An-Najah National University
Faculty of Graduate Studies

DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF PYRAZOLE-CARBOXAMIDE DERIVATIVES AS COX INHIBITORS

By
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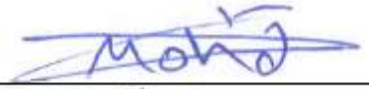
Dr. Mohammed Hawash	
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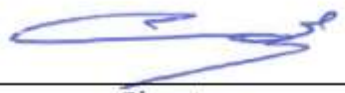
Dr. Mohyeddin Assali	
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Dedication

This Thesis is the result of countless and toughest sacrifices. This work is wholehearted and gratefully dedicated.

This study is dedicated to our beloved parents, who have been our source of inspiration, guidance, and strength when we thought of giving up, they continually provide their moral, spiritual, emotional, and financial support.

To our friends who became our supporters and helped us with any problems we faced.

To my supervisor and doctors at An-Najah University who believed that we would finish this research in time, helping us to improve our research and inspiring us and their inspirational stories when they are.

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Finally, I am grateful to my family for their constant support, patience, and understanding during the course of this research. Their encouragement and belief in me were vital in helping me complete this thesis.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF PYRAZOLECARBOXAMIDE DERIVATIVES AS COX INHIBITORS

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name Nisreen Najeh Shweiki

Signature: 

Date: 03/06/2025

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DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF PYRAZOLE-CARBOXAMIDE DERIVATIVES AS COX INHIBITORS

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most utilized line of drugs as a factor for reducing swelling, uncomfortable feel, and fever. They act as competitive inhibitors of the cyclooxygenase enzyme (COX). Here, in this thesis, eight novel pyrazole-carboxamide derivatives were produced, specified, and increased for their selectivity and potency at COX-1 and COX-2 by an *in vitro* COX inhibition assay kit. MTS assay was used to estimate the cytotoxicity of these compounds against human normal cell lines (Hek293T) and hepatic cell lines (LX-2). All recently synthesized compounds were identified utilizing FTIR, HRMS, ^{1}H-NMR, and ^{13}C-NMR techniques. HRMS results were rational and all derivatives were verified and masses corresponding to the calculated masses. NMR results indicate a successful synthesis and were found to be consistent with the research outputs. Based on the results, it was determined that **3b**, with an IC_{50} value of 0.46±0.25 µM, was the most potent inhibitory agent against COX-1 enzyme. Additionally, it showed a potent COX-2 inhibitory effect with an IC_{50} value of 3.82 ±1.36 µM. On the other hand, the highest selectivity against COX-2 ratio (1.68) was for compound 3g, with the highest effectiveness against COX-2 also (IC_{50} = 2.65±1.55 µM) in comparison to ketoprofen selectivity ratio was (0.21) ,and it's IC_{50} against COX-2 = 0.164±0.12 µM. The 3d compound demonstrated strong COX-2 selectivity as well. (1.14) with an IC_{50} of 4.92±2.29 µM. Negligible cytotoxic activities against the utilized normal cell lines while 3a showed cytotoxicity against CaCo2, MCF-7, and HepG2 cancer cell lines. Finally, we recommend doing docking studies of the synthesized compounds for better understanding of the structure-activity relationship and improve COX2 selectivity.

Keywords: Cyclo-oxygenase-enzyme; Anti-inflammatory; NSAIDs; COX inhibitors; Heterocyclic; Pyrazole-carboxamide.

Chapter One

Introduction

1.1 Introduction

A minimum of 2,500 years ago, Hippocrates suggested using an extract of willow barb to treat fever and ease the agony of childbirth. This led to the first prescriptions for items with analgesic, anti-inflammatory, and antipyretic effects. After salicin was found to be the plant's active component, salicylic acid was produced in large quantities in 1860. Later, acetylsalicylic acid, a more tolerable version of the medication, was created and continues to be utilized today (Burton, 2006). The history of nonsteroidal anti-inflammatory medications (NSAIDs) is lengthy, and this is only the beginning. In summary, Vane identified in 1971 the mechanism of action of these medications: the suppression of prostaglandin formation through the cyclooxygenase mechanism (Simmons, Botting, & Hla, 2004).

About 20 years later, cyclooxygenase (COX) was found to have a novel isoform termed COX-2, whose expression is stimulated through inflammatory mediators in different tissues. While this was going on, a sizable number of NSAIDs with varying makeups of chemicals were brought to market; nonetheless, it wasn't long before the gastrointestinal system toxicity of these medications was recognized as one of their main side effects. Due to the rationale that COX-2 selective inhibition would preserve the therapeutic effects of these medications while preventing gastrointestinal damage, the identification of COX-2 in this context prompted the rapid creation of COX-2 selective inhibitors or coxib (Pereira-Leite, Nunes, & Reis, 2013). Some coxibs were taken off the market as a result of the quick emergence of cardiovascular side effects linked to these selective inhibitors. This also prompted concerns about the non-selective NSAIDs safety in the cardiovascular system. Due to growing concerns about NSAID toxicity, the use of these medications has decreased globally. This has prompted additional research into the pharmacological activities of NSAIDs to find safer and more effective medications to treat inflammatory disorders (Lichtenberger, et al., 1995). The idea that surface-active phospholipids served as a cytoprotective mechanism on the stomach mucosa initially came forward in the 1980s. The same study briefly discussed the negative impact of aspirin on the hydrophobicity of the stomach mucosa's surface, which they connected to

the medication's interaction with the hydrophobic or non-wettable phospholipid lining (Hills, 1996).

The evaluation of the NSAID-phospholipid interaction started with this and has been ongoing since the 1990s. With major contributions from several labs worldwide, the scientific field has witnessed an increase in the in vitro assessment of NSAID membrane interaction in the 2000s (Conaghan, 2012). Nonsteroidal anti-inflammatory drugs (NSAIDs) are a heterogenous class of pharmaceuticals that are widely used to treat several diseases and reduce pain, like menstrual pain, migraine, arthritis, rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis, as well as inflammatory conditions. According to in vitro evaluations using cell cultures, membrane mimetic models, and molecular dynamic modeling systems, some of these effects appear to be connected to the capability of NSAIDs to cross biological membranes. In this area, those interfere with regular signaling events and modify vital processes, such as cell adhesion, necessary for cellular function (Pereira-Leite, Nunes, & Reis, 2013).

NSAIDs are categorized based on various factors, such as their chemical characteristics and COX selectivity. These medications fall into many chemical classes. But generally speaking, those are weak organic acids with hydrophobic characteristics, which help them approach COX, a monotopic membrane protein, and penetrate inflammatory tissues with a lower pH because the cyclooxygenase active site is situated at the end of a hydrophobic channel (Perrone, Vitale, Panella, Fortuna, & Scilimati, 2015). Moreover, these medications are categorized as non-selective NSAIDs and COX-2 selective NSAIDs based on the COX-1/COX-2 inhibitory potency ratio (Kolb, Finn, & Sharpless, 2001).

Examples of FDA-Approved NSAIDs:

Table 1.1

Selective and non-selective NSAID examples

Non-Selective NSAID	COX-2 selective	COX-1 selective
Naproxen	celecoxib	Aspirin
Ketoprofen	valdecoxib	TFAP
Fenoprofen	rofecoxib	Mofezolac
Diflunisal	meloxicam	
Ibuprofen		
Oxaprozin		

Note: (Wright, 2002).

1.2 Heterocylic

Heterocyclic substances are unique organic cyclic biomolecules that include atoms of at least two different elements as members of their ring(s). Nitrogen and oxygen are the most common heteroatoms, with sulfur in a lower percentage. Heterocyclic compounds are considered a bridge between chemistry and other sciences, including the medical one. It has the largest and most diverse family of organic compounds. Additionally, those play a vital role in metabolism because they are naturally distributed in many natural products that are isolated from animal and plant tissues, including vitamins, alkaloids, and hormones, as well as dyes, pharmaceuticals, and several others. Moreover, heterocyclic compounds are easily synthesized and have enhanced (Ansari, Ali, & Asif, 2017) bioavailability and solubility. Pharmacological activities enhanced by them, like anti-inflammatory, analgesic, enzyme inhibitors, antifungal, antibacterial, herbicidal, anti-HIV, antioxidant, and anticancer activities (Hawash, et al., 2020). The antifungal drugs influenced the fungal cells which mostly exist on skin, nails, and hair by lowering their reproduction and development (Al-Mulla, 2017). So this nucleus is used as a major component in numerous anti-inflammatory compounds like lonazolac, omeprazole, and celecoxib, as well as other pharmaceutically active compounds with anti-obesity (Rimonabant), H2-receptor agonists (Betazole), and anti-depressant (Fezolamine). Regarding the synthetic importance of pyrazole derivatives, they have diverse therapeutic effects, such as anti-cancer, anti-bacterial, anti-viral, and anti-fungal (Ansari, Ali, & Asif, 2017). Various methods were used in earlier research to synthesize selective COX-2 inhibitors, but they have high production costs and render them unaffordable for the average individual. Therefore, novel COX-2 inhibitors that are safe, affordable, selective, and efficacious are needed.. Pyrazole heterocycles have been utilized to create novel molecules such as selective COX-1 inhibitors (Petrou, Fesatidou, & Geronikaki, 2021). They have also demonstrated intriguing biological action, particularly in the area of anti-inflammatory behavior. These could also be made quickly and easily in a one-step reaction with a high yield (Kolb, Finn, & Sharpless, 2001; Petrou, Fesatidou, & Geronikaki, 2021).

1.3 Pyrazole

An essential tri-substituted planar heterocyclic ring with five members that is joined to lipophilic substituted phenyl rings is a feature shared by all selective COX-2 inhibitors. Because it is regarded as a fundamentally helpful structure in heterocyclic and medicinal chemistry, so we decided to make the pyrazole ring the focal point of our innovative molecules. Along with COX-2 inhibitory activity, it also plays a significant role in various other bioactivities, most notably analgesic and anti-inflammatory actions. Several selective COX-2 inhibitors, including celecoxib and SC-558, contain the pyrazole nucleus as one of its central components (Magda, Abdel-Aziz, Alaa, El-Azab, & ElTahir, 2012). Research is being done on all of these pyrazoles as COX inhibitors, anti-viral, antibacterial, anti-inflammatory agents, and potential treatments for diseases like cancer (Priya, et al., 2022; Faria, et al., 2017).

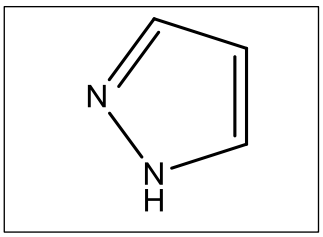
A pyrazole ring is present in several drugs. The standard medications that contain the pyrazole ring include fezolamide (antidepressant), rimonabant (anti-obesity), difenamizole (analgesic), celecoxib and lonazolac (anti-inflammatory), rimonabant (cytoprotective), and pyrazofurin (anticancer), and the other as shown in (figure 1.7), All of these medications offer plenty of opportunities for ongoing research and analysis of novel analogs.

