of N-(4-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3yl) phenyl)stearamide (3.6d)



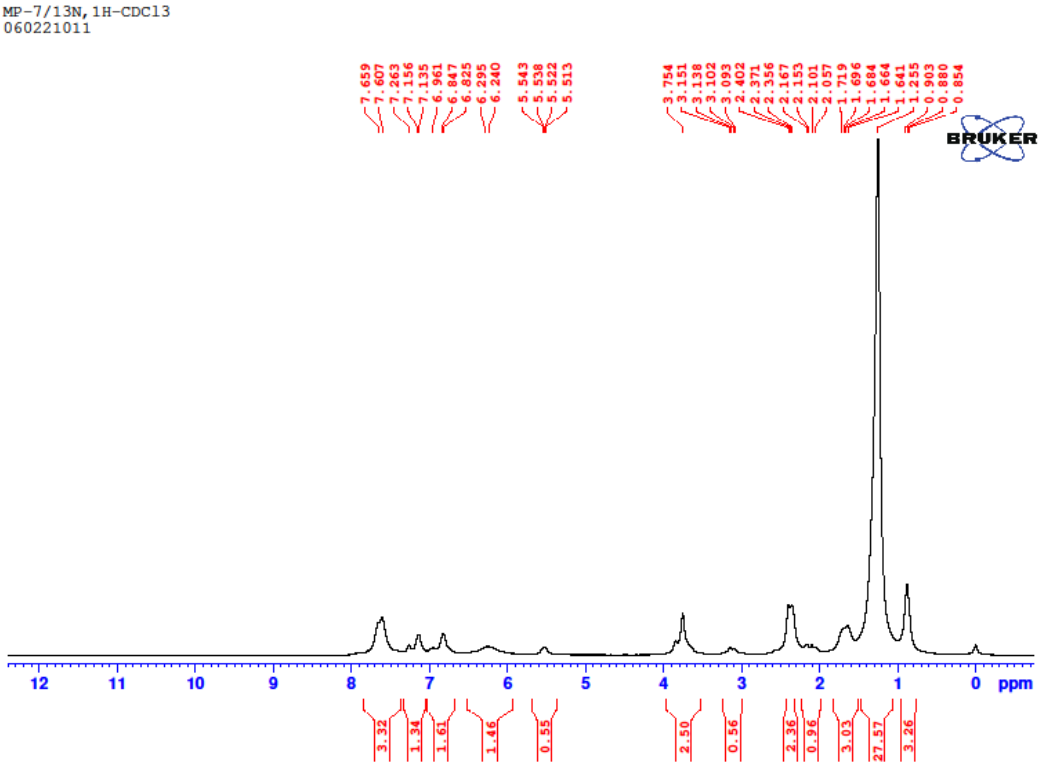
3.6.15 FT-IR Spectrum of N-(4-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3yl) phenyl) stearamide (3.6d)



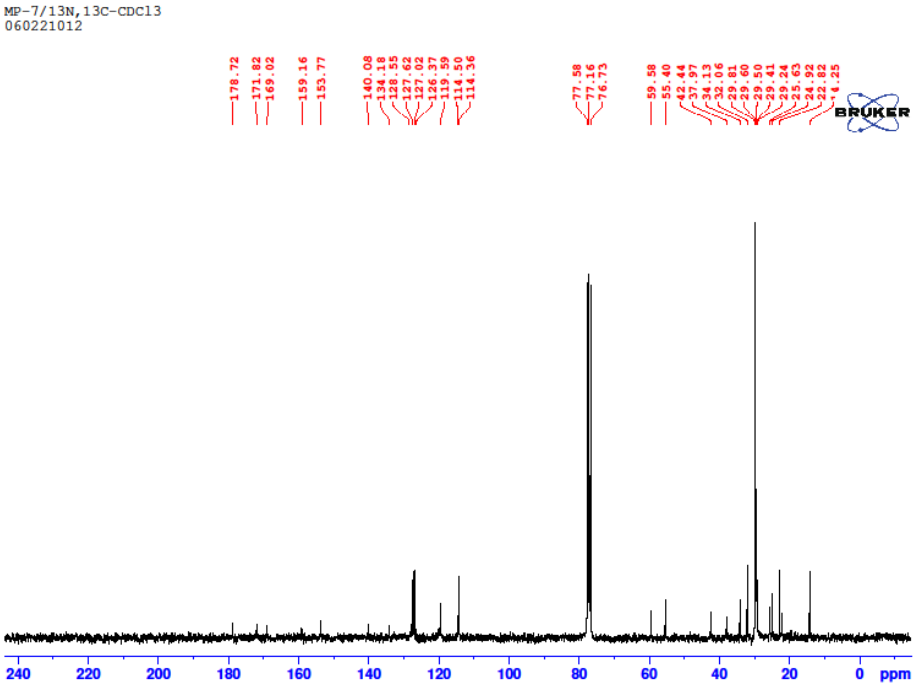
3.6.16 Mass Spectrum of N-(4-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3yl) phenyl) stearamide (3.6d)



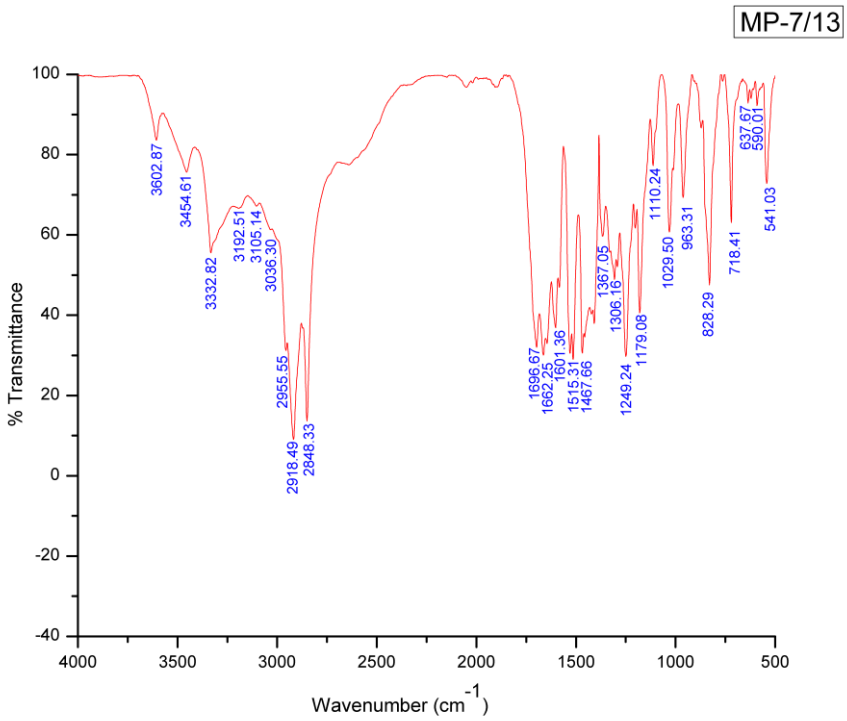
3.6.17 1H NMR Spectrum of N-(4-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3.6e)



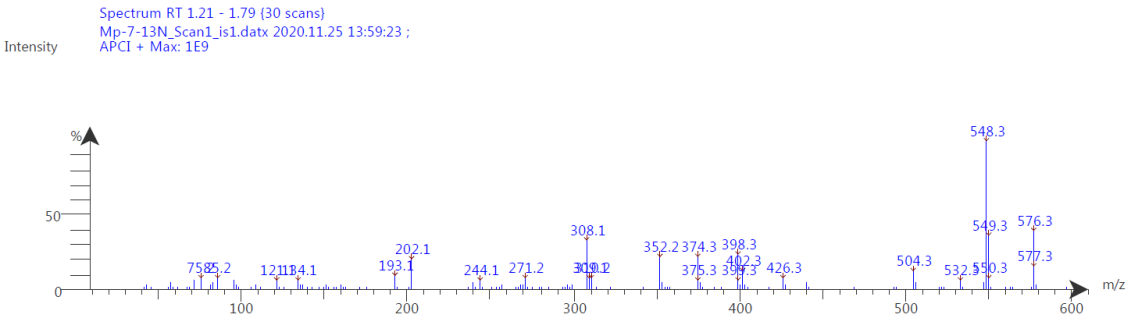
3.6.18 13C NMR Spectrum of N-(4-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3.6e)



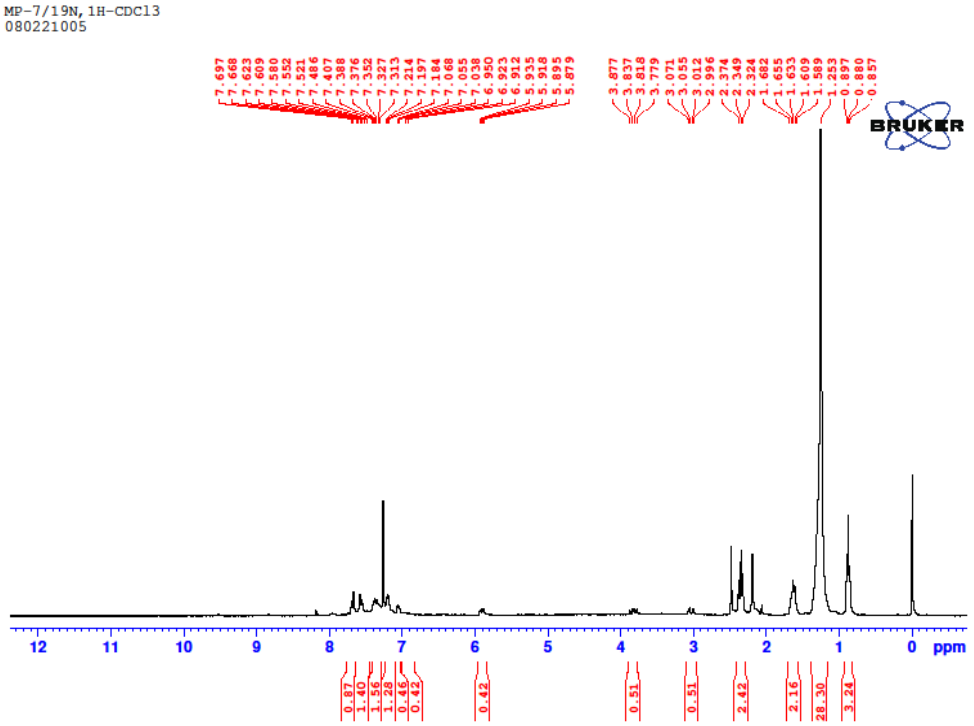
3.6.19 FT-IR NMR Spectrum of N-(4-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3.6e)



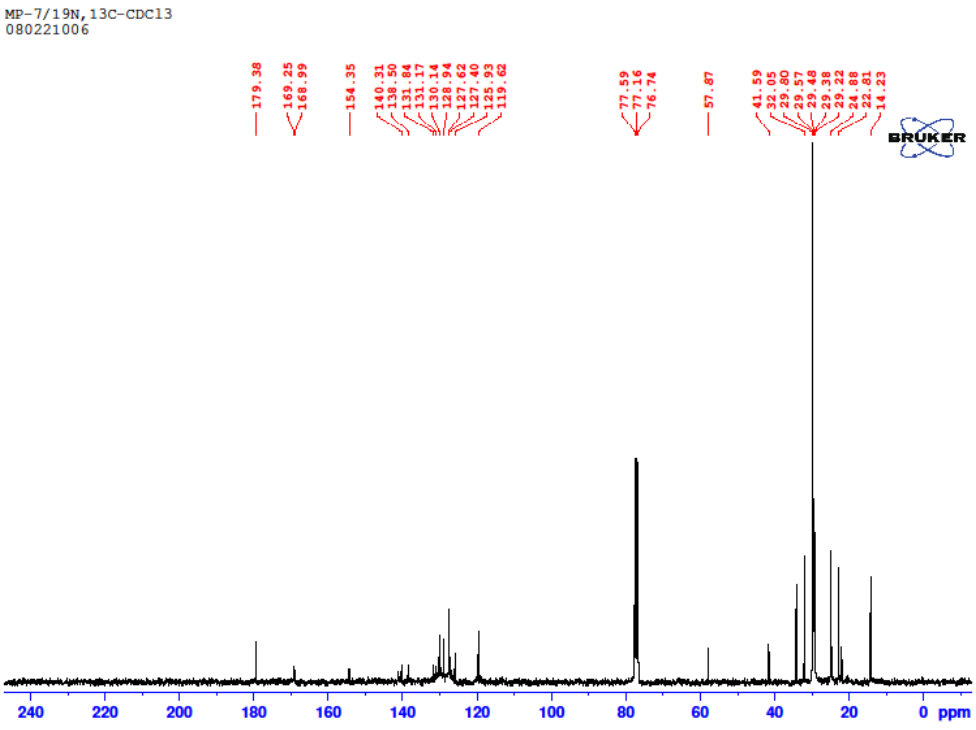
3.6.20 Mass NMR Spectrum of N-(4-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3.6e)



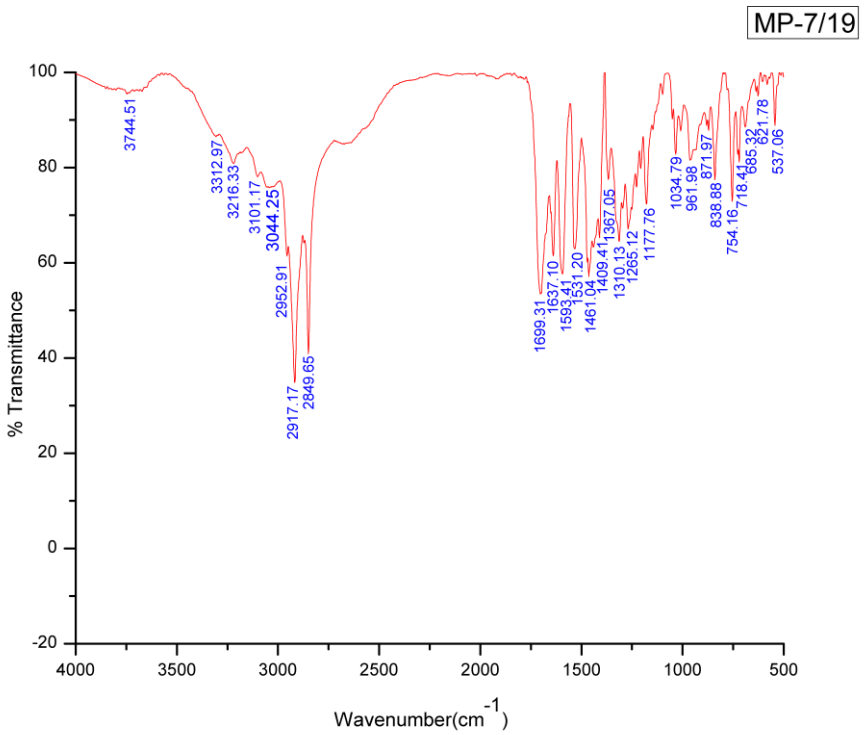
3.6.21 1H NMR Spectrum of N-(4-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3.6f)