1.4 The Major Strategies to Obtain the Pyrazole Nucleus

Pyrazole has too much π and is an aromatic heterocycle. Positions 3 and 5 are where nucleophilic attacks take place, whereas position 4 is usually where electrophilic replacement reactions happen.

Figure 1.1

Pyrazole nucleus



The pyrazoles are interesting because they have a variety of biological functions and are diversely substituted by heteroaromatic and aromatic groups. Since the initial syntheses, multiple changes have been made to the different pathways leading to the pyrazole nucleus. The following are common techniques for obtaining substituted pyrazoles:

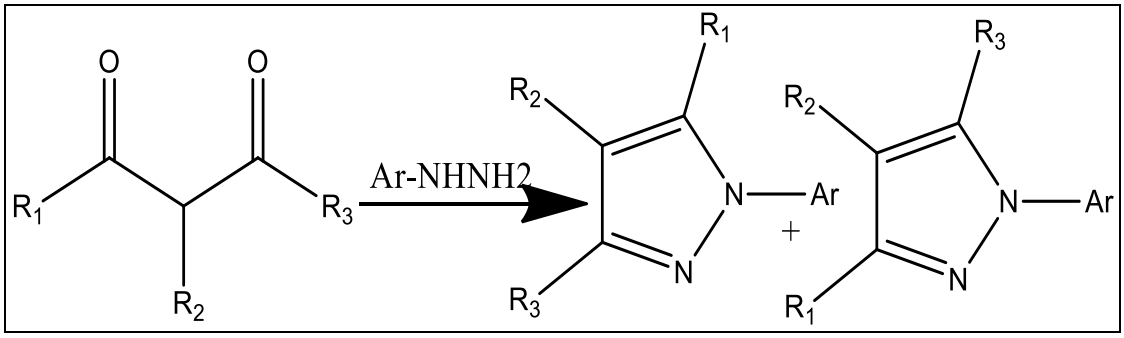
1. Cyclocondensation of hydrazine and its derivatives with the carbonyl systems.
2. Dipolar cycloadditions.
3. Multicomponent reactions (Karrouchi, et al., 2018).

1. Cyclocondensation of the Carbonyl systems with Hydrazine and its Derivatives

A cyclocondensation reaction between a suitable hydrazine acting as a bidentate nucleophile and a carbon unit, such as a 1, 3-dicarbonyl molecule, a 1, 3-dicarbonyl derivative, or α, β-unsaturated ketone, is the most widely used technique for creating substituted pyrazoles. A- From 1, 3-Diketones.

Figure 1.2

Poly-substituted pyrazole synthesis results from 1, 3-dicarbonyl molecules



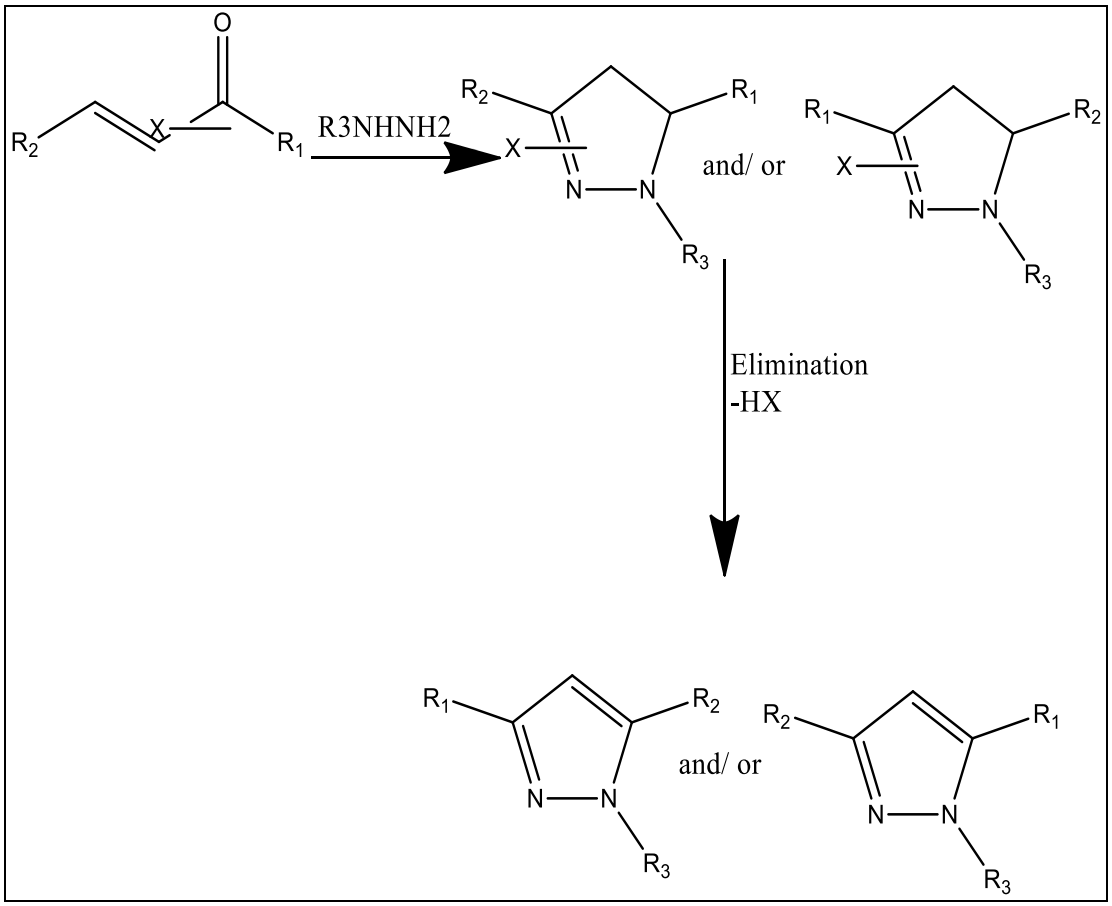
A. From Acetylenic Ketone: For over a century, it has been known that hydrazine derivatives cyclocondensate with acetylenic ketones to create pyrazoles (Ramadan, Aly, El-Haleem, Alshammari, & Bräse, 2021).

B. From Vinyl Ketone: A, β-ethylenic ketone, and a hydrazine derivative react cyclocondensatorily to produce pyrazolines, which oxidize to produce the pyrazole ring.

C. From Vinyl Ketones (Karrouchi, et al., 2018).

Figure 1.3

The cyclo-condensation of α, β-ethylenic ketones has a leaving group, leading to the synthesis of Pyrazoles



2. Dipolar Cycloadditions

Additional techniques for reaching the pyrazole nucleus entail [3 + 2] cycloaddition processes between an alkyne (or an olefin) and 1, 3-dipolar compounds like nitrilimines, sydnones, or diazo compounds.

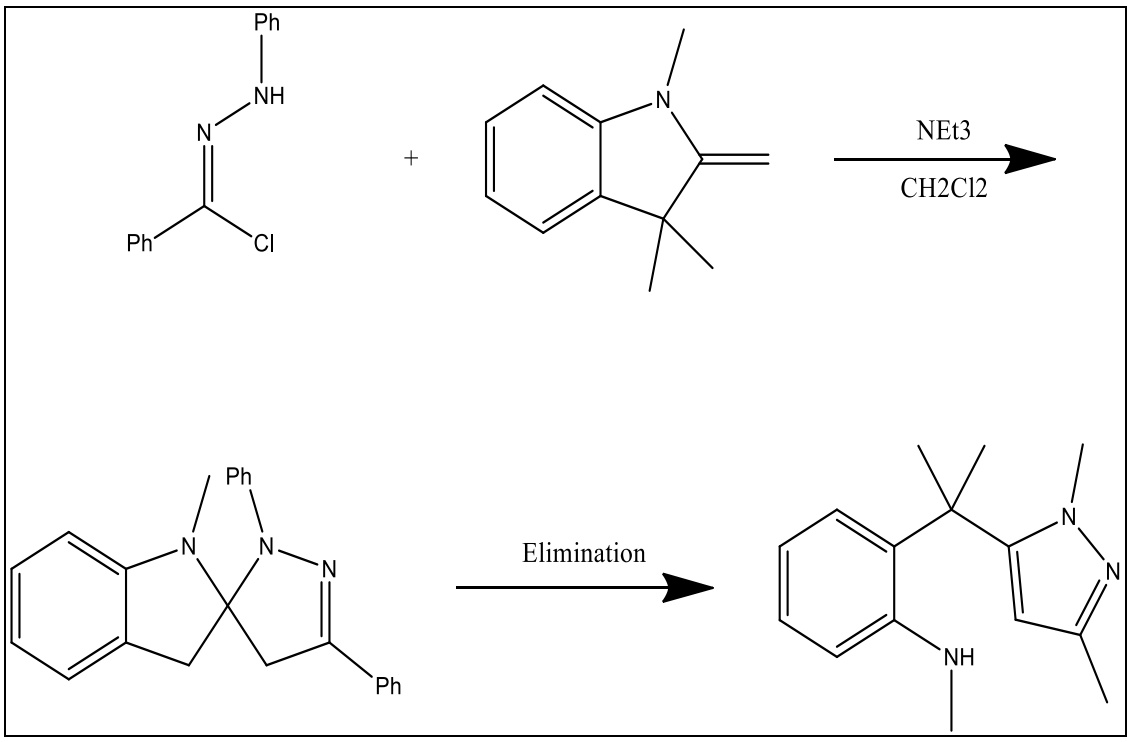
A. Cycloaddition of Diazocarbonyl Components: Using zinc trifolate as a catalyst, researchers studied the interaction between ethyl α-diazoacetate and phenylpropargyl in triethylamine (He, Chen, Niu, Wu, & Liang, 2009).

B. A cycloaddition process of sydnones can yield the pyrazoles. Researchers used a cycloaddition process between a sydnones and an alkyne to synthesize the two Regio isomeric 1, 3, 4, and 5-substituted pyrazole (Delaunay, et al., 2010).