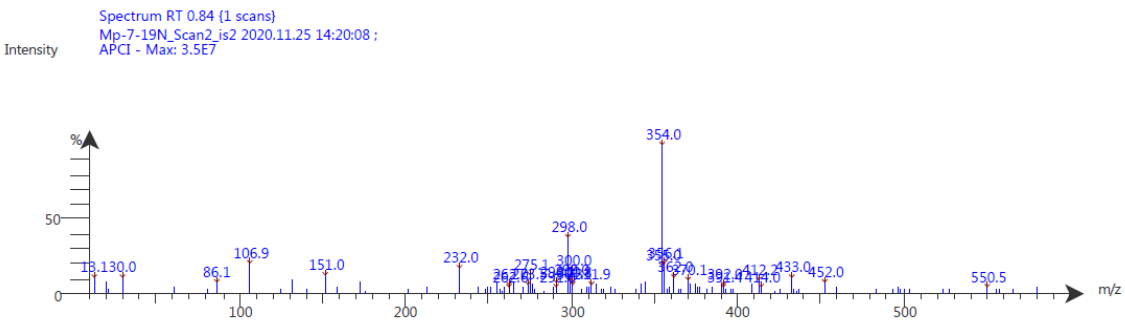
3.6.22 13C NMR Spectrum of N-(4-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3.6f)



3.6.23 FT-IR Spectrum of N-(4-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3.6f)



3.6.24 Mass Spectrum of N-(4-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl) stearamide (3.6f)



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Chapter 4

Molecular docking studies of Octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl] amide Chalcones and octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl) phenyl]-amide pyrazolines as Antimicrobial Targets

Abstract

In this chapter, molecular docking studies was incorporated to elucidate the possible interactions of the synthesized compounds (**2.6a-e** and **3.6a-f**) with bacterial and fungal protein**.** The studies demonstrated the binding mode of the synthesized compounds (ligand) with the three target protein structures of enzymes dihydrofolate reductase (Pdb id: 3SRW) as gram-positive bacteria target, DNA Gyrase B (Pdb id: 1KZN) as gram-negative bacteria target and dihydrofolate reductase (Pdb id: 1AI9) as fungi target.

4.1 INTRODUCTION

The path to drug discovery is a time consuming, challenging and arduous task. Fortunately, computational tools have played a vital role by using computer aided techniques in the discovery of drugs and development process. Molecular docking is one such computational method which has tremendously contributed in structure-based drug design.[1] It aims at predicting a possible mode of interaction between two molecules particularly known as the ligand and the protein generating a binding model. Molecular docking generally involves small molecule and a macromolecule (protein-ligand docking) but more recently it is also applied between two macromolecules (protein-protein docking).[2] It predicts the binding orientation of the ligand and receptor. The information gained from the binding orientation predicts the energy profiling such as binding free energy, strength and stability such as binding affinity and binding constant by using the scoring function.[3] In the early days, the process of development of drug relied upon hit and trial approach by testing a number of compounds and observing their antimicrobial and pharmacological properties. After its appearance in the mid-1970s, the advancement in the biological, biochemical and the prospective of drug research developed enormously. Over the years, with the advancement and the improved knowledge of cell structure, molecular biology, biochemistry, microbiology and recombinant DNA technology, it eases the possibility to identify specific targets for drug like molecules. Therefore, the current approach to drug discovery particularly focus on identifying effective and specific

targets usually proteins in the pathogens and thus find the interactions of the active site of those targets with drug like molecules that binds and inactivate the function of the target protein through structure-based drug design.[4–18] Henceforth, Molecular docking studies were performed to understand the possible interaction between the receptor protein and the synthesized compounds. Bacterial protein DNA gyrase belongs to the topoisomerase family which is involved in replication and transcription by catalysing the negative supercoiling of bacterial circular DNA. Since its blockage induces bacterial death it is considered a suitable target for antibacterial agents. Dihydrofolate reductase (DHFR) is a vital target in many therapeutic areas like cancer and antiinfectives where it is used to generate antifungal, antibacterial and antiparasitic agents.[19-20]

4.2 EXPERIMENTAL SECTION

4.2.1 Molecular docking studies of octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (2.6a-e)

Molecular docking studies were conducted using Molegro Virtual Docker (MVD). In this study, the methodology is based on grid cavity prediction algorithm to determine the potential binding sites. The compounds or ligands (**2.6a-e**) were docked against three target protein structures of enzymes dihydrofolate reductase (Pdb id: 3SRW) as gram-positive bacteria target, DNA Gyrase B (Pdb id: 1KZN) as gram-negative bacteria target and dihydrofolate reductase (Pdb id: 1AI9) as fungi target. All the ligands were drawn using ChemDraw Ultra 12.0 and Chem3D pro 12.0 optimized by MM2 force field method. For molecular docking simulation, all the water molecules were removed and charges assigned. MVD was used for predicting the cavities and the binding cavity set at the site X: -0.03, Y: -31.41, Z: 7.45 with a sphere radius 14 Å, X: 19.91, Y: 27.04, Z: 39.32 with a sphere radius 12 Å, X: 30.09, Y: -6.71, Z: 4.35 with a sphere radius 19 Å for enzymes dihydrofolate reductase (Pdb id: 3SRW), DNA Gyrase B (Pdb id: 1KZN) and dihydrofolate reductase (Pdb id: 1AI9) respectively. All the ligands were docked against the three-target protein with 30 independent runs for each ligand and the top pose of the ligand from the docking score was selected for molecular interaction study.[21-22]

4.2.2 Molecular docking studies of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl) phenyl] -amide pyrazoline derivatives (3.6a-f)

The same above procedure was followed with the binding cavity set at the site X: -1.03, Y: -31.45, Z: 7.57 with a sphere radius 13 Å, X: 19.88, Y: 26.73, Z: 39.56 with a sphere radius 15 Å, X: 30.13, Y: -6.63, Z: 4.28 with a sphere radius 19 Å for enzymes dihydrofolate reductase (Pdb id: 3SRW), DNA Gyrase B (Pdb id: 1KZN) and dihydrofolate reductase (Pdb id: 1AI9) respectively. All the ligands (**3.6a-f**) were docked against the three-target protein with 30 independent runs for each ligand and the top pose of the ligand from the docking score was selected for molecular interaction study.[21-22]

4.3 RESULTS AND DISCUSSION

From the molecular docking studies, the binding mode of ligand (**2.6a-e**) with the three target protein structures of enzymes dihydrofolate reductase (Pdb id: 3SRW) as gram-positive bacteria target, DNA Gyrase B (Pdb id: 1KZN) as gram-negative bacteria target and dihydrofolate reductase (Pdb id: 1AI9) as fungi target were evaluated. The top pose from each ligand was then selected for ligand-protein interaction energy analysis as shown in Tables 4.1, 4.3 and 4.5. Figure 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6 depicts the possible mode of interaction between the ligand and the active site of the target protein. Table 4.2, 4.4 and 4.6 show the ligand-protein interaction, interaction energy, interaction distance and their hybridization. It can be observed from the results that the compounds possessed favorable ligand-protein interaction energy values of -2.5 to -0.064 kJ/mol, -2.5 to -0.148 kJ/mol and -2.5 to -0.079 kJ/mol at the binding cavity of 1KZN, 3SRW and 1AI9 respectively. A common molecular interaction of the compounds and streptomycin was observed with the Asn46, Arg76, Thr165 for enzymes DNA Gyrase B (Pdb id: 1KZN), Thr122 and Thr47 for enzymes dihydrofolate reductase (Pdb id: 3SRW), and also both compounds and fluconazole revealed a common molecular interaction with Lys57, Arg56, Arg79, Ser78 for enzymes dihydrofolate reductase (Pdb id: 1AI9).

Similarly, from the molecular docking studies, the binding mode of ligand (**3.6a-f**) with the three target protein structures of enzymes dihydrofolate reductase (Pdb id: 3SRW) as gram-positive bacteria target, DNA Gyrase B (Pdb id: 1KZN) as gram-negative bacteria target and dihydrofolate reductase (Pdb id: 1AI9) as fungi target were evaluated. The top pose from each ligand was then selected for ligand-protein interaction energy analysis as shown in Tables 4.7, 4.9 and 4.11. Figure 4.7, 4.8, 4.9, 4.10, 4.11 and 4.12

depicts the possible mode of interaction between the ligand and the active site of the target protein. Table 4.8, 4.10 and 4.12 show the ligand-protein interaction, interaction energy, interaction distance and their hybridization. It can be observed from the results that the compounds possessed favorable ligand-protein interaction energy values of -2.5 to -0.069 kJ/mol, -2.5 to -0.242 kJ/mol and -2.5 to -0.043 kJ/mol at the binding cavity of 1KZN, 3SRW and 1AI9 respectively. A common molecular interaction of the compounds and streptomycin was observed with the Gly77 for enzymes DNA Gyrase B (Pdb id: 1KZN), Gly95, Thr47 and Asn19 for enzymes dihydrofolate reductase (Pdb id: 3SRW), and also both compounds and fluconazole revealed a common molecular interaction with Leu77, Arg56, Glu116, Lys57 and Ile117 for enzymes dihydrofolate reductase (Pdb id: 1AI9).

Table 4.1. Docking score of compounds **(2.6a-e)** with 1KZN.

Ligand	MolDock Score	Rerank Score	Interaction	Internal	HBond	LE1	LE3
3a	-138.425	-51.0216	-147.319	8.89317	-0.06466	-3.74123	-1.37896
3b	-146.234	-85.7918	-149.43	3.19578	-0.87088	-3.95227	-2.3187
3c	-144.355	-75.7005	-158.241	13.8855	-2.58127	-3.90149	-2.04596
3d	-129.022	-62.4226	-150.201	21.1785	-2.58062	-3.48708	-1.6871
3e	-137.286	-38.728	-155.323	18.0365	-3.40544	-3.61279	-1.01916
Streptomycin	-115.466	-87.4057	-151.75	36.2843	-7.3073	-2.88664	-2.18514

Table 4.2. Molecular interaction analysis of compounds **(2.6a-e)** with the active site of 1KZN.

Compound	Interaction (Protein-Ligand)	Interaction Energy (kJ/mol)	Interaction Distance(Å)	Hybridization (Protein Atom)	Hybridization (Ligand Atom)
3a	Asn46(O)---N(19)	-0.064	3.34	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr165(OG1)---O(18)	-2.5	3.00	Sp^3 (Both)	Sp^2 (Acceptor)
3b	Arg136(NH1)---O(29)	-0.823	3.44	Sp^2 (Donor)	Sp^2 (Acceptor)
	Gly77(N)---O(29)	-0.184	3.15	Sp^2 (Donor)	Sp^2 (Acceptor)
3c	Arg76(NH1)---O(29)	-0.809	3.44	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg76(NE)---O(29)	-0.424	3.30	Sp^2 (Donor)	Sp^2 (Acceptor)
	Asp73(OD1)---N(19)	-1.947	2.88	Sp^3 (Acceptor)	Sp^2 (Donor)
	Thr165(OG1)---O(18)	-0.643	3.17	Sp^3 (Both)	Sp^2 (Acceptor)
3d	Arg76(NH1)---O(29)	-1.225	3.35	Sp^2 (Donor)	Sp^2 (Acceptor)

	Arg76(NE)---O(29)	-0.096	3.47	Sp^2 (Donor)	Sp^2 (Acceptor)
	Asp73(OD1)---N(19)	-2.314	2.92	Sp^3 (Acceptor)	Sp^2 (Donor)
3e	Asn46(ND2)---O(18)	-2.421	2.59	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg136(NH1)---O(36)	-2.5	2.76	Sp^2 (Donor)	Sp^3 (Acceptor)
Streptomycin	Asp49(OD1)---O(35)	-2.313	3.14	Sp^3 (Acceptor)	Sp^3 (Both)
	Asn46(ND2)---O(10)	-2.166	3.17	Sp^2 (Donor)	Sp^3 (Both)
	Val43(O)---N(2)	-2.262	3.15	Sp^2 (Acceptor)	Sp^2 (Donor)
	Arg76(NH1)---O(33)	-2.5	2.67	Sp^2 (Donor)	Sp^3 (Both)
	Val71(O)---N(O)	-1.813	3.24	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr165(O)---N(O)	-2.5	3.04	Sp^2 (Acceptor)	Sp^2 (Donor)
	Asp73(OD2)---N(38)	-1.362	2.46	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr165(OG1)---N(38)	-1.251	2.65	Sp^3 (Both)	Sp^2 (Donor)
	Thr165(OG1)---N(36)	-2.5	2.85	Sp^3 (Both)	Sp^2 (Donor)
	Thr165(OG1)---O(11)	-2.5	2.76	Sp^3 (Both)	Sp^3 (Both)
	Asn46(O)---O(12)	3.139	1.94	Sp^2 (Acceptor)	Sp^3 (Both)

Table 4.3. Docking score of compounds **(2.6a-e)** with 3SRW.

Ligand	MolDock Score	Rerank Score	Interaction	Internal	HBond	LE1	LE3
3a	-181.109	-43.1287	-210.042	28.9325	-3.32596	-4.89484	-1.16564
3b	-187.937	-138.678	-220.282	32.345	-4.37414	-5.07938	-3.74805
3c	-184.064	-136.079	-203.623	19.5586	-3.68455	-4.97471	-3.67781
3d	-160.318	-130.048	-197.226	36.9082	-4.24794	-4.33291	-3.51481
3e	-175.047	-71.7291	-205.323	30.2761	-2.53655	-4.6065	-1.88761
Streptomycin	-139.362	-82.4942	-179.706	40.3446	-19.7147	-3.48404	-2.06235

Table 4.4. Molecular interaction analysis of compounds **(2.6a-e)** with the active site of 3SRW.