D. C- Nitrilimines. (Dadiboyena, 2009)

Figure 1.4

Pyrazole is produced by 1, 3-dipolar cycloaddition of an alkene and diphenyl nitrilimine

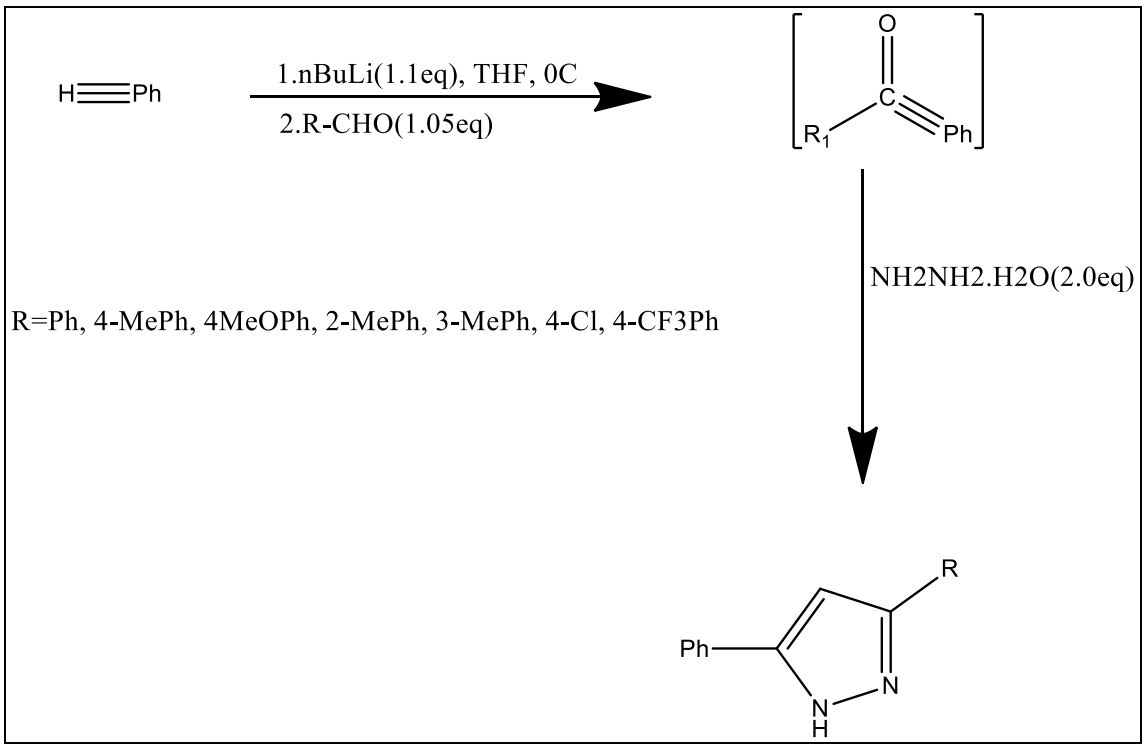


3. Multicomponent Reactions

A- Researchers found that reacting terminal alkynes with aromatic aldehydes, hydrazines, and molecular iodine, they were able to produce 3, 5-substituted pyrazole with good yields (68–99%), with great regioselectivity in just one pot (In Situ Formation of Carbonyl Derivatives) (Harigae, Moriyama, & Togo, 2014).

Figure 1.5

Pyrazole is synthesized using hydrazine and α, β-unsaturated carbonyl



B- In situ β-Aminoenones Form by combining an oxime and an alkynein dimethylformamide, which yields the β-aminoenone 6, they created a unique method for the synthesis of 3, 5-substituted pyrazoles (Kovacs & Novak, 2013).

C- The scientists reported a unique process for the In Situ Formation of a Hydrazone: which involves the one-pot cyclization of hydrazone dianions with diethyl di-oxalate to produce pyrazole-3-carboxylates (Dang, Fischer, Görls, & Langer, 2008).

D- The research team has developed a multi-component procedure for the (in-situ) generation of diazo derivatives: from different aldehydes and tosylhydrazines, which reduces the hazards involved in the isolation of these molecules. These are subsequently utilized to produce equivalent pyrazoles (Aggarwal, de Vicente, & Bonnert, 2003).

1.5 Similar Structures

1.5.1 Imidazole

For the production of pharmaceuticals, medicinal and synthetic chemists find heterocyclic scaffolds like pyrazole and imidazole to be appealing choices (Tabassum, Ekta, & Kavitkumar, 2018). Thus, the imidazole is a planar, heterocyclic ring with five members that includes an in-ring N and two atoms each of C and N. Nucleic acid, purine, histamine, histidine, and other significant natural compounds all include the imidazole ring. This chemical is extremely polar. The chemical is categorized as aromatic because it has six π-electrons, which are made up of two electrons. Because it is amphoteric, imidazole can act as a base as well as an acid. Most research demonstrates that imidazole derivatives have several pharmacological activities:

- Anti-depressant activities
- Anti-fungal and anti-bacterial activities
- Anti-HIV activity (Verma et al., 2013)
- Anti-cancer activity
- Anti-inflammation
- Anti-virus activity (Shalini, Sharma, & Kumar, 2010).

1.4.2 Isoxazole

Isoxazole is one of the five-membered heterocycles that has two heteroatoms in the neighboring positions: a nitrogen atom and an oxygen atom. Two carbon-carbon double bonds are responsible for the molecule's unsaturated state (Dai, et al., 2017). The structure of isoxazole allows for a variety of non-covalent interactions, such as hydrophilic contacts, pi-pi stacks (unsaturated five-membered rings), and hydrogen bonds (acceptors N and O). Improved pharmacokinetic profiles, lower toxicity, and enhanced efficacy could result from the addition of isoxazole to the market (Rao, et al., 2014). Owing to their diverse biological activity and possible to interact with a large range of proteins that target, these activities include:

- Anticancer.
- Antifungal, antiviral.

- Anti-bacterial organisms such as Staphylococcus aureus and Corynebacterium diphtheria.
- Anti-TB, anti-inflammatory.

Numerous medications are now on the market as a result of the successful applications of creating isoxazole compounds. By blocking the enzyme dihydropteroate synthetase, sulfisoxazole has received approval for the medical intervention of severe, recurrent, or persistent urinary tract infections. Risperidone has a license to treat schizophrenia in adults and adolescents 13–17 years old, as well as transient manic or mixed episodes in children and teenagers with bipolar I disorder (ages 10 to 17). Blocking the limbic system's dopaminergic D2 receptors reduces the positive symptoms of schizophrenia (Zhu et al., 2018)

1.5.3 Thiazole

Structurally, thiazole are identical to imidazole, except instead of sulfur, they have nitrogen instead. Thiazoles are heterocyclic compounds. With the chemical formula C_3 H_3 NS, Thiazole is a pale-yellow liquid with an odor similar to pyridine. Planar aromatic thiazole rings are present. Proton NMR spectroscopy shows a significant diamagnetic ring current due to the chemical shift of the ring protons, showing that thiazole are more aromatic than equivalent oxazole due to a stronger pi-electron delocalization. The thiazole ring is a component of the vitamin called thiamine B_1 (Rouf & Tanyeli, 2015; Gupta & Kant, 2013).

The thiazole scaffold is found in over eighteen FDA-approved medications.

Cefiderocol, also known as Fetroja®, is one of them. In 2019, the FDA approved it as the first siderophore antibiotic. In the absence of other therapeutic options, this thiazole derivative is used to treat severe UTIs. It is effective against Gram-negative bacteria that are resistant to many drugs, including Pseudomonas aeruginosa. Alpelisib, also marketed under the brand name Pigray®, is another medication based on thiazoles that was approved in 2019 to treat specific forms of breast cancer. In 2018, the drug lusutrombopag was licensed to treat thrombocytopenia, including thrombocytopenia linked to chronic liver disease, by stimulating platelet production (Petrou, Fesatidou, & Geronikaki, 2021).

1.5.4 Pyridine

Pyridine, having the chemical formula C5H5N, is a fundamental heterocyclic organic molecule. It bears many similarities to benzene, a well-known and fundamental aromatic compound in which a nitrogen atom has taken the place of one of the C-H groups. The conjugated system of six π-electrons in pyridine is delocalized over the heterocyclic ring, just like in benzene. The molecule is planar and satisfies the aromaticity requirements set forth by Hückel criteria (Altaf, et al., 2015).

Pyridine derivatives containing thiazolidinones demonstrate antidiabetic actions among their many biological activities, including anti-diabetic effects. A new class of antidiabetic medications that also function as analgesics may be developed as a result of the results, which suggest that some of these compounds had highly effective anti-diabetic properties, although only a small number of these compounds exhibited significant antidiabetic action. Several heterocyclic compounds including pyridine nucleolus were synthesized and evaluated for analgesic action, several of these compounds had antiviral and anti-parasitic properties (Nigade, Chavan, & Deodhar, 2012; Firke, Firake, Chaudhari, & Patil, 2009).