Compound	Interaction (Protein-Ligand)	Interaction Energy (kJ/mol)	Interaction Distance(Å)	Hybridization (Protein Atom)	Hybridization (Ligand Atom)
3a	Thr97(N)---O(29)	-0.825	3.20	Sp^2 (Donor)	Sp^2 (Acceptor)
	Thr97(OG1)---O(29)	-2.5	3.08	Sp^3 (Both)	Sp^2 (Acceptor)
3b	Gly95(N)---O(29)	-1.874	2.66	Sp^2 (Donor)	Sp^2 (Acceptor)
	Leu98(N)---O(29)	-2.5	3.10	Sp^2 (Donor)	Sp^2 (Acceptor)

3c	Thr122(OG1)---O(18)	-2.5	3.08	Sp^3 (Both)	Sp^2 (Acceptor)
	Thr97(N)---O(29)	-1.809	3.21	Sp^2 (Donor)	Sp^2 (Acceptor)
	Thr97(OG1)---O(29)	-1.885	2.53	Sp^3 (Both)	Sp^2 (Acceptor)
3d	Thr47(OG1)---O(18)	-1.851	3.23	Sp^3 (Both)	Sp^2 (Acceptor)
	Gly96(N)---O(29)	-0.148	3.17	Sp^2 (Donor)	Sp^2 (Acceptor)
	Thr97(N)---O(29)	-2.329	3.13	Sp^2 (Donor)	Sp^2 (Acceptor)
	Thr97(OG1)---O(29)	-1.770	3.25	Sp^3 (Both)	Sp^2 (Acceptor)
3e	Ala8(N)---O(29)	-1.712	3.00	Sp^2 (Donor)	Sp^2 (Acceptor)
	Thr47(OG1)---O(18)	-1.099	3.38	Sp^3 (Both)	Sp^2 (Acceptor)
	Gly96(N)---O(18)	-0.824	3.10	Sp^2 (Donor)	Sp^2 (Acceptor)
Streptomycin	Asn19(OD1)---N(2)	-2.5	2.63	Sp^2 (Acceptor)	Sp^2 (Donor)
	Asp121(OD1)---N(O)	-2.5	2.99	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr122(OG1)---N(O)	-1.437	2.47	Sp^3 (Both)	Sp^2 (Donor)
	Asp28(OD2)---N(30)	-1.673	3.07	Sp^2 (Acceptor)	Sp^3 (Donor)
	Asp28(OD2)---N(35)	-1.705	3.26	Sp^2 (Acceptor)	Sp^3 (Both)
	Trp23(NE1)---O(35)	-2.405	3.05	Sp^2 (Donor)	Sp^3 (Both)
	Thr47(OG1)---O(12)	-2.274	3.15	Sp^3 (Both)	Sp^3 (Both)
	Thr47(OG1)---O(22)	-2.5	2.95	Sp^3 (Both)	Sp^2 (Acceptor)
	Phe93(O)---O(20)	-2.5	2.94	Sp^2 (Acceptor)	Sp^3 (Both)
	Ala8(O)---O(34)	-2.5	2.66	Sp^2 (Acceptor)	Sp^3 (Both)
	Ile(15)---O(34)	-2.5	3.08	Sp^2 (Acceptor)	Sp^3 (Both)
	Ile(15)---O(11)	-2.5	2.66	Sp^2 (Acceptor)	Sp^3 (Both)
	Gln20(O)---O(11)	-1.116	3.32	Sp^2 (Acceptor)	Sp^3 (Both)
	Asn19(O)---O(10)	-2.5	2.82	Sp^2 (Acceptor)	Sp^3 (Both)
	Ser50(OG)---O(10)	-1.610	3.28	Sp^3 (Both)	Sp^3 (Both)
	Ser50(OG)---O(12)	-2.5	3.00	Sp^3 (Both)	Sp^3 (Both)

Table 4.5. Docking score of compounds **(2.6a-e)** with 1AI9.

Ligand	MolDock Score	Rerank Score	Interaction	Internal	HBond	LE1	LE3
3a	-147.54	-108.893	-169.726	22.1856	-5.86546	-3.98757	-2.94304
3b	-154.115	-121.728	-182.917	28.8018	-2.92557	-4.16527	-3.28994
3c	-156.009	-106.569	-190.672	34.6624	-6.06279	-4.21647	-2.88023
3d	-170.491	-133.238	-193.86	23.3694	-4.80214	-4.60786	-3.60104

3e	-150.034	-112.319	-195.353	45.319	-7.49744	-3.94826	-2.95576
Fluconazole	-118.699	-77.7577	-124.825	6.12616	-15.914	-5.3954	-3.53444

Table 4.6. Molecular interaction analysis of compounds **(2.6a-e)** with the active site of 1AI9.

Compound	Interaction (Protein-Ligand)	Interaction Energy (kJ/mol)	Interaction Distance(Å)	Hybridization (Protein Atom)	Hybridization (Ligand Atom)
3a	Thr58(OG1)---O(18)	-2.038	2.54	Sp^3 (Both)	Sp^2 (Acceptor)
	Thr58(N)---O(18)	-2.153	2.99	Sp^2 (Donor)	Sp^2 (Acceptor)
	Gly114(N)---O(18)	-1.694	2.96	Sp^2 (Donor)	Sp^2 (Acceptor)
3b	Thr58(OG1)---O(18)	-2.389	3.12	Sp^3 (Both)	Sp^2 (Acceptor)
	Arg56(N)---O(29)	-0.425	2.94	Sp^2 (Donor)	Sp^2 (Acceptor)
	Lys57(N)---O(29)	-2.5	3.01	Sp^2 (Donor)	Sp^2 (Acceptor)
3c	Thr58(OG1)---O(29)	-2.5	2.76	Sp^3 (Both)	Sp^2 (Acceptor)
	Thr58(N)---O(29)	-1.774	3.22	Sp^2 (Donor)	Sp^2 (Acceptor)
	Gly55(N)---O(29)	-0.079	3.55	Sp^2 (Donor)	Sp^2 (Acceptor)
	Gly114(N)---O(29)	-1.865	2.89	Sp^2 (Donor)	Sp^2 (Acceptor)
3d	Thr58(OG1)---O(29)	-2.5	2.89	Sp^3 (Both)	Sp^2 (Acceptor)
	Gly55(N)---O(29)	-0.601	3.22	Sp^2 (Donor)	Sp^2 (Acceptor)
	Gly114(N)---O(29)	-2.205	2.82	Sp^2 (Donor)	Sp^2 (Acceptor)
3e	Ala115(N)---O(18)	-0.996	3.33	Sp^2 (Donor)	Sp^2 (Acceptor)
	Thr58(OG1)---O(29)	-1.625	3.27	Sp^3 (Both)	Sp^2 (Acceptor)
	Gly114(N)---O(29)	-2.5	2.79	Sp^2 (Donor)	Sp^2 (Acceptor)
	Gly55(N)---O(29)	-0.498	3.27	Sp^2 (Donor)	Sp^2 (Acceptor)
	Ser78(OG)---O(36)	-0.327	3.53	Sp^3 (Both)	Sp^3 (Acceptor)
	Arg79(N)---O(36)	-2.5	2.90	Sp^2 (Donor)	Sp^3 (Acceptor)
	Arg79(NH1)---O(36)	-2.5	2.92	Sp^2 (Donor)	Sp^3 (Acceptor)
Fluconazole	Lys57(N)---O(3)	-2.5	3.01	Sp^2 (Donor)	Sp^3 (Both)
	Arg56(N)---O(3)	-0.487	2.65	Sp^2 (Donor)	Sp^3 (Both)
	Arg79(NH2)---N(12)	-2.5	2.78	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg79(NH1)---N(12)	-1.627	3.27	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg79(NH1)---N(7)	-2.5	2.90	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg79(N)---N(7)	-2.276	3.02	Sp^2 (Donor)	Sp^2 (Acceptor)
	Ser78(OG)---N(7)	-1.200	3.36	Sp^3 (Both)	Sp^2 (Acceptor)

	Ser78(OG)---N(5)	-2.424	3.12	Sp^3 (Both)	Sp^2 (Acceptor)
	Ser78(OG)---N(4)	-1.393	3.32	Sp^3 (Both)	Sp^2 (Acceptor)
	Arg56(NE)---N(4)	-0.748	3.36	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg56(NE)---N(5)	-2.462	2.69	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg56(NH2)---N(5)	-1.247	3.15	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg56(NH2)---N(10)	-0.960	2.94	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg56(NH2)---N(9)	-0.086	3.52	Sp^2 (Donor)	Sp^2 (Acceptor)
	Lys57(NZ)---N(9)	-2.5	2.71	Sp^3 (Donor)	Sp^2 (Acceptor)
	Lys57(NZ)---N(10)	-2.5	2.85	Sp^3 (Donor)	Sp^2 (Acceptor)

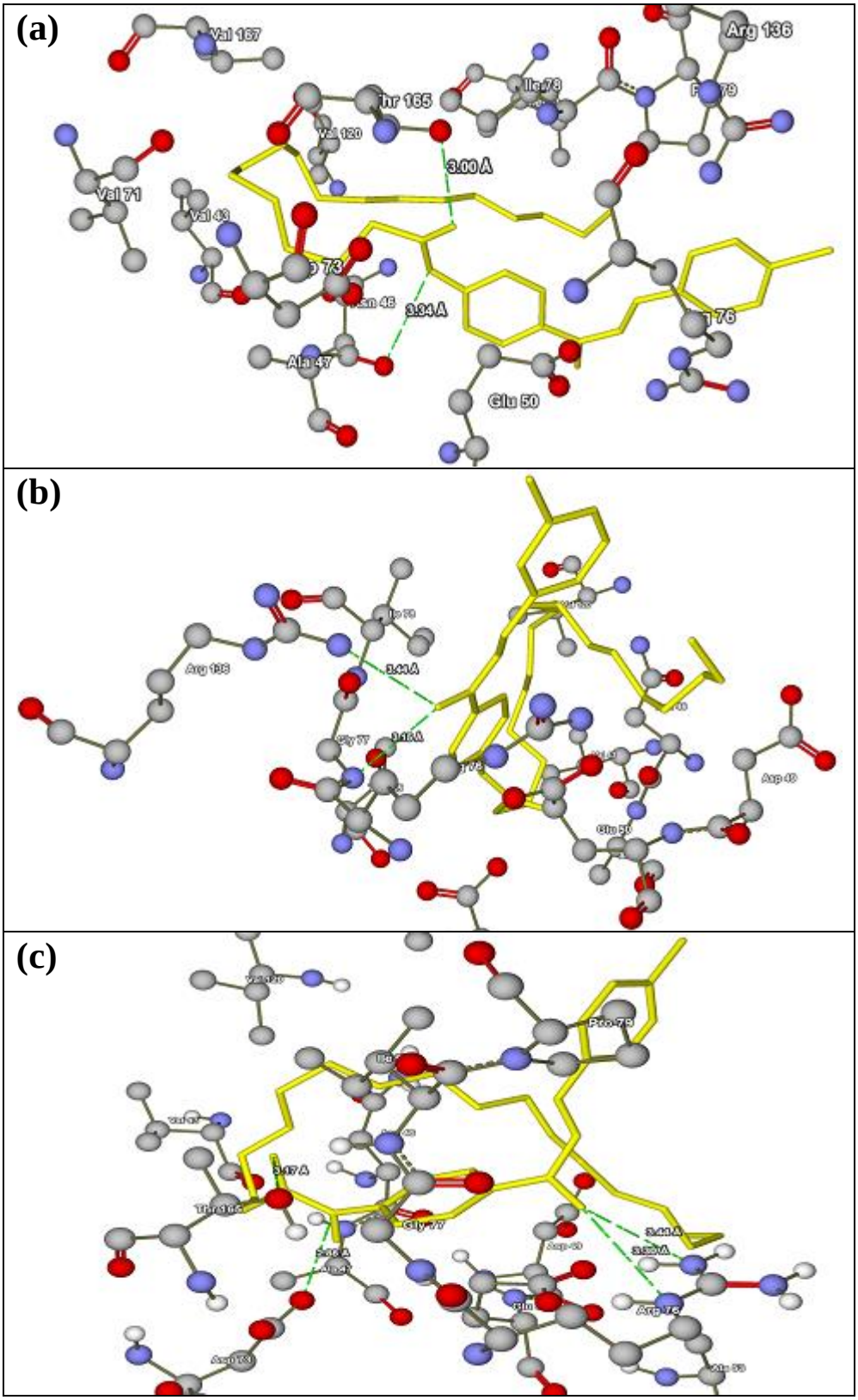


Figure 4.1. Molecular interaction of the compounds **2.6a(a)**, **2.6b(b)** and **2.6c(c)** at the active site pocket of the protein 1KZN.