Figure 1.6

The Pyridine, Imidazole, Isoxazole, and Thiazole structures

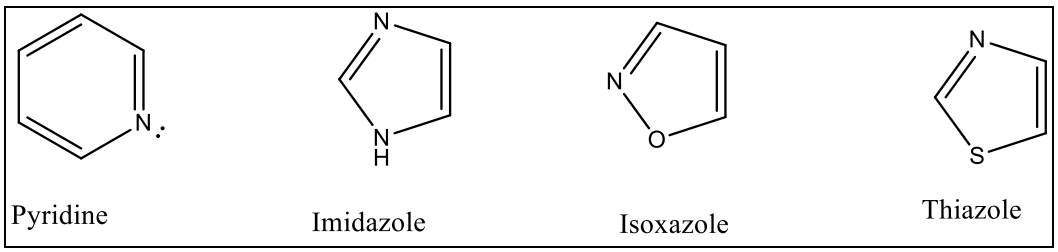
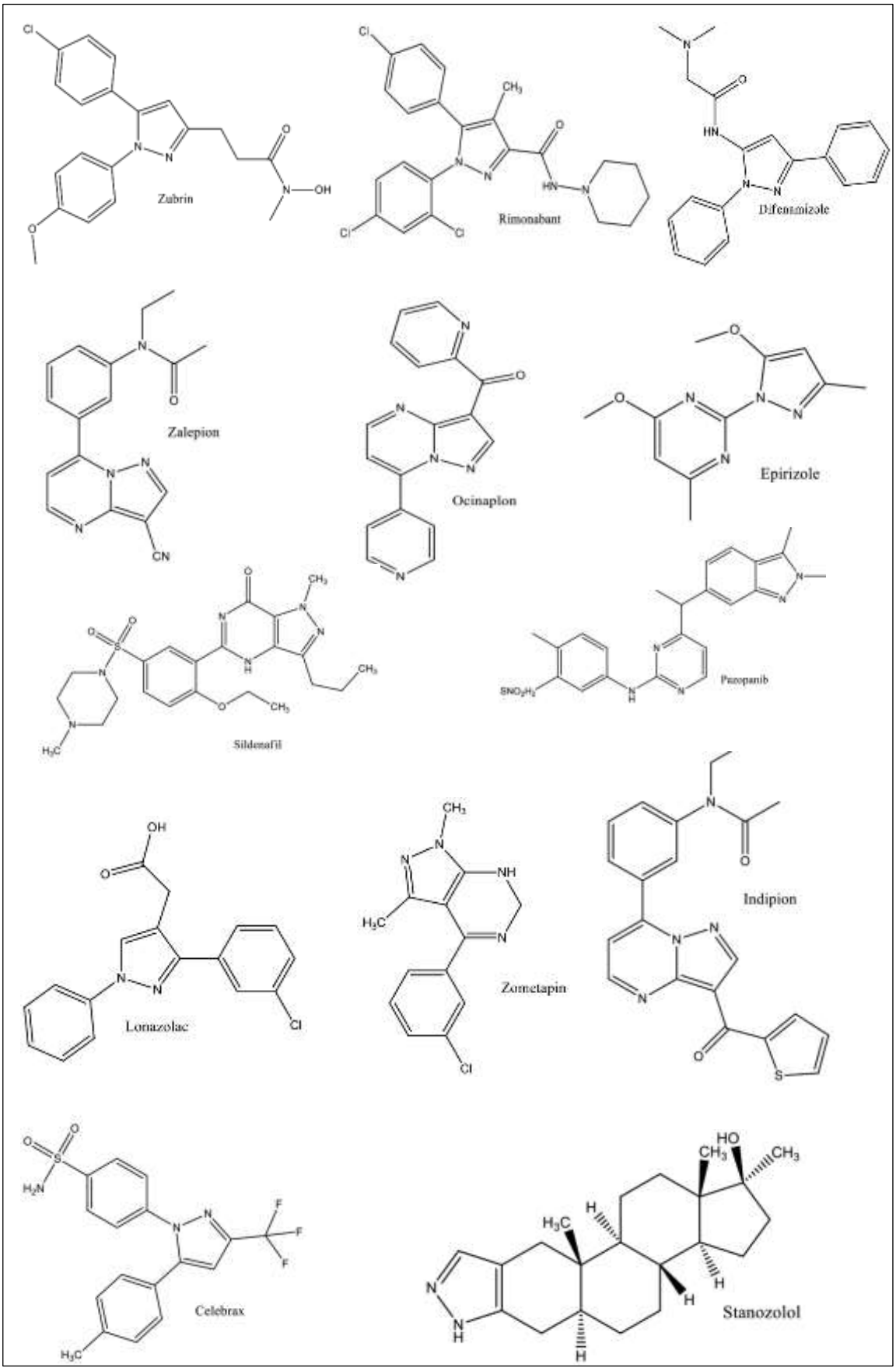


Figure 1.7

Some examples of pyrazole-containing medicines



Because of its vast range of biological actions, pyrazole and its derivatives are generally acknowledged as a pharmacologically relevant scaffold. Numerous well-known medications from a variety of therapeutic disciplines include this heterocyclic structure. For instance, the pyrazole nucleus is incorporated into the powerful anti-inflammatory medicine celecoxib, the antipsychotic CDPPB, the anti-obesity medication rimonabant, the analgesic difenamizole, the H2-receptor agonist betazole, and the antidepressant fezolamide. Pyrazole derivatives demonstrate broad therapeutic potential through their diverse pharmacological applications, making them effective and versatile therapeutic agents. Furthermore, the pyrazole group represents a promising option in drug development and discovery, given its ability to interact with a wide range of biological targets.(Faisal, Saeed, Hussain, Dar, & Larik, 2019).

The pyrazole compound has attracted widespread interest from researchers worldwide due to its proven pharmacological potential. Researchers have also extensively studied the chemical and biological properties of pyrazole derivatives, contributing to the development of molecules that perform diverse pharmacological functions. Researchers have developed pyrazole derivatives using diverse synthetic strategies aimed at enhancing their pharmacological properties, ranging from traditional condensation methods to modern catalytic techniques. These derivatives have demonstrated promising therapeutic potential against various diseases, exhibiting anti-inflammatory, analgesic, anti-obesity, antipsychotic, antidepressant, and anticancer properties (Karrouchi et al., 2018).

Recent research in the field of non-steroidal anti-inflammatory drugs (NSAIDs) has focused on developing safer medications. Researchers have concentrated on designing compounds that simultaneously inhibit the COX-2 and 5-LOX enzymes, aiming to enhance anti-inflammatory efficacy while minimizing side effects. This approach differs from that of conventional anti-inflammatory drugs, which can cause gastrointestinal issues due to their inhibition of the COX-1 enzyme. The dual-inhibition strategy relies on simultaneously targeting the **5-LOX** and **COX-2** enzymes. The **5-LOX** enzyme plays a crucial role in producing leukotrienes—which contribute to inflammation—while the **COX-2** enzyme promotes the inflammatory response. Targeting both enzymes facilitates the development of more effective anti-inflammatory compounds with fewer side effects, making them a safer alternative to conventional anti-inflammatory drugs. Researchers employed a hybridization approach to combine the

properties of **Celecoxib**—a selective **COX-2** inhibitor that also demonstrates **5-LOX** inhibitory activity—and **Sulindac**—a non-selective **COX** inhibitor—to develop a new series of **pyrazole sulfonamide** derivatives. This strategy aimed to yield compounds that combine anti-inflammatory efficacy with reduced side effects. The researchers prepared the new compounds and tested their ability to alleviate pain, reduce inflammation, and induce gastric ulcers. They then tested the compounds that yielded promising results to determine their ability to inhibit the **COX-1**, **COX-2**, and **5-LOX** enzymes in vitro. Compound **5b St.1** (Figure 1.8) demonstrated the best results among all the compounds studied, as it was the most effective in alleviating pain and reducing inflammation.

Compound 5b, a benzothiophene-2-yl pyrazole carboxylic acid derivative, exhibited enhanced efficacy and selectivity relative to celecoxib and indomethacin. It demonstrated substantial suppression of COX-1 (IC50 = 5.40 μM), COX-2 (IC50 = 0.01 μM), and 5-LOX (IC50 = 1.78 μM). The selectivity index of 344.56 demonstrated a pronounced preference for COX-2 over COX-1. The absence of significant ulcerogenicity in compound 5b underscores its superior safety profile compared to traditional NSAIDs. Although equally effective as 5b, the prodrug ester derivatives 6c and 6d also exhibited no gastrointestinal toxicity. The compounds were developed by molecular docking investigations, which validated their effective binding to COX-2 and 5-LOX, thereby establishing their potential as dual inhibitors (Gedawy, Kassab, & El Kerdawy, 2020).

To discover more effective anti-inflammatory medications, novel chemical families have been synthesized, including 1, 5-diphenyl pyrazoles and arylhydrazone derivatives. These compounds were synthesized by utilizing 1-(4-chlorophenyl)-4, 4, 4-trifluorobutane-1.3-dione as a precursor, and their potential anti-inflammatory effects were evaluated. The carrageenan-induced rat paw edema model demonstrated that several produced compounds had promising anti-inflammatory activities, suggesting substantial therapeutic promise for inflammatory disorders.

The researchers focused on studying the ability of the compounds to inhibit the **COX-1** and **COX-2** enzymes, as these play a significant role in inflammation. Compounds **2f**, **6a**, and **6d** demonstrated a high capacity to inhibit the **COX-2** enzyme. Compound **2f** demonstrated the best results, exhibiting a selectivity index of **111.1** and an **IC50** value of **0.45 μM**, indicating that it

inhibits the **COX-2** enzyme to a greater extent than **COX-1**. These results suggest that this compound could be a promising candidate for the development of drugs targeting **COX-2** while minimizing side effects compared to non-selective inhibitors. The researchers conducted molecular docking and virtual screening experiments to investigate how compounds **2f**, **6a**, and **6d** bind to the **COX-2** enzyme. The results showed that these compounds bind to the enzyme's active site in a manner similar to the well-known selective inhibitor **SC-558**.

The results indicate that the new compounds may act as selective **COX-2** inhibitors, as they bind to the enzyme in a manner similar to the compound **SC-558**. Compound **2f** (Figure 1.8) was the most effective among them, demonstrating high selectivity for **COX-2** and potent anti-inflammatory activity, making it a promising candidate for the development of new drugs to treat inflammatory diseases. (El-Sayed, et al., 2011).

To minimize the side effects associated with conventional non-steroidal anti-inflammatory drugs (NSAIDs)—particularly gastric and gastrointestinal issues—researchers have focused on developing new pyrazole compounds that act as selective **COX-2** inhibitors. Since this enzyme drives inflammation, targeting it selectively helps reduce inflammation and alleviate pain while minimizing impact on the **COX-1** enzyme, which protects the stomach lining; this approach may reduce the occurrence of gastrointestinal side effects.

The researchers synthesized several pyrazole derivatives (compounds 2–5) and evaluated their ability to inhibit COX-1 and COX-2 enzymes in vitro. Most of these compounds demonstrated greater inhibitory activity against COX-2 than COX-1, indicating good selectivity for COX-2. Some compounds also exhibited potent activity even at very low concentrations, making them promising candidates for the development of new anti-inflammatory drugs. Compounds 2a, 3b, 4a, 5b, and 5e were the most effective, recording the lowest IC50 values, which indicates high efficacy in inhibiting the COX-2 enzyme.

Compounds **3b**, **4a**, **5b**, and **5e** demonstrated high selectivity for the **COX-2** enzyme, meaning they target this enzyme more than **COX-1**. This result is significant because inhibiting **COX-2** helps reduce inflammation, while preserving **COX-1** activity minimizes the risk of side effects—such as gastric ulcers—often associated with non-selective anti-inflammatory drugs.

Researchers also tested the best of these compounds in in vivo experiments to verify their ability to combat inflammation. The results demonstrated that these compounds were highly effective, matching or—in some cases—surpassing **Celecoxib**, one of the most well-known **COX-2** inhibitor drugs.

Researchers tested the best of these compounds on living organisms to determine their ability to reduce inflammation. The results showed that these compounds were highly effective, matching or even surpassing—in some cases—the drug **Celecoxib**, a well-known medication used to inhibit the **COX-2** enzyme and treat inflammation. Compound **3b** (Figure 1.8) was the best of the compounds tested by the researchers; it demonstrated high selectivity for the **COX-2** enzyme, potent anti-inflammatory efficacy in *in vivo* experiments, and significant inhibitory activity against the enzyme. These results indicate that it is a promising candidate for the development of new anti-inflammatory drugs.

Molecular dynamics simulation results supported the efficacy of compound **3b**, demonstrating that it binds stably within the active site of the **COX-2** enzyme. These results confirm that compound **3b** exhibits high selectivity for this enzyme, making it a promising candidate for the development of new anti-inflammatory drugs. This molecule is considered the best in the series, as it demonstrated high efficacy and significant selectivity for the **COX-2** enzyme, and yielded excellent results in *in vivo* experiments.(Hassan, et al., 2019).

Researchers have focused on developing drugs that selectively target the **COX-2** enzyme, as this approach helps reduce inflammation while minimizing the gastrointestinal side effects associated with non-selective anti-inflammatory drugs. The **COX-2** enzyme is a significant target in the treatment of inflammation because it plays a key role in the inflammatory response.

In recent years, researchers have focused on developing new **pyrazole** and **pyrazolo[1,2-a]pyridazine** compounds aimed at selectively inhibiting the **COX-2** enzyme while minimizing effects on **COX-1**. This approach facilitates the development of effective anti-inflammatory drugs with reduced side effects, particularly those affecting the stomach.

The researchers prepared these compounds using a method based on acid catalysis and ultrasonication—a technique that enhances reaction efficiency and improves the yield of the resulting compounds. The results demonstrated that compounds **11** (Figure 1.8) and **16** exhibited the highest inhibitory activity against the **COX-2** enzyme among all the newly synthesized compounds, suggesting they are prime candidates for the development of selective inhibitors for this enzyme.

Both compounds demonstrated a high capacity to target the COX-2 enzyme over COX-1—a significant advantage, as this may help reduce side effects such as gastric ulcers, which are often associated with non-selective anti-inflammatory drugs. Researchers also evaluated their anti-inflammatory effects in *in vivo* studies; the results showed significant efficacy, positioning them as promising candidates for the development of new anti-inflammatory medications.

The researchers investigated how compounds **11** and **16** bind to the active site of the **COX-2** enzyme using molecular docking. The results showed that these two compounds bind to the enzyme in a manner similar to **Celecoxib**, a known **COX-2** inhibitor. The researchers also calculated the Mulliken charges and the electrostatic surface potential of compound **11** and the drug **Celecoxib** using **Density Functional Theory (DFT)**, aiming to understand the basis of their activity and their mode of interaction at the molecular level. These results indicate the potential for developing new compounds that target the **COX-2** enzyme with higher efficiency and selectivity. Compound **11** stands out as the leading candidate, as it demonstrated high selectivity for the **COX-2** enzyme and a very low **IC50** value, making it a promising candidate for the development of new anti-inflammatory drugs.(Ghareb, Elshihawy, Abdel-Daim, & Helal, 2017).

The researchers evaluated the pain-relieving potential of the compounds using the hot-plate and formalin tests. Compound **AD 532** (Figure 1.8) demonstrated promising results: it effectively alleviated pain, did not cause gastric ulcers, and had a minimal impact on renal function in both short- and long-term toxicity tests. Moreover, in vitro studies revealed that compound AD 532 was a less potent COX-2 inhibitor than celecoxib, which may point to a decreased risk of cardiovascular harm. All things considered, the AD 532 compound seems to be a secure and reliable course of treatment for persistent inflammatory diseases (Mohy El-Din, et al., 2021).

A different study created and assessed new anti-inflammatory pyrazole as coxib analogs, synthesized, and assessed for their inhibitory effects both in vivo and in vitro. When compared to the COX-2 selective reference drug celecoxib (Selectivity Index = 313.12), the most selective COX-2 compounds were 6a and 6b St.6 (fig 1.8) (Selectivity Index = 462.91, 334.25).

Furthermore, compounds 6a and 6b demonstrated the strongest anti-inflammatory activity (ED50 = 136 and 126 μMol/kg), despite having the lowest ulcerogenic liability (Ulcer Index = 1.25 and 1.00, respectively), which indicates their anticipated safe GI profiles. Additionally, SO2NH2, a selective COX-2 pharmacophore, is present in these compounds. Furthermore, based on the different binding types of interactions, molecular docking research indicated a connection between the synthesized compounds and the COX-2 active site (Abdellatif, et al., 2021).

The standard acute carrageenan-induced paw edema method was used in the study to assess the anti-inflammatory qualities of a newly designed and synthesized series of 1, 3-diaryl pyrazole derivatives.

These derivatives connected pyrazoles, isoxazoles, pyridines, and pyrimidines, among other nitrogenous heterocyclic ring systems, at the C–4 position. Based on the findings, six compounds (2a, 2b, 3a, 6b, 7b, and 9b) exhibited consistently good anti-inflammatory action four hours after the carrageenan injection (84.39–89.57% inhibition), which was similar to that of the popular drugs Celebrex and indomethacin (72.99% and 83.76%, respectively). The most effective product seems to be the cyanopyridone derivative 6b St.7 (fig 1.8), which outperformed both celecoxib and indomethacin (reference standards) in terms of activity (89.57% inhibition of edema). This product may be seen as a promising lead for the future development of more potent anti-inflammatory drugs due to its selective anti-inflammatory properties (Nossie, Fahmy, Khalifa, El-Eraky, & Baset, 2017).

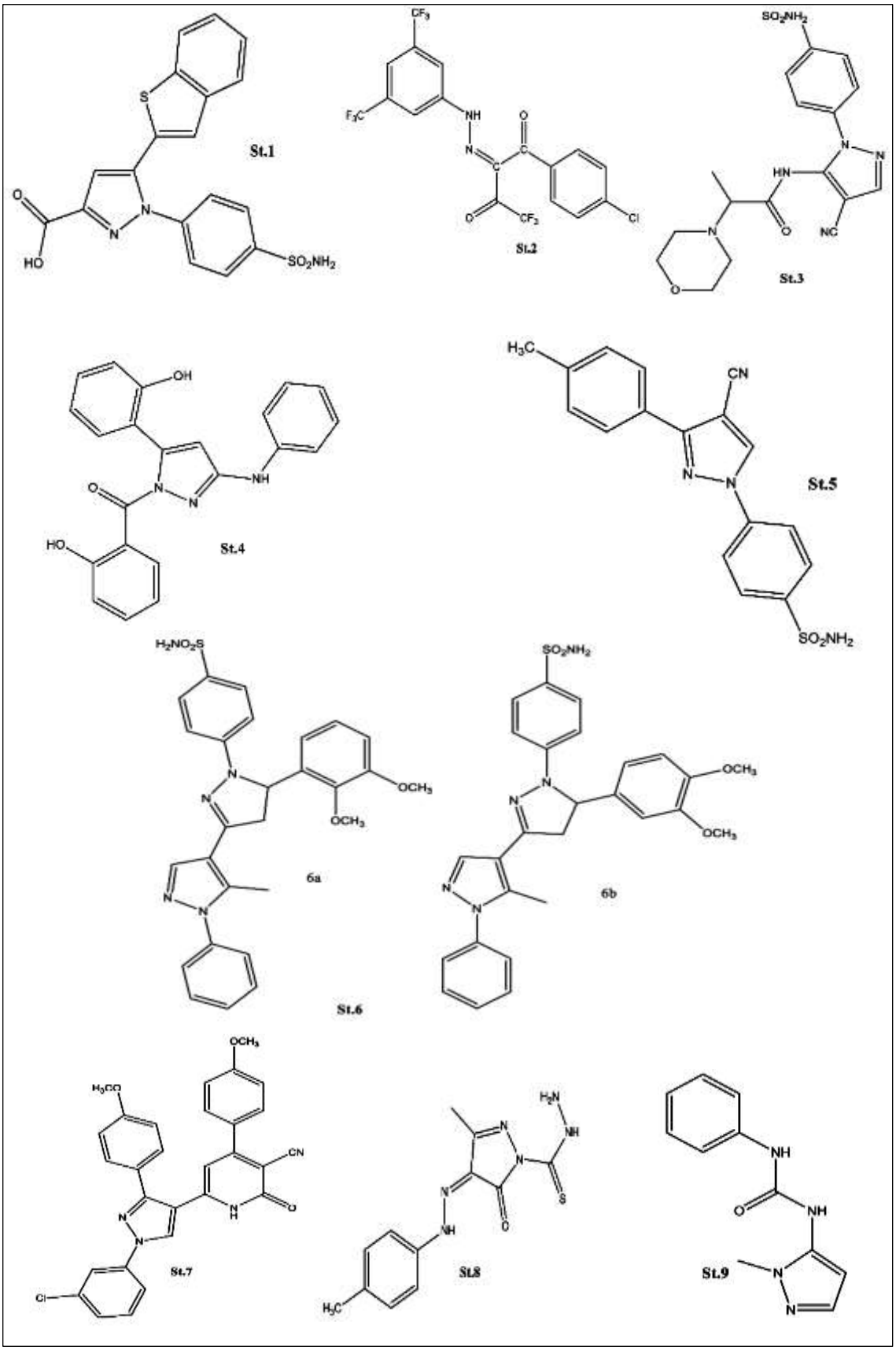
One more investigation, the first of a new class of pyrazole and pyrazolone derivatives that have been produced in good yields is the pyrazole-1-carbothiohydrazide 1a, b. multiple synthesized compounds exhibited moderate to good antibacterial activity. 4-(2(p-tolyl) hydrazineylidene)-pyrazole-1-carbothiohydrazide 21a St.8 (fig 1.8) exhibited the strongest antibacterial and antifungal activity, with minimum inhibitory concentrations

(MIC) that were lower than those of the widely used drugs clotrimazole and chloramphenicol. When the free carbothiohydrazide moiety is present, the antibacterial action is enhanced (Radini, 2018).

Over the past 20 years, the coupling of the pyrazole nucleus with a urea moiety has facilitated the creation and synthesis of numerous molecules with interesting biological properties in numerous other investigations. Many novel compounds have been developed and investigated for their potential to combat infections (including those caused by bacteria, plasmodium, toxoplasma, and other pathogens), lessen inflammation, and particularly combat cancer. The urea moiety has been identified as a versatile scaffold for the synthesis of kinase inhibitors due to its unique binding mechanism and kinase inhibition profile. Specifically employed in type II kinase inhibitors, the urea moiety forms one or two hydrogen bonds with the conserved glutamic acid and the aspartic acid backbone amide of the DFG motif. High-affinity DNA binders and pharmacophores are also connected by this structure. Fitting the urea function at position 5 of the pyrazole scaffold produced the most interesting results (possibly because the synthesis process was simpler). 5. Numerous studies have been conducted on 5-pyrazolyl-ureas St.9 (fig 8); BIRB 796, a p38MAPK inhibitor that can be used to treat autoimmune diseases, has received the most attention of these compounds.

Figure 1.8

Some chemical structure of pyrazole compounds has different activities



In the field of anticancer research, Prostaglandin biosynthesis in vascular smooth muscle cells via the cyclooxygenase pathway catalyzed by cyclooxygenase-1 and -2 (COX-1/2), prostaglandin production begins with this step. Their significance in carcinogenesis has shown more clearly in the recent past (Rayar, et al., 2018). They are important in the synthesis of carcinogens and have an impact on invasion, angiogenesis, and apoptosis. Typically, cancer cells express COX-2 at a high level. Large epidemiological trials have demonstrated the potential advantage of cyclooxygenase inhibitors and non-steroidal anti-inflammatory medicines (NSAIDs) in preventing the initiation and progression of cancer. Therefore, it seems that focusing on the COX-2 pathway is a good way to prevent and treat solid tumors (Khan, et al., 2011; Ghosh, Chaki, Mandal, & Mandal, 2010).