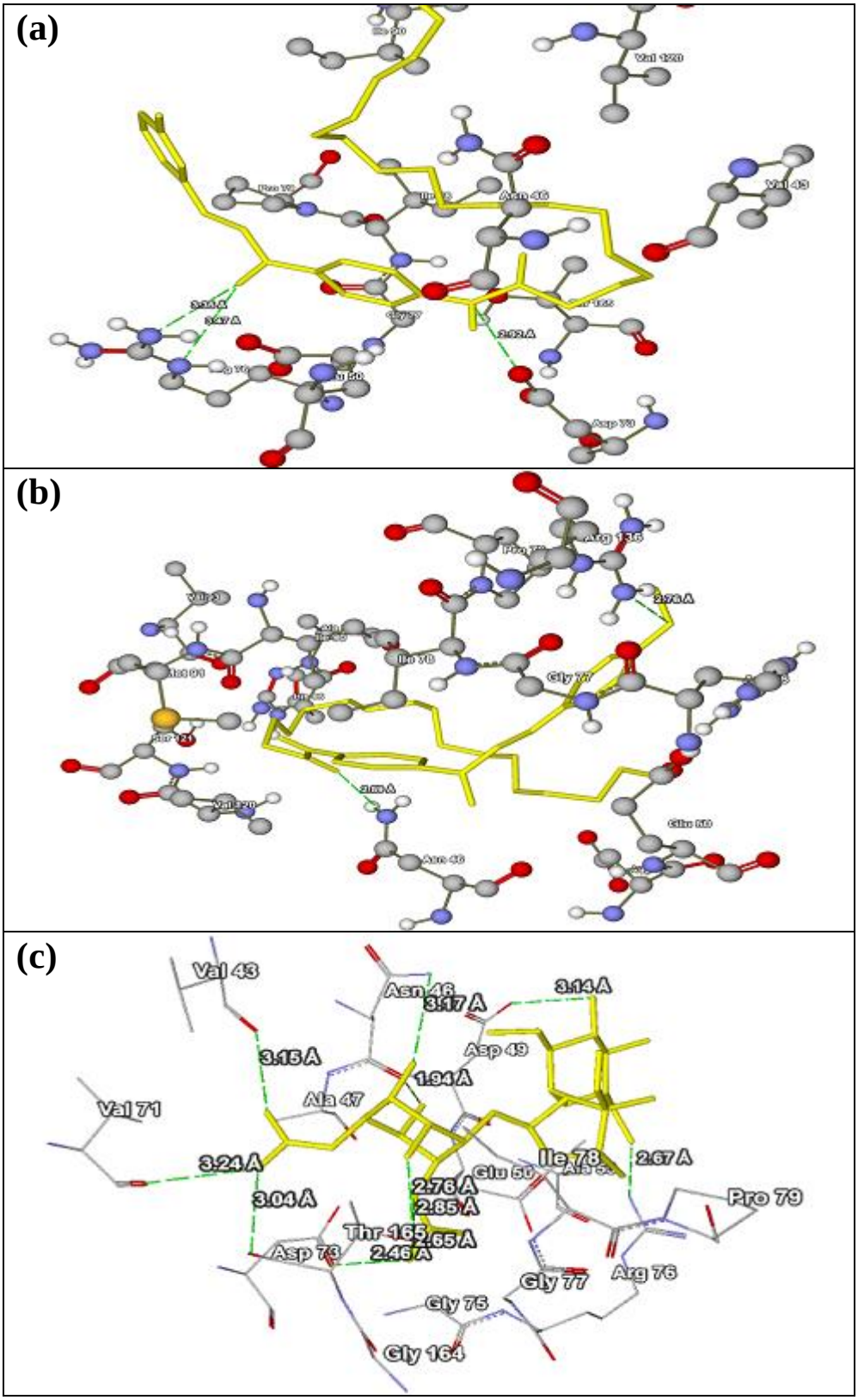


Figure 4.2. Molecular interaction of the compounds **2.6d(a)**, **2.6e(b)** and streptomycin**(c)** at the active site pocket of the protein 1KZN.

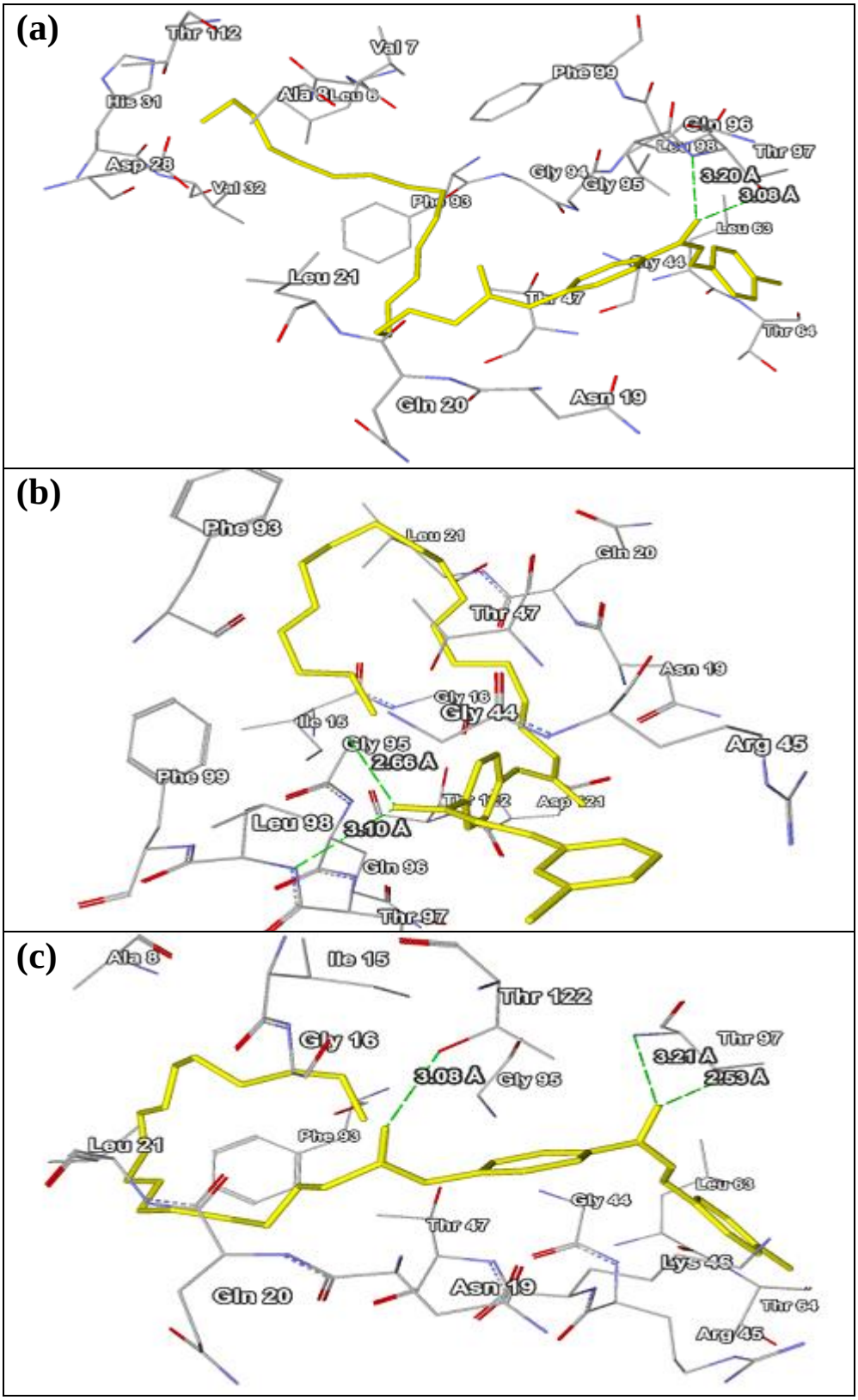


Figure 4.3. Molecular interaction of the compounds **2.6a(a)**, **2.6b(b)** and **2.6c(c)** at the active site pocket of the protein 3SRW.

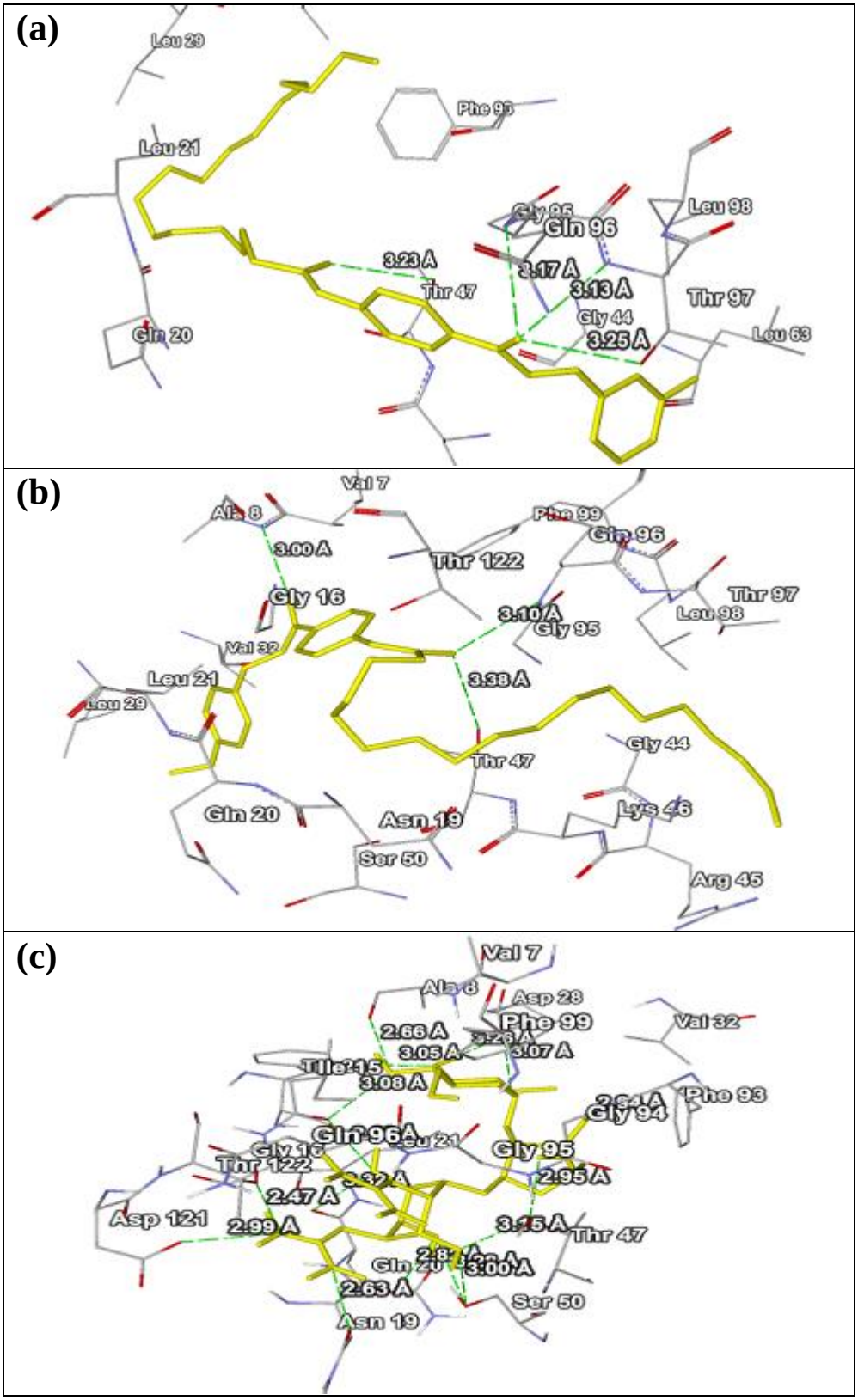


Figure 4.4. Molecular interaction of the compounds **2.6d(a)**, **2.6e(b)** and streptomycin**(c)** at the active site pocket of the protein 3SRW.

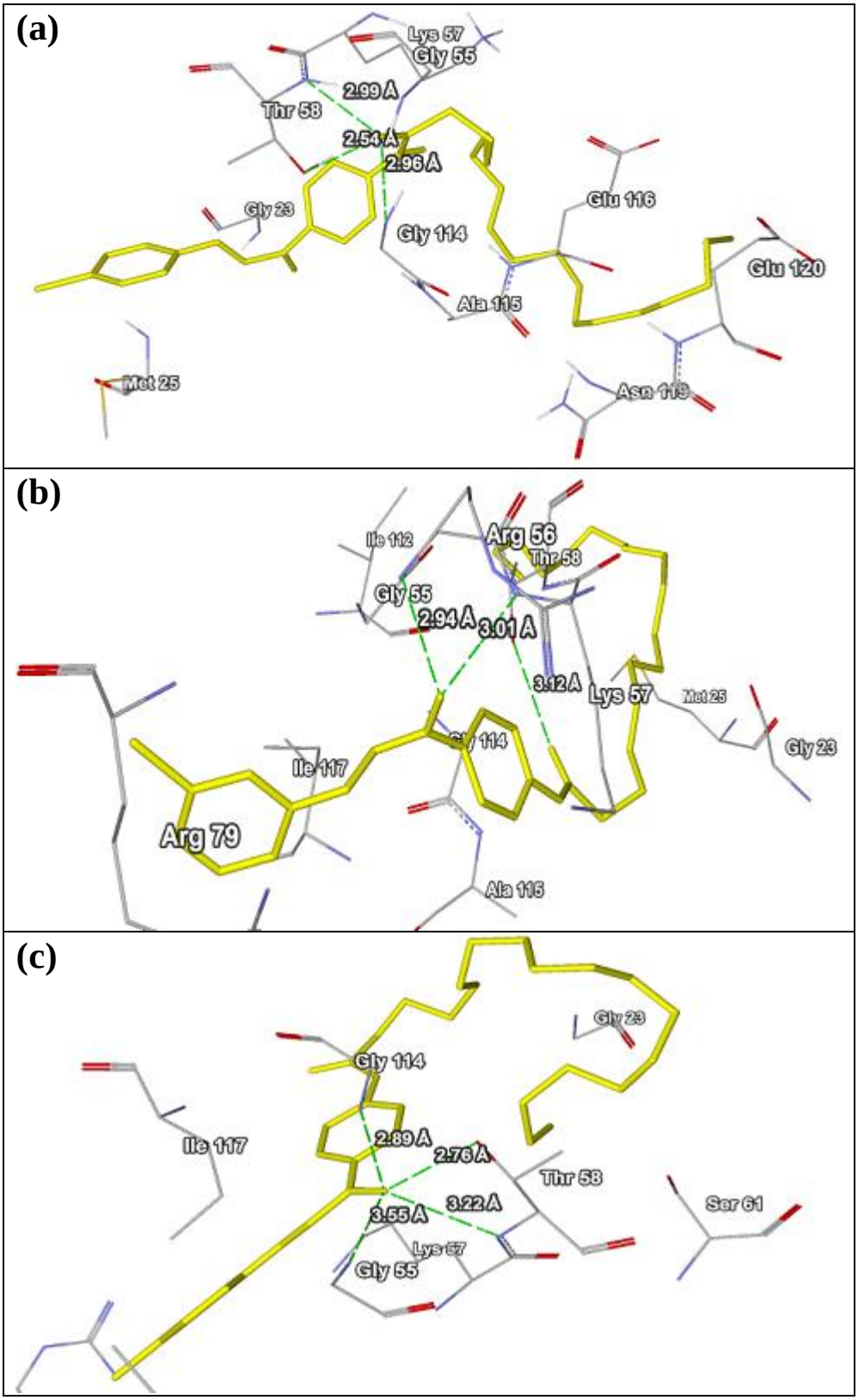


Figure 4.5. Molecular interaction of the compounds **2.6a(a)**, **2.6b(b)** and **2.6c(c)** at the active site pocket of the protein 1AI9.

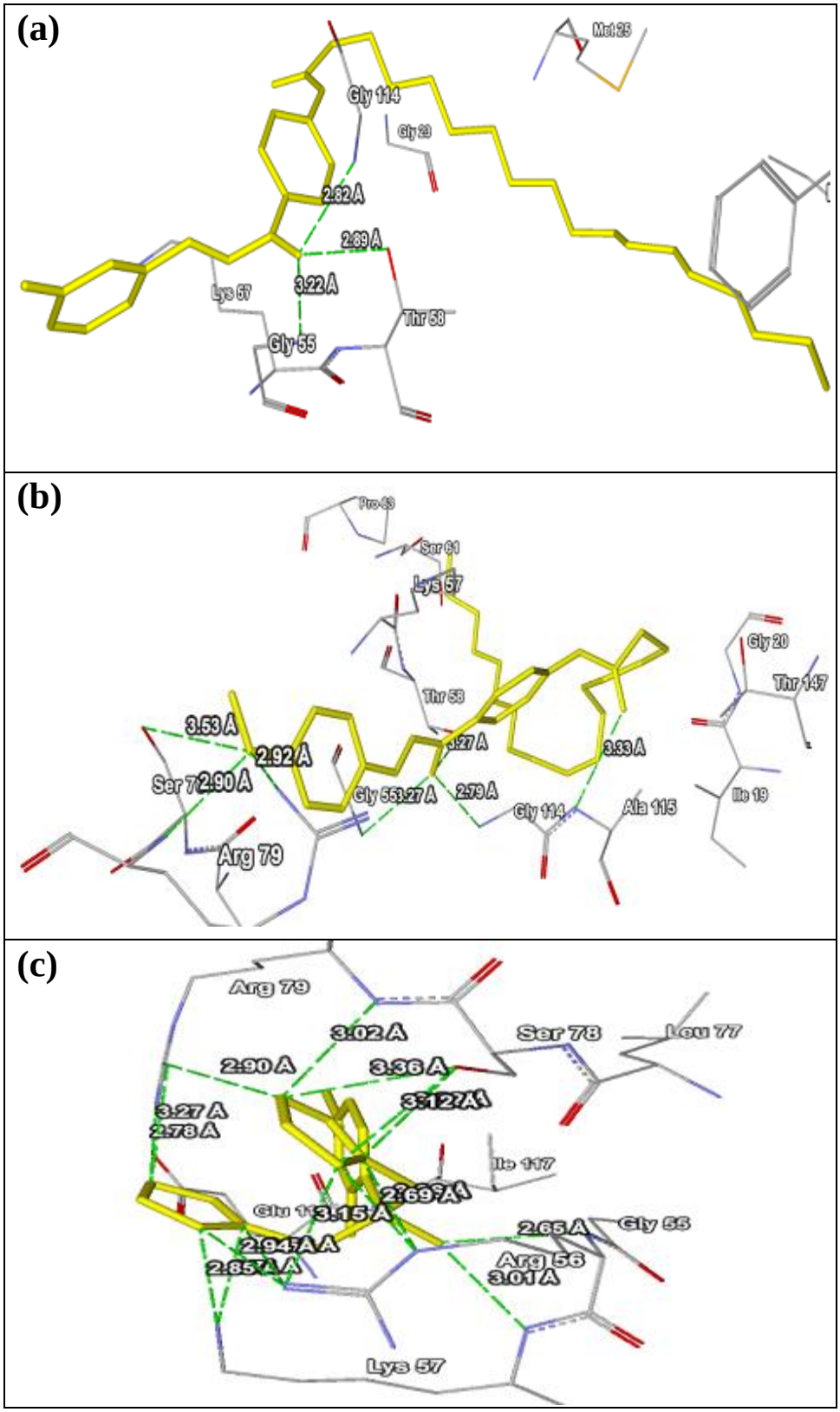


Figure 4.6. Molecular interaction of the compounds **2.6d(a)**, **2.6e(b)** and fluconazole**(c)** at the active site pocket of the protein 1AI9.

Table 4.7. Docking score of compounds **(3.6a-f)** with 1KZN.

Ligand	MolDock Score	Rerank Score	Interaction	Internal	HBond	LE1	LE3
3a	-145.476	-71.2202	-164.777	19.3016	-1.40248	-3.93178	-1.92487
3b	-150.295	-33.9811	-170.541	20.2463	-3.52455	-3.95513	-0.89424
3c	-152.504	-40.9473	-173.785	21.2812	-2.54496	-4.01326	-1.07756
3d	-149.581	-9.48633	-162.948	13.3674	-3.25912	-3.93634	-0.24964
3e	-106.747	-41.5115	-125.545	18.7989	-2.97058	-2.73709	-1.0644
3f	-147.282	-79.7796	-175.631	28.3486	-1.37037	-3.87585	-2.09946
Streptomycin	-111.289	-103.256	-156.693	45.4042	-9.19529	-2.78222	-2.58141

Table 4.8. Molecular interaction analysis of compounds **(3.6a-f)** with the active site of 1KZN.

Compound	Interaction (Protein-Ligand)	Interaction Energy (kJ/mol)	Interaction Distance(Å)	Hybridization (Protein Atom)	Hybridization (Ligand Atom)
3a	Gly77(O)---N(26)	-0.402	3.16	Sp^2(Acceptor)	Sp^2(Donor)
	Arg136(NH1)---N(27)	-1.231	3.35	Sp^2(Donor)	Sp^2(Acceptor)
	Gly77(N)---N(27)	-0.080	3.38	Sp^2(Donor)	Sp^2(Acceptor)
3b	Gly77(O)---N(26)	-2.498	2.66	Sp^2(Acceptor)	Sp^2(Donor)
	Gly77(N)---N(27)	-1.026	3.09	Sp^2(Donor)	Sp^2(Acceptor)
3c	Gly77(O)---N(26)	-1.475	2.84	Sp^2(Acceptor)	Sp^2(Donor)
	Gly77(N)---N(27)	-1.069	2.96	Sp^2(Donor)	Sp^2(Acceptor)
3d	Gly77(O)---N(26)	-0.342	3.34	Sp^2(Acceptor)	Sp^2(Donor)
	Gly77(N)---N(27)	-0.069	3.54	Sp^2(Donor)	Sp^2(Acceptor)
3e	Arg136(NH1)---O(37)	-2.5	2.89	Sp^2(Donor)	Sp^3(Acceptor)
	Arg136(NH2)---O(37)	-1.097	3.23	Sp^2(Donor)	Sp^3(Acceptor)
3f	Gly77(N)---N(27)	-0.249	3.35	Sp^2(Donor)	Sp^2(Acceptor)
	Gly77(O)---N(26)	-0.832	3.12	Sp^2(Acceptor)	Sp^2(Donor)
	Asp73(OD1)---N(19)	-0.289	2.66	Sp^3(Acceptor)	Sp^2(Donor)
Streptomycin	Asn46(O)---O(11)	-2.5	2.69	Sp^2(Acceptor)	Sp^2(Both)
	Glu50(N)---O(11)	-0.069	3.55	Sp^2(Donor)	Sp^3(Both)
	Gly77(O)---N(2)	-0.555	3.18	Sp^2(Acceptor)	Sp^2(Donor)
	Asn46(ND2)---O(33)	-2.5	2.77	Sp^2(Donor)	Sp^3(Both)
	Asn46(ND2)---O(14)	-2.5	2.66	Sp^2(Donor)	Sp^3(Acceptor)

	Lle90(O)---O(34)	-1.392	3.32	Sp^2(Acceptor)	Sp^3(Both)
	Val120(N)---O(34)	-0.759	2.87	Sp^2(Donor)	Sp^3(Both)
	Ser121(N)---O(34)	-2.5	2.85	Sp^2(Donor)	Sp^3(Both)
	Ser121(OG)---O(34)	-1.668	3.27	Sp^3(Both)	Sp^3(Both)
	Ala96(N)---O(35)	-1.346	2.94	Sp^2(Donor)	Sp^3(Both)
	Val93(O)---O(35)	-0.793	3.44	Sp^2(Acceptor)	Sp^3(Both)

Table 4.9. Docking score of compounds **(3.6a-f)** with 3SRW.

Ligand	MolDock Score	Rerank Score	Interaction	Internal	HBond	LE1	LE3
3a	-173.371	-73.9621	-186.148	12.7778	-3.49061	-4.68569	-1.99898
3b	-159.917	-100.526	-169.882	9.96486	-1.62162	-4.20834	-2.64543
3c	-153.707	-93.6604	-183.33	29.6229	-2.19789	-4.04492	-2.46475
3d	-157.351	-50.9853	-179.902	22.5502	-1.61141	-4.14082	-1.34172
3e	-156.937	-28.6536	-179.205	22.2676	-3.19522	-4.02403	-0.73471
3f	-161.157	-111.949	-181.687	20.5294	-2.5	-4.24098	-2.94602
Streptomycin	-125.724	-113.962	-183.652	57.9273	-15.6688	-3.14311	-2.84905

Table 4.10. Molecular interaction analysis of compounds **(3.6a-f)** with the active site of 3SRW.

Compound	Interaction (Protein-Ligand)	Interaction Energy (kJ/mol)	Interaction Distance(Å)	Hybridization (Protein Atom)	Hybridization (Ligand Atom)
3a	Ala8(O)---N(26)	-1.393	3.26	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr47(N)---O(18)	-0.670	3.17	Sp^2 (Donor)	Sp^2 (Acceptor)
	Thr47(OG1)---O(18)	-1.201	2.44	Sp^3 (Both)	Sp^2 (Acceptor)
	Gly95(N)---O(18)	-0.366	3.33	Sp^2 (Donor)	Sp^2 (Acceptor)
3b	Thr47(OG1)---O(18)	-0.273	3.28	Sp^3 (Both)	Sp^2 (Acceptor)
	Ser50(OG)---O(18)	-2.5	2.85	Sp^3 (Both)	Sp^2 (Acceptor)
3c	Thr47(OG1)---N(19)	-2.197	3.16	Sp^3 (Both)	Sp^2 (Donor)
3d	Thr122(OG1)---O(18)	-2.5	3.00	Sp^3 (Both)	Sp^2 (Acceptor)
	Thr47(OG1)---N(19)	-1.611	3.25	Sp^3 (Both)	Sp^2 (Donor)
3e	Ala8(O)---N(26)	-2.151	3.17	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr122(OG1)--- O(18)	-1.938	3.01	Sp^3 (Both)	Sp^2 (Acceptor)
3f	Asn19(N)---O(18)	-1.043	3.30	Sp^2 (Donor)	Sp^2 (Acceptor)

	Ser50(OG)---O(18)	-2.5	2.76	Sp^3 (Both)	Sp^2 (Acceptor)
Streptomycin	Thr47(O)---N(39)	-1.340	3.29	Sp^2 (Acceptor)	Sp^2 (Donor)
	Asn19(OD1)---O(20)	-1.769	2.51	Sp^2 (Acceptor)	Sp^3 (Both)
	Asn19(N)---O(22)	-0.441	3.42	Sp^2 (Donor)	Sp^2 (Acceptor)
	Lle15(O)---O(11)	-2.5	2.64	Sp^2 (Acceptor)	Sp^3 (Both)
	Thr97(OG1)---O(35)	-1.283	3.34	Sp^3 (Both)	Sp^3 (Both)
	Lys46(NZ)---O(35)	-1.166	3.37	Sp^3 (Donor)	Sp^3 (Both)
	Phe93(O)---N(2)	-1.095	3.38	Sp^2 (Acceptor)	Sp^2 (Donor)
	Phe93(O)---O(12)	-1.331	3.33	Sp^2 (Acceptor)	Sp^3 (Both)
	Thr47(OG1)---O(12)	-2.5	2.66	Sp^3 (Both)	Sp^3 (Both)
	Thr47(OG1)---O(13)	-0.242	3.55	Sp^3 (Both)	Sp^3 (Acceptor)
	Thr47(OG1)---O(14)	-2.5	2.93	Sp^3 (Both)	Sp^3 (Acceptor)
	Leu98(N)---O(33)	-2.5	2.92	Sp^2 (Donor)	Sp^3 (Both)
	Thr97(N)---O(33)	-0.515	3.29	Sp^2 (Donor)	Sp^3 (Both)
	Thr97(N)---O(24)	-0.526	3.49	Sp^2 (Donor)	Sp^3 (Acceptor)
	Gly95(N)---O(24)	-0.394	3.30	Sp^2 (Donor)	Sp^3 (Acceptor)
	Gly96(N)---O(24)	-0.514	2.69	Sp^2 (Donor)	Sp^3 (Acceptor)

Table 4.11. Docking score of compounds **(3.6a-f)** with 1AI9.

Ligand	**MolDock Score**	**Rerank Score**	**Interaction**	**Internal**	**HBond**	**LE1**	**LE3**
3a	-160.069	-115.946	-208.812	48.7422	-2.6774	-4.3262	-3.13368
3b	-153.085	-119.312	-175.238	22.1533	-2.5	-4.02854	-3.13978
3c	-179.668	-127.828	-205.144	25.4766	-3.69616	-4.7281	-3.36389
3d	-174.102	-134.016	-208.953	34.8501	-2.50859	-4.58164	-3.52674
3e	-148.034	-112.771	-174.822	26.7877	-4.1113	-3.79575	-2.89157
3f	-165.004	-74.1873	-180.004	15.0006	-2.69913	-4.34221	-1.9523
Fluconazole	-125.68	-96.2464	-134.019	8.33914	-13.0705	-5.71273	-4.37483

Table 4.12. Molecular interaction analysis of compounds **(3.6a-f)** with the active site of 1AI9.