Due to their ability to self-renew, cancer stem cells (CSCs) are seed cells that resist the effects of traditional chemotherapy and radiation therapy and instead promote tumor recurrence. It has recently been discovered that NSAID-metal complexes block HMLER, breast CSCs, and HMLER-shEcad, suggesting that they may have applications as anti-CSC medications for the treatment of especially metastatic malignancies (Khan, Parveen, Yousuf, Tabassum, & Arjmand, 2022).

Previous trial studies using mofezolac were observed to inhibit colon tumors Induced by azoxymethane affecting tumor multiplicity, and volume in male F344 rats (Hawash, et al., 2024). Nimesulide treatment showed a decrease in both the number and size of polyps in the gut of APC1309 mice. Tumor growth is tightly correlated with changes in angiogenesis and alterations in cell proliferation and apoptosis. Sulindac has been shown to reduce the size and quantity of intestinal polyps in Min mice with elevated apoptosis; however, concurrent administration of EP receptor agonists also reduces apoptosis, indicating that PGE 2 may play a critical role in the development of intestinal polyps, potentially through modulating cell turnover. The actions of COX-1 and COX-2 are known to cause intestinal polyps to produce more PGE-2. Moreover, their role in tube formation from endothelial cells co-cultured with colon carcinoma cells and angiogenesis in gastrointestinal cancer xenografts in nude mice has been demonstrated. Also, cervical carcinomas and Hela cells have elevated expression of both COX-1 and COX-2. Artificial overexpression of COX-1 causes prostaglandin E synthase and COX2 to be upregulated, which is followed by increased PGE2 synthesis and upregulation of angiogenic factors. Based on these findings, it is likely that COX-1 and COX-2 affect cell turnover and

increase PGE-2 levels, which may eventually help intestinal polyps form, either separately or in combination.

Additionally, it's likely that the COX isoforms influence EP 1 and EP 2 receptors, for example, and are involved in prostaglandin synthesis, promoting carcinogenesis through several receptor-mediated pathways (Cingolani, et al., 2017; Kitamura, et al., 2002). In particular, pyrazole-based NSAIDs like celecoxib have an evident anticancer effect in ovarian cancer. Hawash et al. (2024) 5-pyrazolyl-ureas have been shown to interact at the intracellular level on multiple pathways, specifically on different kinases like Src, Bcr-Abl, Flt3, TrkA, and others. This has been corroborated by numerous patents that have been reported in the literature since 2000. Various 5-pyrazolyl ureas also showed promise for antiangiogenic and anti-proliferative effects that warrant further investigation. Although they have been studied less, 4- and 3-pyrazolyl ureas have demonstrated several of pharmacological characteristics, containing the inhibition of epoxide hydrolase and carbon anhydrase as well as actions on potassium channels and cannabinoid receptors. Due to their comparable biological activities, 4- and 5-pyrazolyl ureas have both been covered by similar patents. For all these reasons, research into medicinal chemistry is still being advanced through the design and synthesis of pyrazolyl-ureas (Brullo, Rapetti, & Bruno, 2020).

1.6 Objectives of the Study

The main aim of our research is to create active molecules with a broad range of structures by introducing a chemical variety into the molecular domain. Following the problem introduction, we go into great detail about the thesis' objectives.

- To develop and synthesized an overall framework to find novel compounds with active inhibition of COX-1 and COX-2 inhibitors.
- To determining the physical and chemical properties of the newly created derivatives was one of the main goals of this effort.
- COX-1 and COX-2 enzyme screening kits are used for in vitro testing.
- To do In vitro testing multiple cell lines and determining the cytotoxicity of each product.

Chapter Two

Methodology

2.1 Reagents and Materials

All chemicals were bought from C.S. Chemicals Company, Alfa Aesar, and Sigma-Aldrich without any additional purification.1-(4nitrophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (lot # 10226909),1-(3Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI) (lot # Y09E044), 4-(Methylthionine (lot # L04950), 4-chloro-2,5dimethoxyaniline (lot # 10122927), and (methoxy phenoxy)aniline (lot # 10161129) were bought from Alfa Aesar Company.

4-(Dimethyl amino) pyridine (DMAP) (lot # 39405-50G), 3, 4, 5(Trimethoxy aniline) (lot # T68209-10G), Silica gel (lot # S74874), Sodium sulphate anhydrous, 4-Tertbutylaniline (lot # 209864-5G), 3, 4-Dimethoxyaniline (lot # A83008-10G), 3,5Dimethoxyaniline (lot #D130001-5G), and 4-aminobiphenyl (lot #A2898-5G) were purchased from the Sigma-Aldrich Company. HCl 32%, COX inhibitor screening assay (Cayman Chemical, USA) kit No. 560131, RPMI 1640 media[-](L-Glutamine), DMEM media, Trypsin EDTA solution B, MTS reagent. Fetal Bovine Serum and L-Glutamine solution were both also bought from Sigma-Aldrich Company (Brazil Origin). All other chemicals like methanol, acetone, hexane, ethanol, DCM, and Ethyl acetate have been also bought from C.S Chemicals Company.

2.2 Instruments

To determine the melting temperatures Accompanying the GALLENKAMP melting point apparatus were the Vacuubrand rotary evaporator with complete set, RO UV lamp, LABOMED inverted fluorescence microscope, ESCO laminar flow cabinet, BIOBASE medical CO2 incubator, Lab Tech digital water bath, J.P. SELECTA digital vortex, mommert oven, and SHIMADZU balance. Infrared spectra were acquired for IR spectroscopy work at Najah University using the Perkin Elmer Spectrum 400

FTIR/FTNR spectrometer, the auto-baseline correction tool was used, which is a feature of important FTIR software that allows for the restoration of basic baseline faults without the need to define regions. See Appendix A.

The Bruker DPX-500 High-Performance Digital FT-NMR Spectrometer was used to obtain 1H- and 13C-NMR spectra. The frequency markings for the 1H- and 13C-NMR spectra were 500 MHz and 125 MHz, respectively. DMSO-d6 was the solvent used; chemical shift values (δ) were obtained and recorded in ppm, along with coupling constants in Hz. Using a water LCT Premier XE mass spectrometer and the ESI (+) method, high-resolution mass spectra (HRMS) were observed. At Gazi University in Ankara, Turkey, the Pharmacy Faculty conducted both NMR and HRMS analyses. To determine the inhibitory activity of the ovine COX-1 and human recombinant COX-2 enzymes, the COX inhibitor screening assay kit No. 560131 (Cayman Chemical, USA) was employed. A UV spectrophotometer (Bio-Rad Japan) with a wavelength of 415 nm and a microplate reader is used to determine the yellow result of this enzymatic reaction.

2.3 Synthesis and Biological Evaluation of Products

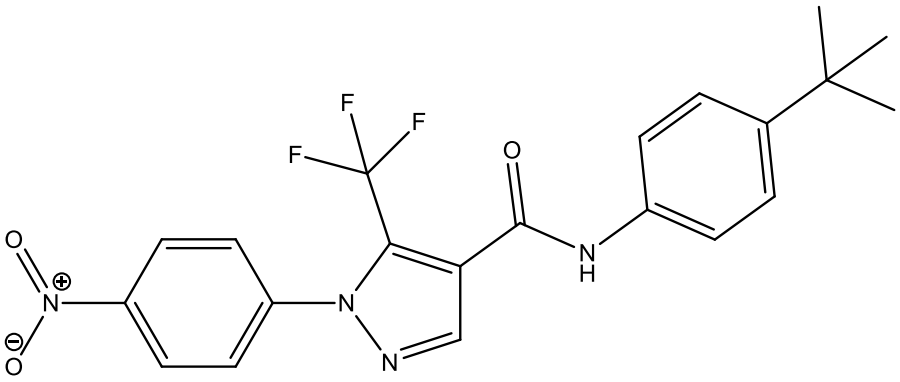
The total synthetic procedures were done at the laboratories of An-Najah National University.

2.4 General Synthetic Procedures

Inside a sanitized round bottom after the addition DMAP (36.284mg, 0.297mmol) to1(4-nitrophenyl) -5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (300 mg, 0.99 mmol) in 15 milliliters of DCM, a EDCI (246.717 mg, 1.287 mmol),added as coupling reagent, . Next, an aniline derivative was added to the reaction mixture was stilled for 30 minutes, after the addition of the aniline derivative to the flask, the reaction mixture was stirred with a stirrer for 48 to 72 hours under argon gas to prevent oxidation. To identify aniline, TLC paper was pigmented with ninhydrin, to ensure the product was pure, and the other one used bromo-cresol to detect the presence of acid. Subsequently, an extraction funnel was used to separate the aniline derivative from the reaction mixture containing 32% HCl. The lower layer leaked within a conical flask. The filter paper was used in the filtration step, and the flask was filled with sodium sulfate anhydrous, a drying agent. The filtrate was mixed with three or four spatulas to add silica gel, keeping in mind the DCM boiling point of 39.6 °C. The product-loaded silica remained in the flask after the reaction mixture was extracted using a rotary vacuum evaporator. Several solvent systems were used in silica gel column chromatography to purify the product-loaded silica. Then the product-containing tubes were gathered and evaporated in accordance with the reaction.

2.4.1 Synthesis of N-(4-(tertbutyl phenyl)-1-(4-nitrophenyl)-5-(trifluoromethyl) 1H-pyrazole-4-carboxamide (3a)

The same as the previous procedure, and the aniline derivative used here was 4-tert butyl aniline (173.43µl, 1.089 mmol), Silica gel column chromatography was used to purify product-loaded silica using a 3:2 solvent system of hexane to ethyl acetate.



N-(4-(tert-butyl)phenyl)-1-(4-nitrophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide

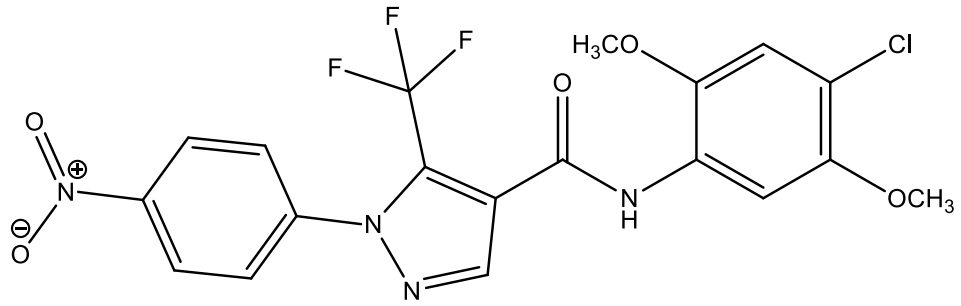
Beige powder product with $\mathbf{R_f}$: 0.66(EA: Hexane 2:3). The percentage yield is 88.6%.