Compound	Interaction (Protein-Ligand)	Interaction Energy (kJ/mol)	Interaction Distance(Å)	Hybridization (Protein Atom)	Hybridization (Ligand Atom)
3a	Leu77(O)---N(26)	-0.678	2.49	Sp^2 (Acceptor)	Sp^2 (Donor)
	Arg56(N)---N(27)	-1.594	3.08	Sp^2 (Donor)	Sp^2 (Acceptor)
	Glu116(N)---O(18)	-0.405	3.23	Sp^2 (Donor)	Sp^2 (Acceptor)
3b	Ser61(OG)---N(19)	-2.5	2.88	Sp^3 (Both)	Sp^2 (Donor)
3c	Glu116(OE1)---N(26)	-0.101	3.43	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr58(OG1)---N(19)	-1.094	2.73	Sp^3 (Both)	Sp^2 (Donor)
	Ala115(N)---O(18)	-2.5	2.89	Sp^2 (Donor)	Sp^2 (Acceptor)
3d	Glu116(N)---N(27)	-0.985	3.18	Sp^2 (Donor)	Sp^2 (Acceptor)
	Ile117(N)---N(27)	-1.522	3.28	Sp^2 (Donor)	Sp^2 (Acceptor)
3e	Ile19(O)---N(19)	-1.604	2.97	Sp^2 (Acceptor)	Sp^2 (Donor)
	Asp146(OD1)---N(26)	-2.286	2.73	Sp^3 (Acceptor)	Sp^2 (Donor)
	Thr147(OG1)---N(26)	-0.221	3.00	Sp^3 (Both)	Sp^2 (Donor)
	Thr147(OG1)---N(27)	-2.5	2.72	Sp^3 (Both)	Sp^2 (Acceptor)
3f	Thr58(OG1)---N(27)	-2.5	2.67	Sp^3 (Both)	Sp^2 (Acceptor)
	Arg56(N)---N(27)	-0.222	3.22	Sp^2 (Donor)	Sp^2 (Acceptor)
	Lys57(N)---N(27)	-2.476	3.10	Sp^2 (Donor)	Sp^2 (Acceptor)
Fluconazole	Gly114(N)---N(12)	-0.043	3.57	Sp^2 (Donor)	Sp^2 (Acceptor)
	Ile117(N)---N(12)	-1.318	3.34	Sp^2 (Donor)	Sp^2 (Acceptor)
	Glu116(N)---N(12)	-1.431	2.79	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg56(N)---O(3)	-1.557	2.82	Sp^2 (Donor)	Sp^3 (Both)
	Leu77(O)---O(3)	-1.427	3.31	Sp^2 (Acceptor)	Sp^3 (Both)
	Arg79(NH1)---N(7)	-2.450	3.11	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg79(NH2)---N(7)	-2.369	2.99	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg56(NH2)---N(5)	-0.592	2.98	Sp^2 (Donor)	Sp^2 (Acceptor)
	Lys57(NZ)---N(5)	-2.303	3.14	Sp^3 (Donor)	Sp^2 (Acceptor)
	Lys57(NZ)---N(4)	-2.5	2.96	Sp^3 (Donor)	Sp^2 (Acceptor)

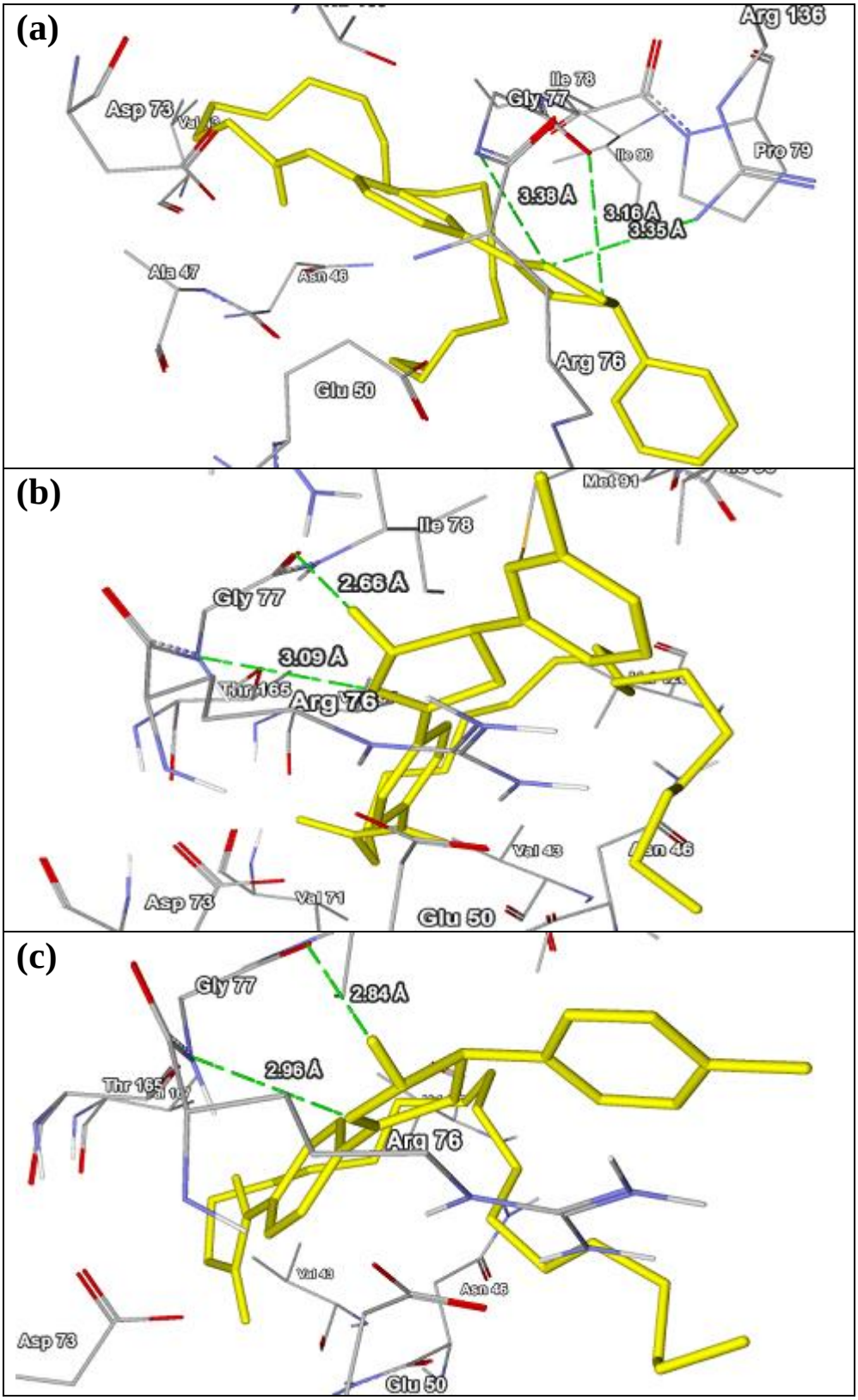
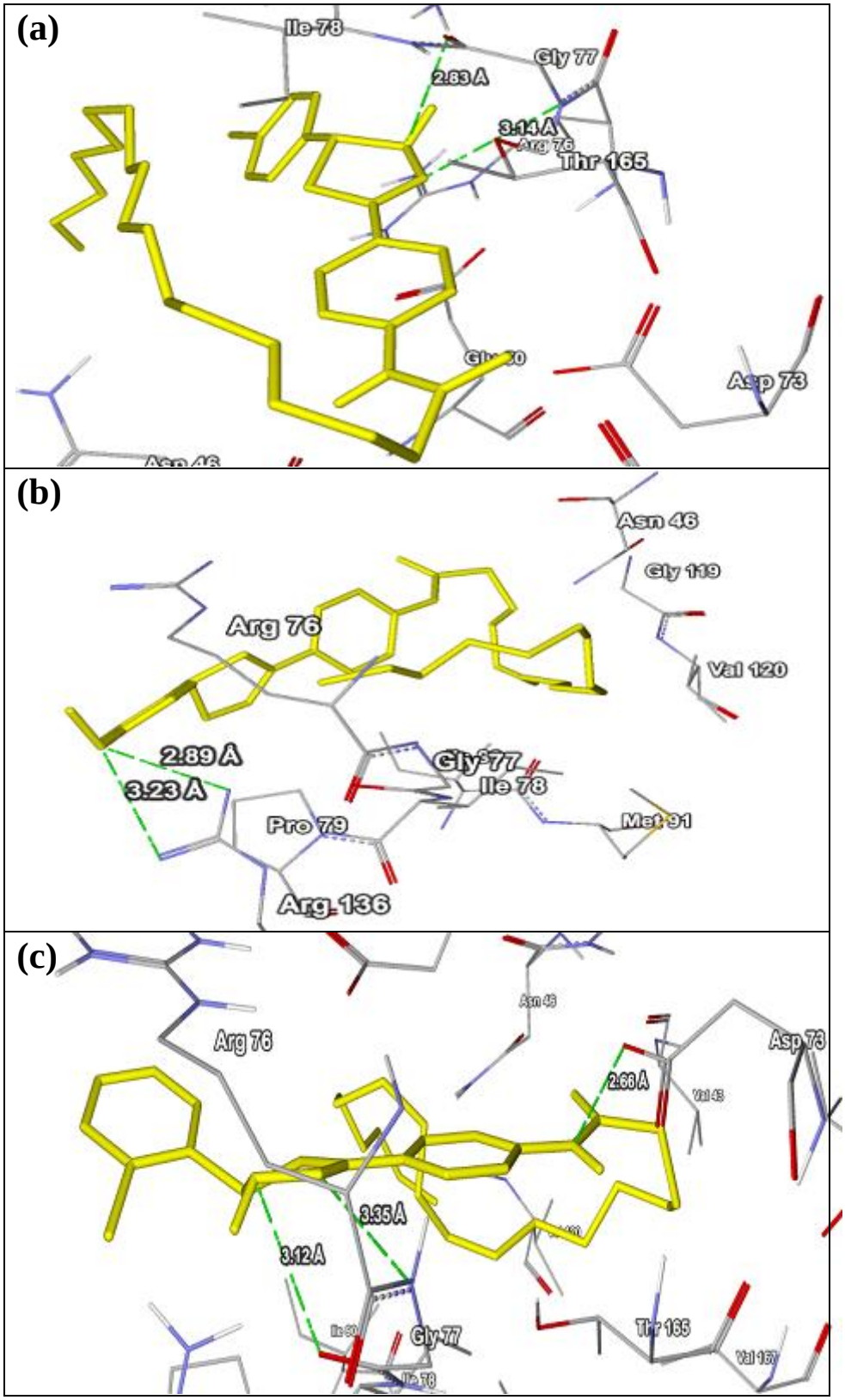


Figure 4.7. Molecular interaction of the compounds **3.6a(a)**, **3.6b(b)** and **3.6c(c)** at the active site pocket of the protein 1KZN.


(a)
Ile 78
Gly 77
2.83 Å
3.14 Å
Arg 76
Thr 165
Asp 73
(b)
Asn 46
Gly 119
Arg 76
Val 120
2.89 Å
3.23 Å
Gly 77
Ile 78
Pro 79
Met 91
Arg 136
(c)
Arg 76
Asp 73
2.66 Å
3.35 Å
3.12 Å
Gly 77
Thr 165

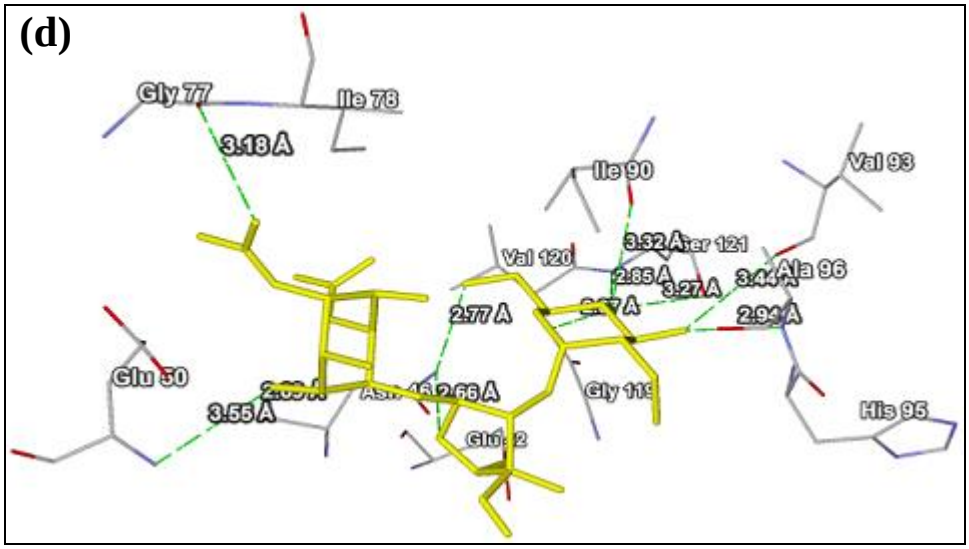
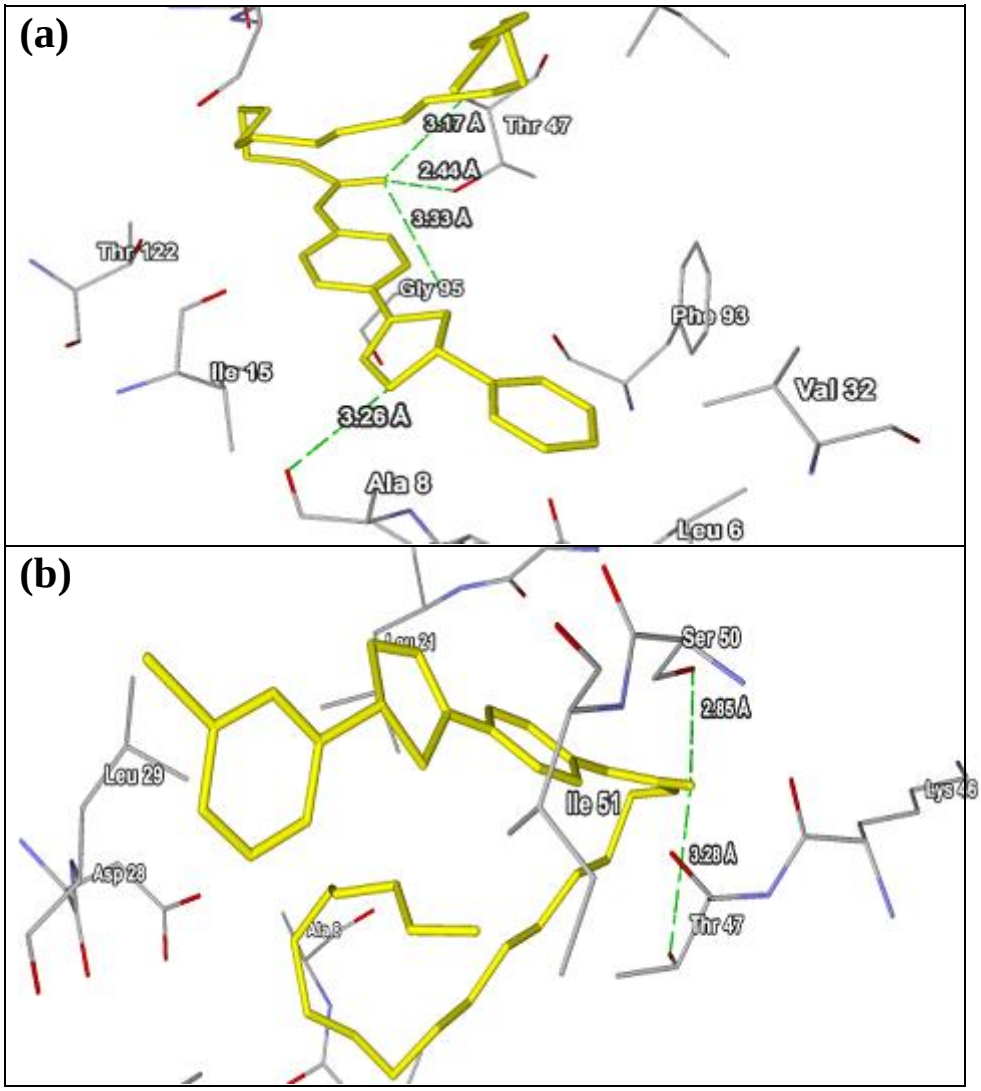


Figure 4.8. Molecular interaction of the compounds **3.6d(a)**, **3.6e(b)**, **3.6f(c)** and streptomycin**(d)** at the active site pocket of the protein 1KZN.