^{1}H-NMR (DMSO-d$_6$, 500 MHz) δ ppm: 10.56 (s, 1H), 8.54 – 8.48 (m, 2H), 8.46 (s, 1H), 7.97 – 7.90 (m, 2H), 7.71 – 7.64 (m, 2H), 7.47 – 7.41 (m, 2H), 1.33 (s, 9H).

^{13}C-NMR (DMSO-d$_6$, 125 MHz) δ ppm: 158.87, 148.39, 146.94, 143.83, 141.21, 136.50, 130.60, 130.29, 127.67, 125.90, 125.41, 123.02, 122.91, 120.76, 120.09, 118.61, 34.57, 31.65, 1.57. See Appendix B for the spectrum.

2.4.2 Synthesis of N-(4-chloro-2, 5dimethoxyphenyl)-1-(4-nitrophenyl)-5 (triflouromethyl)-1H-pyrazole-4-carboxamide (3b)

The aniline derivative, in this case, was 4-chloro-2, 5 dimethoxyaniline (204.32 mg, 1.089 mmol), and a solvent system of hexane: ethyl acetate (3:2) was used to purify the product-loaded silica through silica gel column chromatography.



N-(4-chloro-2, 5dimethoxyphenyl)-1-(4-nitrophenyl)-5(triflouromethyl)-1H-pyrazole-4-carboxamide

HRMS (m/z): [M+H] + Calc. for C19H14ClF3N4O5, 471.0683 found471.0684. See appendix C.

Yellow powder product with R_f: 0.6 (EA: Hexane 2:3). The percentage yield is 89%.

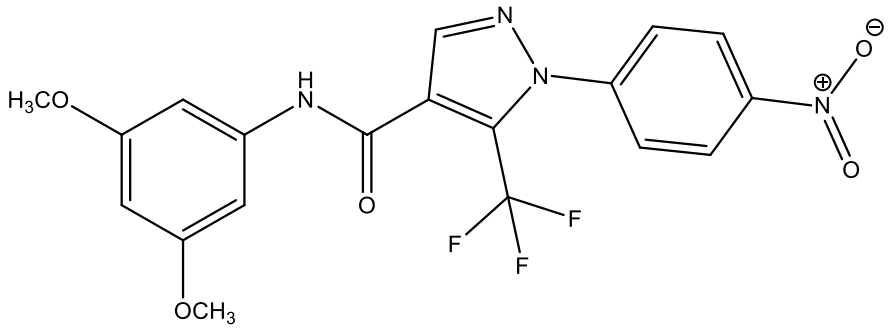
^{1}H NMR (400 MHz, DMSO) δ 9.88 (s, 1H), 8.50 – 8.44 (m, 2H), 7.94 – 7.84 (m, 2H), 7.77 (s, 1H), 7.30 – 7.20 (m, 1H), 3.83 (s, 6H).

^{13}C NMR (101 MHz, DMSO) δ 159.34, 148.57, 148.39, 145.54, 143.88, 141.33, 127.70,

126.50, 125.38, 122.51, 121.00, 118.31, 117.30, 114.12 108.97, 57.12, 5697. 56.97. See Appendix B for the spectrum.

2.4.3 Synthesis of N-(3,5-dimethoxyphenyl)-1-(4-nitrophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (3c)

3, 5-dimethoxy aniline (166.813mg, 1.089mmol) was used, utilizing a 3:2 solvent system, and hexane: ethyl acetate was used to purify the product-loaded silica through silica gel column chromatography.



N-(3,5-dimethoxyphenyl)-1-(4-nitrophenyl)-5-(trifluoromethyl)- 1H-pyrazole-4-carboxamide

Pale yellow powder product with R*f*: 0.43 (EA: Hexane 2:3). The percentage yield is 85.3%.

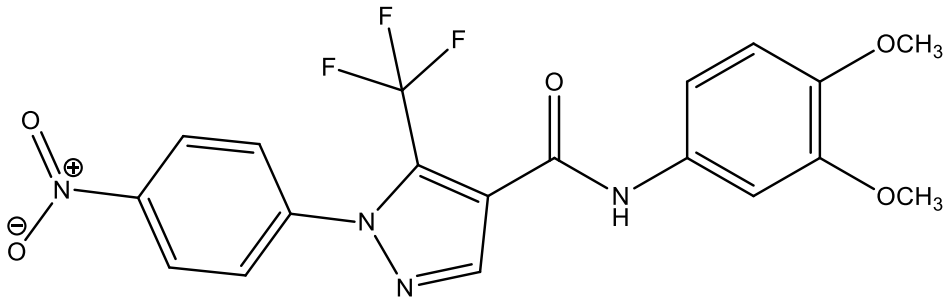
HRMS (m/z): $[M+H]^+$ calcd. For C19H15F3N4O5, 437.1073found 437.1076. See appendix C.

^{1}H NMR (400 MHz, DMSO) δ 10.50 (s, 1H), 8.50 – 8.39 (m, 3H), 7.96 – 7.83 (m, 2H), 6.99 (d, *J* = 2.2 Hz, 2H), 6.32 (s, 1H), 3.76 (s, 7H).

^{13}C NMR (101 MHz, DMSO) δ 161.00, 159.09, 148.42, 143.79, 141.25, 140.72, 127.69, 125.41, 122.88, 120.99, 118.31, 98.59, 96.54, 55.65. See Appendix B for the spectrum.

2.4.4 Synthesis of N-(3,4-dimethoxyphenyl)- [1] -(4-nitrophenyl)-5-(triflouromethyl) 1H-pyrazole-4-carboxamide (3d)

3, 4-dimethoxy aniline was the aniline derivative (166.8mg, 1.089mmol), and a DCM: Ethyl acetate (1:1) solvent system was used to purify the product-loaded silica using silica gel column chromatography. See Appendix B for the spectrum.



N-(3,4-dimethoxyphenyl)- -(4-nitrophenyl)-5-(triflouromethyl)1H-pyrazole-4-carboxamide

Fine beige product with R*f*: 0.71 (DCM: EA 1:1). The percentage yield is 55.4%.

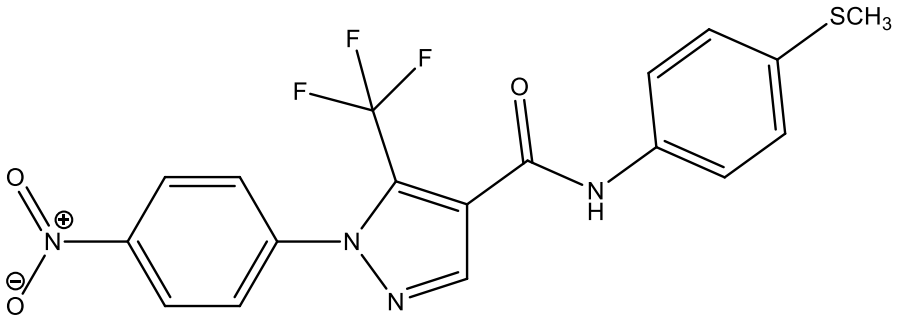
HRMS (m/z): $[M+H]^+$ calcd. For C19H15F3N4O5, 437.1073found 437.1076. See appendix C

^{1}H NMR (400 MHz, DMSO) δ 10.38 (s, 1H), 8.51 – 8.43 (m, 2H), 8.41 (s, 1H), 7.94 – 7.82 (m, 2H), 7.40 (d, *J* = 2.4 Hz, 1H), 7.26 (d, *J* = 8.6, 2.4 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 3.77 (d, *J* = 5.3 Hz, 6H).

13C NMR (101 MHz, DMSO) δ 158.59, 149.03, 148.40, 145.92, 143.85, 141.22, 132.62, 127.68, 125.40, 123.06, 121.04, 118.35, 112.48, 112.36, 105.36, 56.21, 55.91.

2.4.5 Synthesis of N-(4-(methylthio)phenyl)-1-(4-nitrophenyl)-5-(triflouromethyl) 1H-pyrazole-4-carboxamide (3e)

Photosensitive 4-(methylthio) aniline (135.49µL, 1.089mmole) was used, Hexane: Ethyl acetate (3:2) solvent system was used in silica gel column chromatography to purify the product-loaded silica. ……..



N-(4-(methylthio)phenyl)-1-(4-nitrophenyl)-5-(triflouromethyl) 1H-pyrazole-4-carboxamide

Soft white powder product with R*f*: 0.53 (EA: Hexane 2:3). The percentage yield is 92.4%.

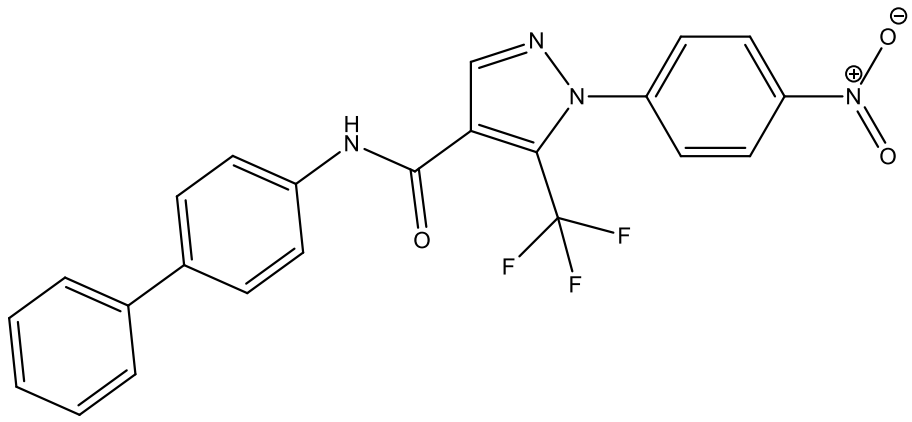
HRMS (m/z): $[M+H]^+$ calcd. For C18H13F3N4O3S, 423.0739found 423.0733. See appendix C.

^{1}H NMR (400 MHz, DMSO) δ 10.58 (s, 1H), 8.53 – 8.39 (m, 3H), 7.93 – 7.87 (m, 2H), 7.69 (d, *J*=8.8 Hz, 2H), 7.34–7.28 (m, 2H), 2.49 (s, 3H).

^{13}C NMR (101 MHz, DMSO) δ 158.91, 148.42, 144.62, 143.81, 141.25, 136.47, 133.42, 130.72, 130.56, 130.33, 127.70, 127.43, 125.40, 120.98, 116.20, 115.82, 15.85. See Appendix B for the spectrum**.**

2.4.6 Synthesis of N-([1, 1'-biphenyl]-4-yl)-[1]-(4-nitrophenyl)-5-(trifluoromethyl)1H-pyrazole-4-carboxamide (3f)

4-aminobiphenyl aniline (184.28 mg, 1.089mmol), and the product-loaded silica was refined using a DCM: Methanol (9:1) solvent system in a silica gel column chromatography.