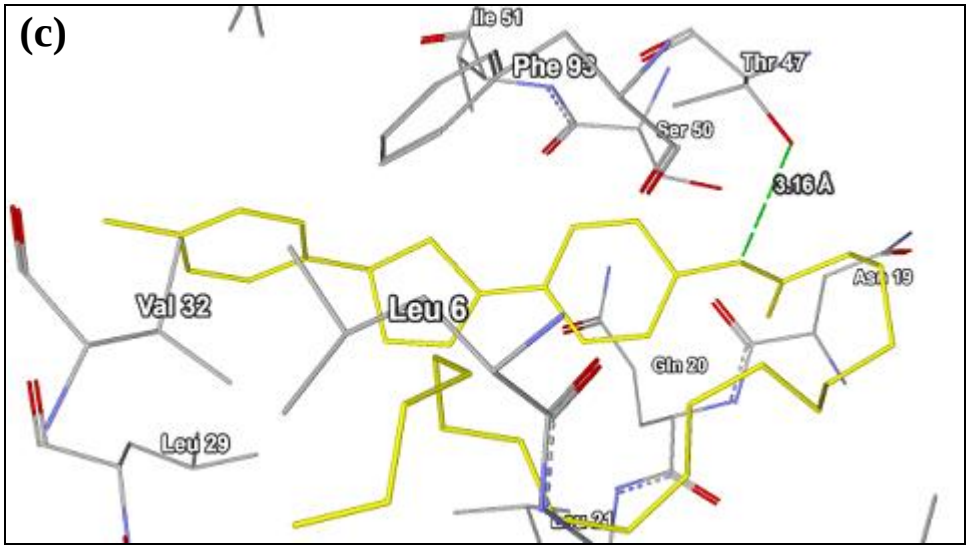
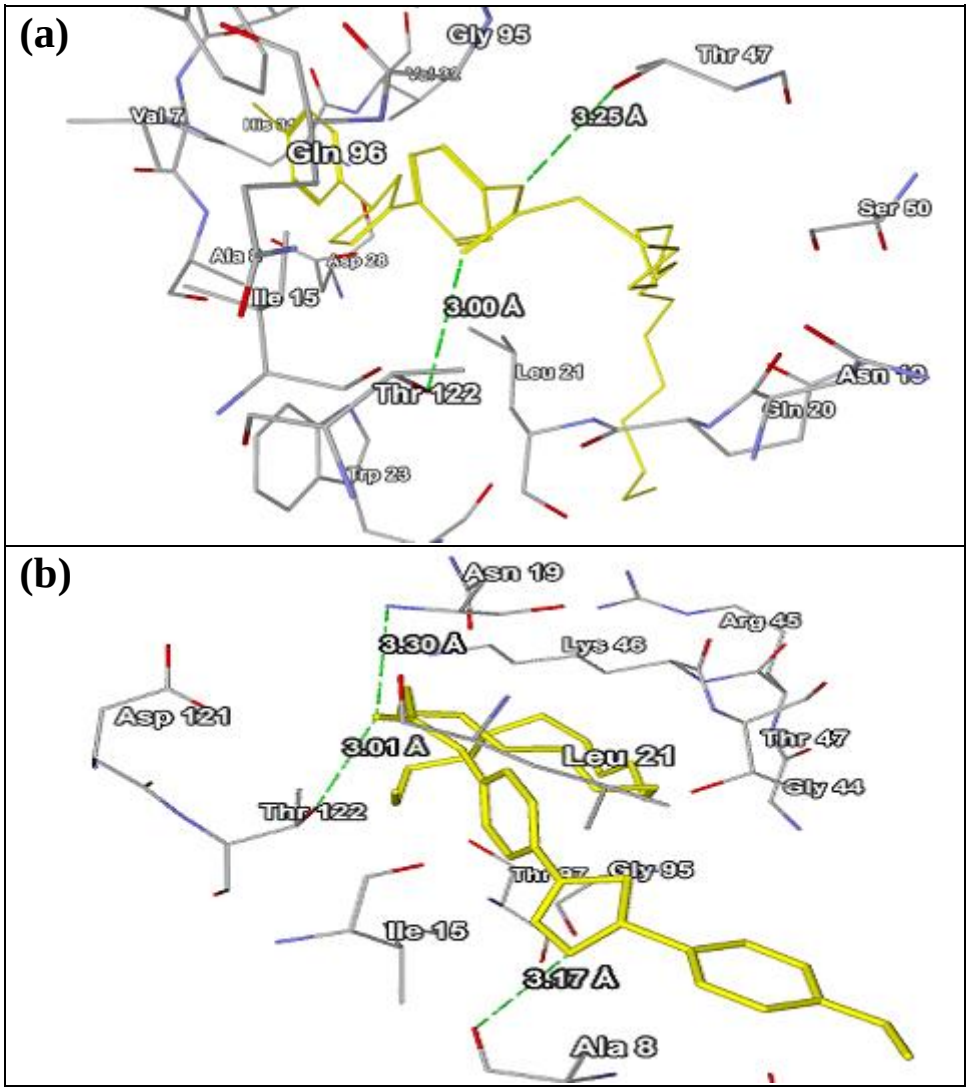


Figure 4.9. Molecular interaction of the compounds **3.6a(a)**, **3.6b(b)** and **3.6c(c)** at the active site pocket of the protein 3SRW.



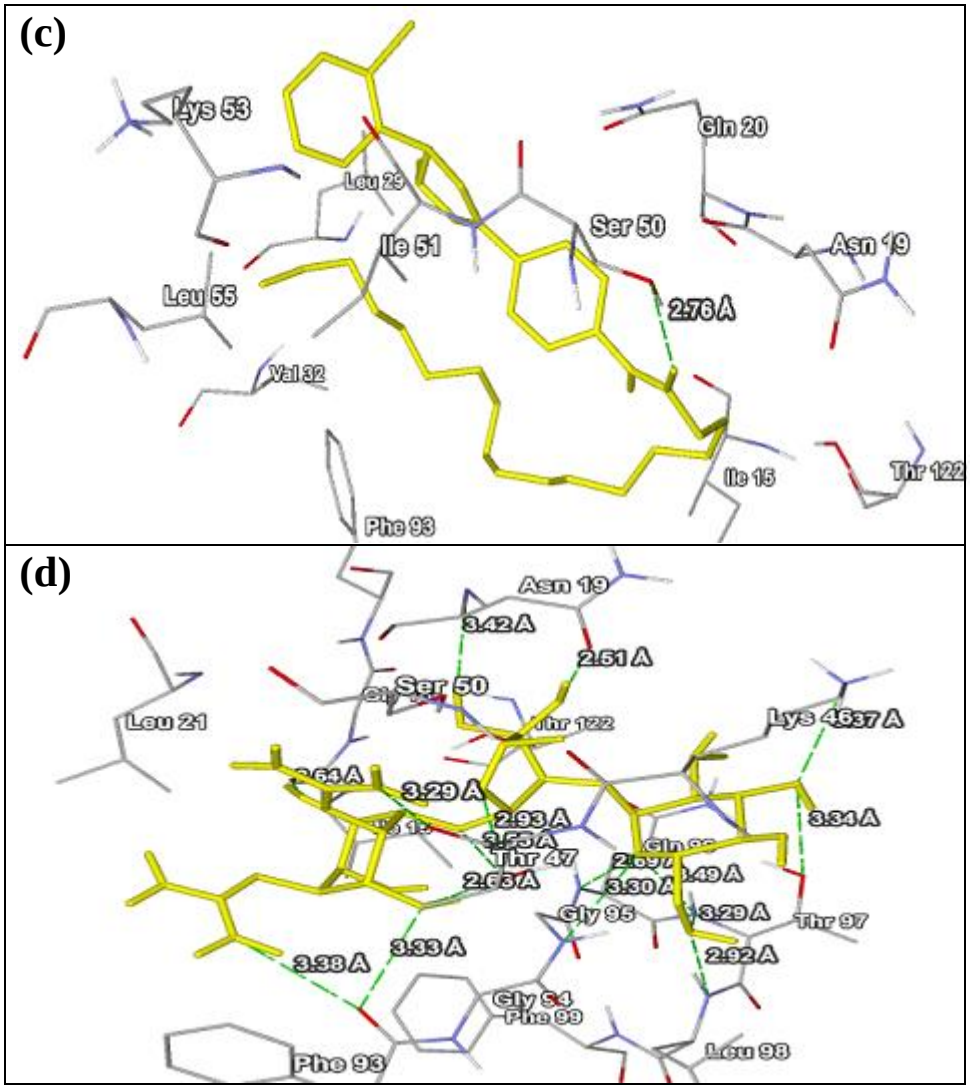
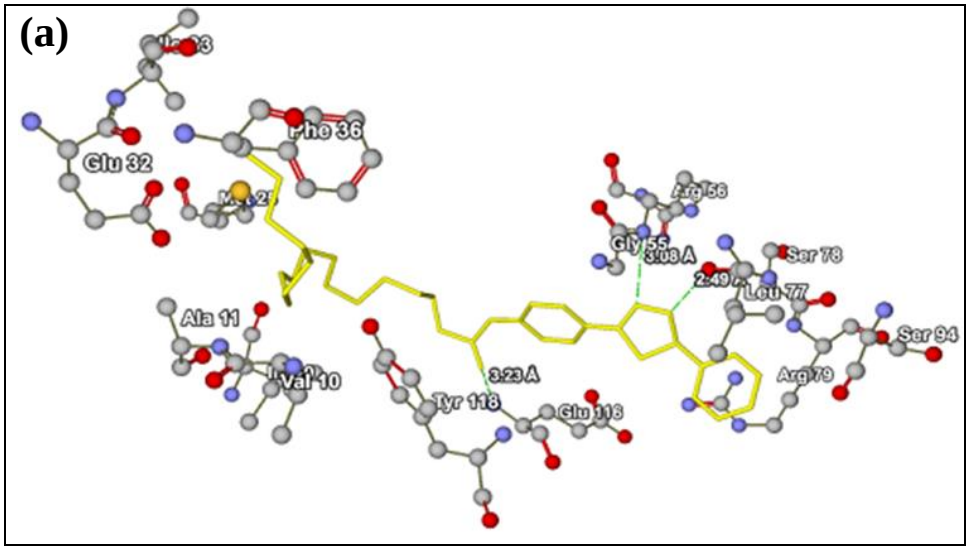


Figure 4.10. Molecular interaction of the compounds **3.6d(a), 3.6e(b), 3.6f(c)** and streptomycin**(d)** at the active site pocket of the protein 3SRW.



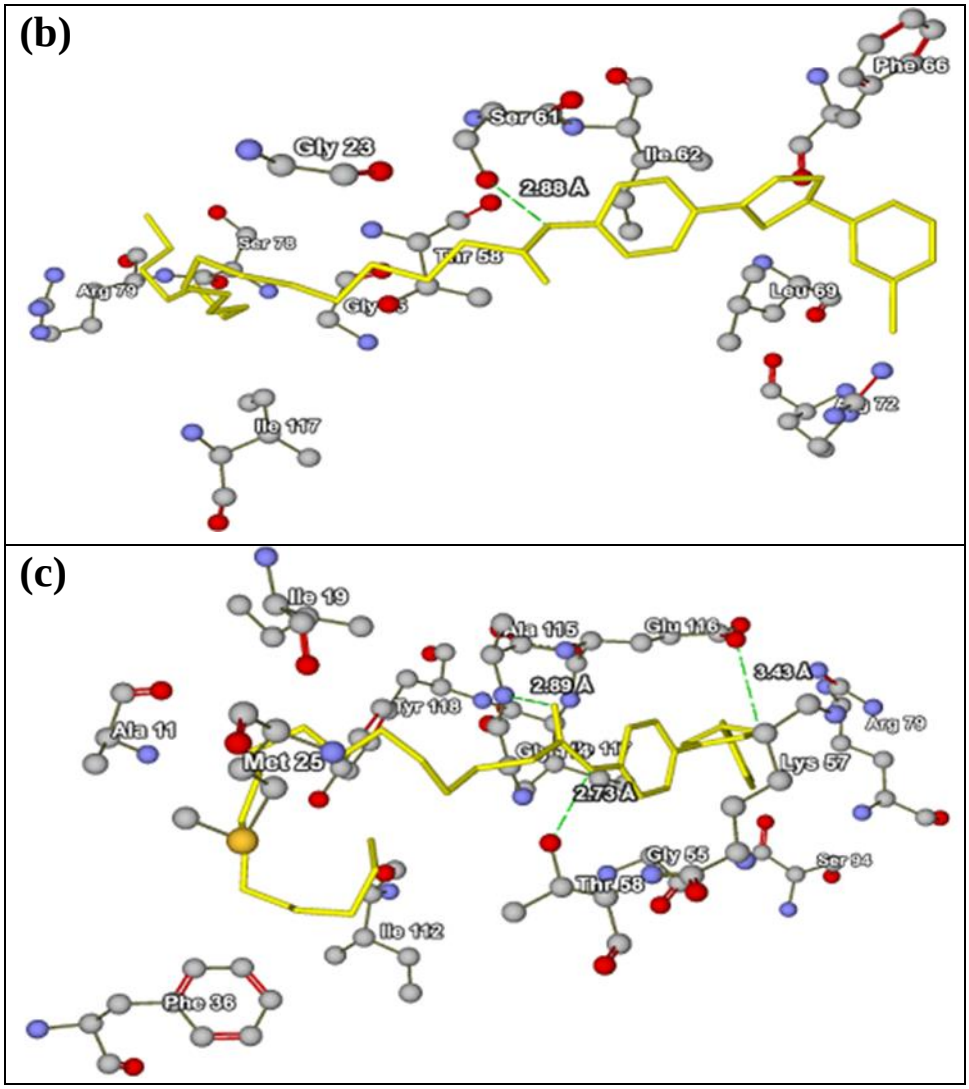
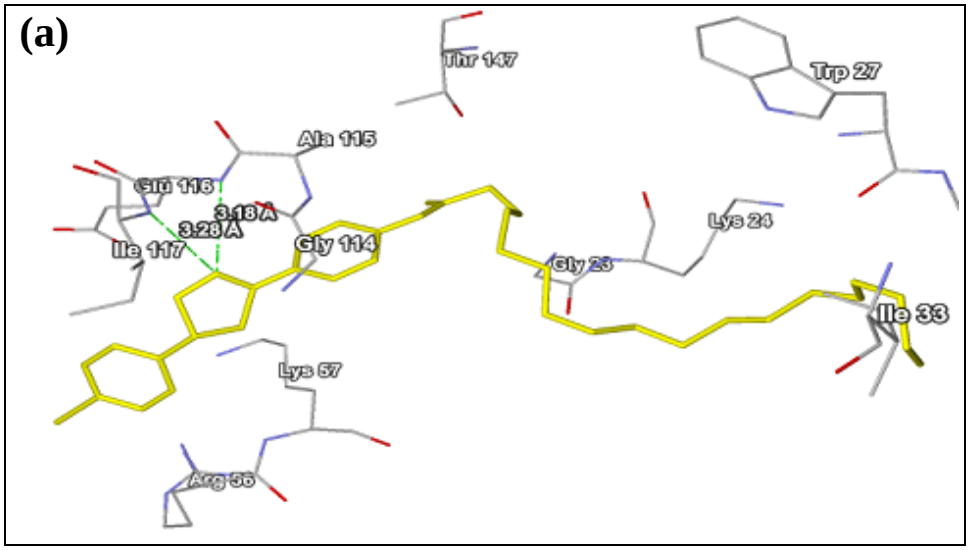


Figure 4.11. Molecular interaction of the compounds **3.6a(a)**, **3.6b(b)** and **3.6c(c)** at the active site pocket of the protein 1AI9.



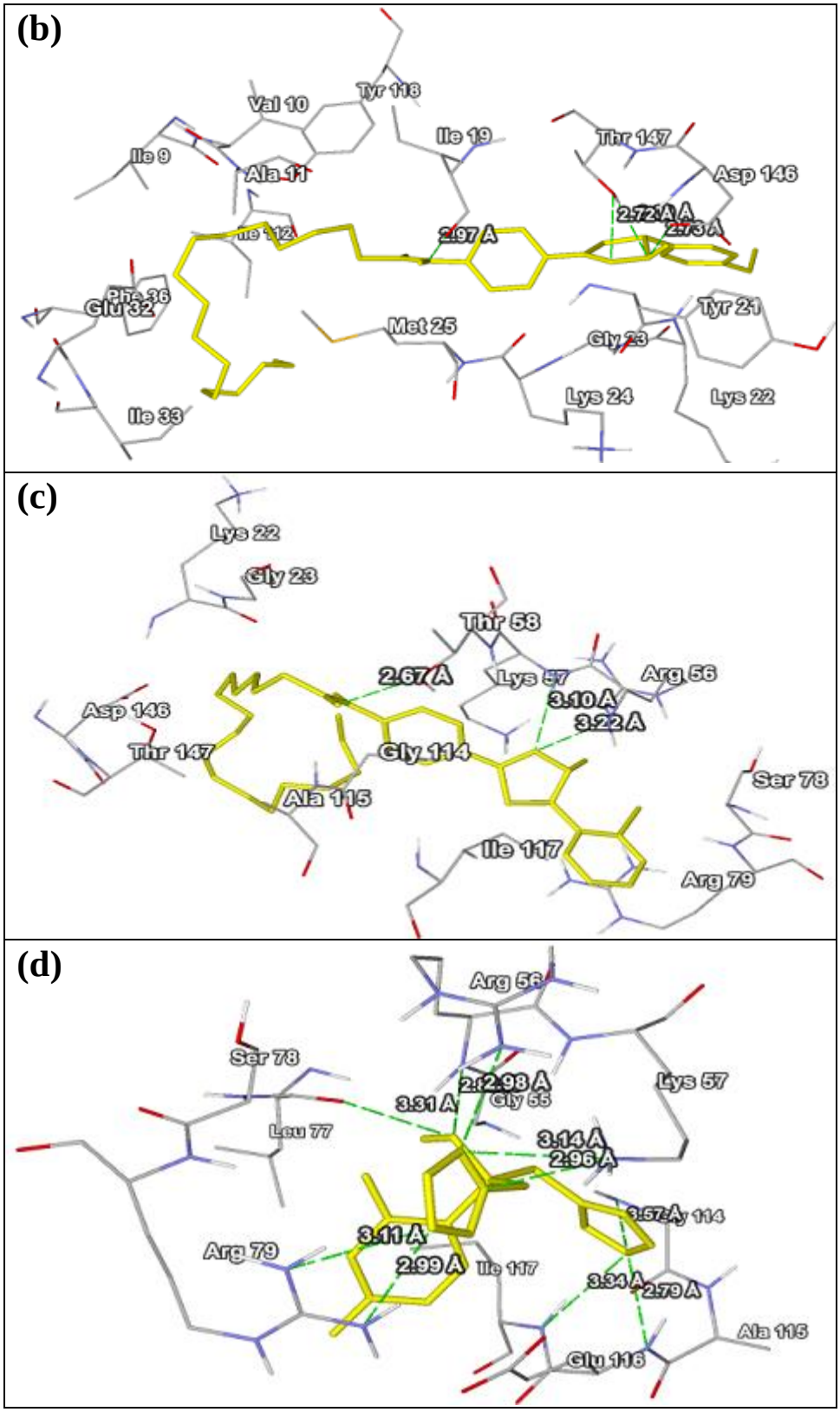


Figure 4.12. Molecular interaction of the compounds **3.6d(a)**, **3.6e(b)**, **3.6f(c)** and fluconazole**(d)** at the active site pocket of the protein 1AI9.

4.4 CONCLUSION

In summary, the molecular docking study provided an insight into the interaction mechanism between the target microbial proteins and the synthesized compounds. The ligand-protein interaction energy values indicated the favorable binding mode of the proteins with the synthesized compounds. Besides, the docking studies revealed the common interaction of the compounds with the standard drugs at the active sites of both the bacterial and fungal proteins. Thus, in-silico driven studies help in understanding the antimicrobial mechanism of the compounds, which would guide in drug-design with potent activities against several pathogens.

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Chapter 5

Summary and conclusions

This chapter presents the overall summary and conclusions of the thesis. The future scope of the work is also highlighted in this chapter.

A series of octadecanamide chalcone and octadecanamide pyrazoline derivatives (**2.6a-3** and **3.6a-f**) were successfully synthesized and tested for their efficacy as an antibacterial and antifungal agent. Besides, molecular docking studies were also conducted to further elucidate the interaction of the synthesized compounds with the bacterial and fungal proteins. A summary of overall thesis work is presented below:

1. A series of novel octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives (**2.6a-e**) were designed and synthesized under base-catalyzed Claisen-Schmidt condensation. The synthesized compounds were characterized using spectroscopic techniques such as Nuclear Magnetic Resonance (1H-NMR and 13C-NMR), Infrared spectroscopy (IR) and Mass Spectroscopy (MS). All the novel synthesized compounds were tested for their *in vitro* anti-microbial activity against two bacterial strains *Escherichia coli* and *Staphylococcus aureus* as well as against two fungal strains *Penicillium italicum and Fusarium oxysporum*. The antimicrobial studies revealed the promising antibacterial activity of compounds **2.6a**, **2.6b**, **2.6c** and **2.6d** against both the bacterial strains, while antifungal studies indicated the antifungal activity of compounds **2.6c** and **2.6d** against both the fungal strains. Thus, this study reveals that novel octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives could be used as a potential candidate to combat various microbial pathogens.

2. Another series of novel octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H -pyrazol-3-yl) phenyl] -amide pyrazoline derivatives (**3.6a-f**) were designed, synthesized and characterized by spectral analysis (IR, MS, 1H-NMR, 13C-NMR). The compounds were screened for in vitro antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. All the newly synthesized compounds (**3.6a-f**) exhibited appreciable antibacterial activity with minimum inhibitory concentrations ranging from 1.25mg/mL to 2.5mg/mL. Besides, the newly synthesized compounds were also found to have good antifungal activity against *Fusarium oxysporum* and *Penicillium italicum* with all the compounds showing MIC of 2.5mg/mL for both the fungal strains. Henceforth, these results may facilitate a promising approach to design newer and novel antimicrobial agents based on the potent compound by structural

modifications of these series of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H -pyrazol-3-yl) phenyl] -amide pyrazoline derivatives.

3. Molecular docking studies were performed to understand the possible interaction between the receptor protein of the microbial pathogens and the synthesized compounds. The studies demonstrated the binding mode of the synthesized compounds (ligand) with the three target protein structures of enzymes dihydrofolate reductase (Pdb id: 3SRW) as gram-positive bacteria target, DNA Gyrase B (Pdb id: 1KZN) as gram-negative bacteria target and dihydrofolate reductase (Pdb id: 1AI9) as fungi target. The results indicated the favorable ligand (**2.6a-e**)-protein interaction energy values of -2.5 to -0.064 kJ/mol, -2.5 to -0.148 kJ/mol and -2.5 to -0.079 kJ/mol at the binding cavity of 1KZN, 3SRW and 1AI9 respectively. A common molecular interaction of the compounds and streptomycin was observed with the Asn46, Arg76, Thr165 for enzymes DNA Gyrase B (Pdb id: 1KZN), Thr122 and Thr47 for enzymes dihydrofolate reductase (Pdb id: 3SRW), and also both compounds and fluconazole revealed a common molecular interaction with Lys57, Arg56, Arg79, Ser78 for enzymes dihydrofolate reductase (Pdb id: 1AI9)**.** Also, the compound (**3.6a-f**) possessed favorable ligand-protein interaction energy values of -2.5 to -0.069 kJ/mol, -2.5 to -0.242 kJ/mol and -2.5 to -0.043 kJ/mol at the binding cavity of 1KZN, 3SRW and 1AI9 respectively. A common molecular interaction of the compounds and streptomycin was observed with the Gly77 for enzymes DNA Gyrase B (Pdb id: 1KZN), Gly95, Thr47 and Asn19 for enzymes dihydrofolate reductase (Pdb id: 3SRW), and also both compounds and fluconazole revealed a common molecular interaction with Leu77, Arg56, Glu116, Lys57 and Ile117 for enzymes dihydrofolate reductase (Pdb id: 1AI9).

Future scope of the work

- The synthesized octadecanamide chalcone and octadecanamide pyrazoline derivatives can be tested for more biological activities such as antioxidants, antitumor, anticancer and anti-inflammatory activities etc.
- In-vivo studies of the synthesized compounds can be carried out to determine the overall efficacy of the synthesized compounds, which would lead to the development of newer drugs against resistant pathogens.
- Diverse natural products-based organic scaffolds can be explored towards the development of high potential drugs with efficient biological activities.

- Newer, simpler and greener synthetic routes can be designed and developed for the synthesis of Natural Products-based Organic Scaffolds.
- The integration of molecular modification strategies and computational approaches can be explored to accelerate the discovery of new chalcone and pyrazoline derivatives.

Appendix 1

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Name & Institutional Address of the Supervisor	Dr. Maddela Prabhakar Department of Chemistry, Nagaland University, Lumami
Name of the Department and School	Department of Chemistry, School of Sciences
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Appendix 2

List of Conferences/Seminars/Webinars/Workshops Attended

- National e-Seminar on "Chemistry in emerging trends of interdisciplinary research (NeSCETIR-2020)" organized by department of Chemistry, Nagaland University, Lumami from 18th-20th November, 2020. **(Participated)**
- One-day Workshop on "Importance of IPR in Academic Institutions" organized by IPR Cell, Nagaland University held on 29th May, 2 019. **(Participated)**
- National Seminar on "Chemistry in interdisciplinary research" (NSCIR-2018) from 9th-10th November, 2018 held at Nagaland University, Lumami. **(Oral Presentation)**
- National Seminar on "Chemistry in interdisciplinary research" (NSCIR-2017) from 16th-17th March, 2017 held at Nagaland University, Lumami. **(Oral Presentation)**
- Workshop on "Liquid Chromatography-Mass Spectrometry and its Applications" organized by at Sophisticated Analytical Instrument Facility (SAIF), North Eastern Hill University (NEHU), Shillong and sponsored by the Department of Science & Technology, Government of India held from 3rd to 5th April, 2018. **(Participated)**
- Training visit at UGC Networking Resource centre, School of chemistry, University of Hyderabad from Aug 2016-Sept 2016 and Jan 2017- Feb 2017. **(Participated)**
- Science Exhibition on "Water Literacy and Innovation in Water Purification & Conservation "organized by Department of Chemistry, Nagaland University catalyzed and supported by National Council for Science and Technology Communication, DST, New Delhi Under "ECO & WaSH Futures" held on 11th November, 2016. **(Participated)**
- "Innovation Exhibition" organized by Nagaland University Innovation Cell in collaboration with Department of Chemistry, Nagaland University & National Innovation Foundation-India held on 17th November,2015. **(Participated)**

Appendix 3

List of publications

Maddela Prabhakar, Thechano Merry, Ruokuosenuo Zatsu, **Shurhovolie Tsurho** and Ramchander Merugu; Microwave-Assisted Fast and Efficient Green Synthesis of 9-Anthracenyl Chalcones and their Anti-Bacterial Activity; *Journal of Pharmacy*, **2017**, *7(12)*, 24-32.

Maddela Prabhakar, Thechano Merry, **Shurhovolie Tsurho**, Ruokuosenuo Zatsu, Nishant Jain, Aaysha Sataniya and Sreenivas Enaganti; Synthesis, Characterization and Biological Evaluation of 9-Anthracenyl Chalcones as Anti-Cancer Agents; *J. Chem. Pharm. Res.*, **2017**, 9(6),185-192.

Shurhovolie Tsurho, Maddela Prabhakar; A Facile Synthesis of Octadecanoic acid [4-(3-phenyl-acrolyl)-phenyl]-amides and their Antimicrobial Activity; *Research Journal of Pharmacy and Technology*., **2022**, *15(8)*. (*Accepted*)

Shurhovolie Tsurho, Maddela Prabhakar; Synthesis, molecular docking and antimicrobial evaluation of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-phenyl]-amide pyrazoline derivatives; *International Journal of Pharmaceutical Sciences and Drug Research*. (*Accepted*)