N-([1, 1'-biphenyl]-4-yl)- -(4-nitrophenyl)-5-(trifluoromethyl)1H-pyrazole-4-carboxamide

White crystals product with R_f: 0.9 (DCM: Methanol 4.5:0.5). The percentage yield is 53.1%.

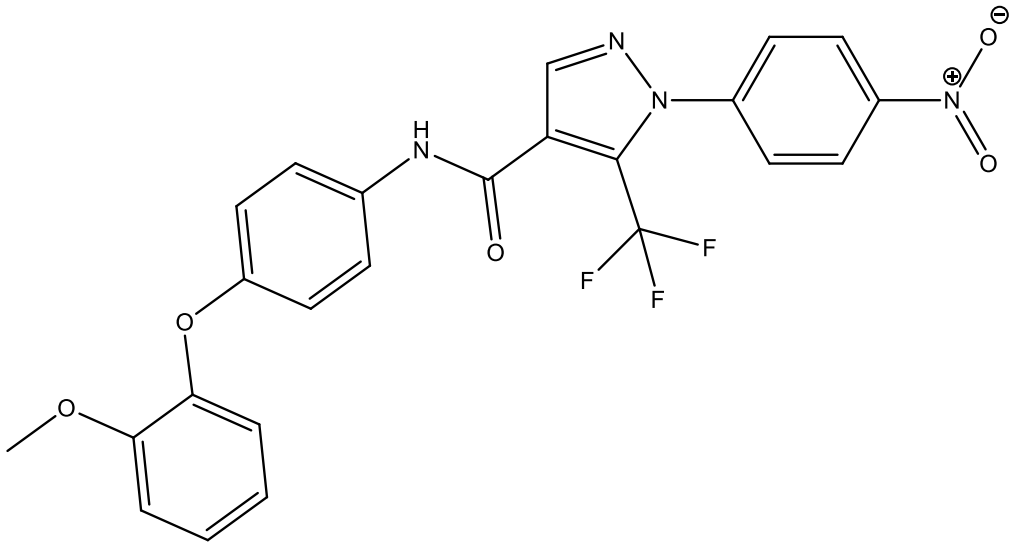
HRMS (m/z): $[M+H]^+$ calcd. For C23H15F3N4O3, 453.1175found 453.1177. See appendix C.

^{1}H NMR (400 MHz, DMSO) δ 10.69 (s, 1H), 8.52 – 8.43 (m, 3H), 7.91 (d, *J* = 8.6 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.75 – 7.66 (m, 4H), 7.53 – 7.45 (m, 2H), 7.40 – 7.33 (m, 1H).

^{13}C NMR (101 MHz, DMSO) δ 159.08, 148.43, 143.81, 141.28, 140.06, 138.54, 136.22, 130.75, 129.41, 127.72, 127.67, 127.49, 126.82, 125.42, 122.90, 121.03, 120.68, 118.34. See Appendix B for the spectrum.

2.4.7 Synthesis of N-(4-(2-methoxyphenyl)phenyl)-1-(4-nitrophenyl)-5 (trifluoromethyl)-1H-pyrazole-4-carboxamide (3g)

4-(2-methoxyphenyl) aniline (234.4mg, 1.089mmol) was used, and using a DCM: Ethyl acetate (1:1) solvent system, the product-loaded silica was purified using silica gel column chromatography.



N-(4-(2-methoxyphenyl)phenyl)-1-(4-nitrophenyl)-5(trifluoromethyl)-1H-pyrazole-4-carboxamide

The fine white and powder product with R_f: 0.83 (DCM: EA 1:1). The percentage yield is 54.8%.

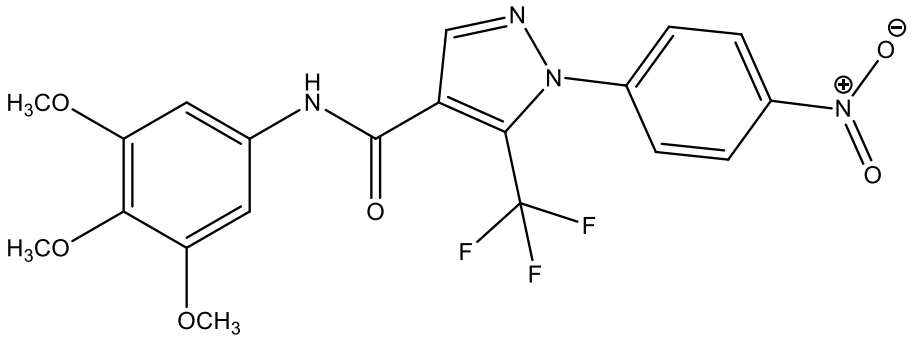
HRMS (m/z): $[M+H]^+$ calcd. For C24H17F3N4O5, 499.1229found499.1228. See appendix C.

^{1}H NMR (400 MHz, DMSO) δ 10.52 (s, 1H), 8.50 – 8.40 (m, 3H), 7.93 – 7.85 (m, 2H), 7.69 – 7.59 (m, 2H), 7.24 – 7.16 (m, 2H), 7.07 – 6.95 (m, 2H), 6.92 – 6.84 (m, 2H), 3.77 (s, 3H).

^{13}C NMR (101 MHz, DMSO) δ 158.75, 154.50, 151.69, 148.40, 144.47, 143.83, 141.23, 133.63, 130.28, 127.69, 125.81, 125.40, 122.95, 121.98, 121.88, 121.65, 121.56, 121.03, 118.34, 117.05, 113.90, 56.10 See Appendix B for the spectrum.

2.4.8 Synthesis of 1-(4-nitrophenyl)-5-(trifluoromethyl)-N-(3, 4, 5-tri methoxyphenyl)-1H-pyrazole-4-carboxamide (3h)

3, 4, 5- trimethoxy aniline (199.5mg, 1.089 mmol) was used, and a DCM: Ethyl acetate (1:1) solvent system was used in silica gel column chromatography to purify the product-loaded silica.



1-(4-nitrophenyl)-5-(trifluoromethyl)-N-(3, 4, 5-tri methoxyphenyl)-1H-pyrazole-4-carboxamide

Pale yellow fine and powder product with R_f: 0.63 (DCM: EA 1:1). The percentage yield is 61.6%.

HRMS (m/z): $[M+H]^+$calcd. For $C_{20}H_{17}F_3N_4O_6$, 467.1178found467.1176. See appendix C.

^{1}H NMR (400 MHz, DMSO) δ 10.48 (s, 1H), 8.53 – 8.38 (m, 3H), 7.96 – 7.83 (m, 2H), 7.13 (d, *J* = 1.4 Hz, 2H), 3.79 (d, *J* = 1.4 Hz, 6H), 3.66 (d, *J* = 1.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 158.84, 153.24, 148.42, 143.82, 141.24, 135.23, 134.50, 130.74, 130.35, 127.69, 125.40, 122.90, 121.01, 118.32, 98.02, 60.62, 56.25. See Appendix B for the spectrum.

2.5 General Procedure of Performing Column Chromatography

A proper column size was chosen, and the column was prepared by adding silica gel after the cotton-capped column and then filling the product-loaded silica. Five layers were added to the cotton-capped column, sand, silica gel, sand, product-loaded silica, and then the sand. Dissolving the product was by mobile gradient phase, which TLC optimized. Finally, tubes were collected after elution for analysis.

2.6 Cell culture and Cytotoxicity Assay

To support the growth of the human hepatic stellate (LX-2) and Hek293T, 1% l-glutamine, 10% fetal bovine serum, and antibiotics (penicillin and streptomycin) were added to RPMI-1640 medium.

Also, we did the cytotoxicity assay of B16F1, HepG2, MCF-7, HeLa, Hep3B, and CaCo-2 cells, the cytotoxicity results of the compounds were compared to the 5-FU positive control.

Typically, the culture flask was gently tilted, and care was taken to avoid damaging the cell monolayer during the perfusion of culture media from the culture vessel. They are being cautious not to rupture the cell monolayer as they remove the DPBS from the culture flask. Next, 1 ml of trypsin was added, and the mixture was gently rotated before being incubated for 5 minutes at 37 °C. Once most of the cells have split, mix in 10 milliliters of uniform culture medium and gently mix or pipette the culture suspension to ensure that all of the neutralization has occurred. In order to prevent disturbing the cell, the media was carefully isolated after a 24hour period. To stop the drug's effect on absorbance, different drug concentrations were added to each well in 100 µl increments, and the mixture was then incubated for 72 hours at 37 °C without messing with the cell. Following the guidelines provided by the manufacturer (Promega Corporation, Madison, WI), The Cell Tilter 96® aqueous one solution cell proliferation (MTS) test was used to determine the cells' viability. An ELISA reader was used to measure the absorbance at 49 nm after adding 20 µl of MTS solution and incubating for two hours in order to determine the IC50. It was twenty degrees Celsius in the freezer.

2.7 Biological Assay on COX Enzyme Screening Kits

This study assessed the inhibitory effect of human recombinant COX-2 and bovine COX-1 on arachidonic acid PGH2 transformation using the COX (human) Inhibitor Screening Test Kit (derived from Cayman Chemicals assay kit No. 560131). Because of their structural resemblance to celecoxib, the prepared products served as the test's positive control. When configuring the reagents and testing procedures, the manufacturer's instructions were adhered to. After dissolving the inhibitors at two different concentrations (40 and 10μM) in dimethyl sulfoxide (DMSO), celecoxib was added. Subsequently, a combination of COX-1 or COX-2 enzymes was incubated for ten minutes at 37°C. The diluted reaction buffer contained a house-related item. Then put 10μl of arachidonic acid and let it sit at 37°C for 30 seconds to start the chemical reaction. 30 μl of stannous chloride was added to stop the enzyme's catalysis, and the mixture was then allowed to sit at room temperature for five minutes. ELISA quantification was performed on the generated prostaglandins. The 96-well plates were incubated at room temperature for eighteen hours on an orbital shaker after being covered with plastic film. The plate was then cleaned five times using a wash buffer after the wells were emptied. Next, each well-received 200μl of Ellman's reagent and 5μl of tracer in addition to the TA wells. The plate was left in the dark at room temperature for 60 to 90 minutes, or until the absorbance of the B0 was between 0.3 and 0.8 at 405 nm. To read the plate, a Microplate Reader 6000 Unilab was employed. The inhibitory response to the concentration curve could be used to determine the IC50. The IC50s of COX-1 and COX-2 were divided in order to calculate the selectivity index (SI).

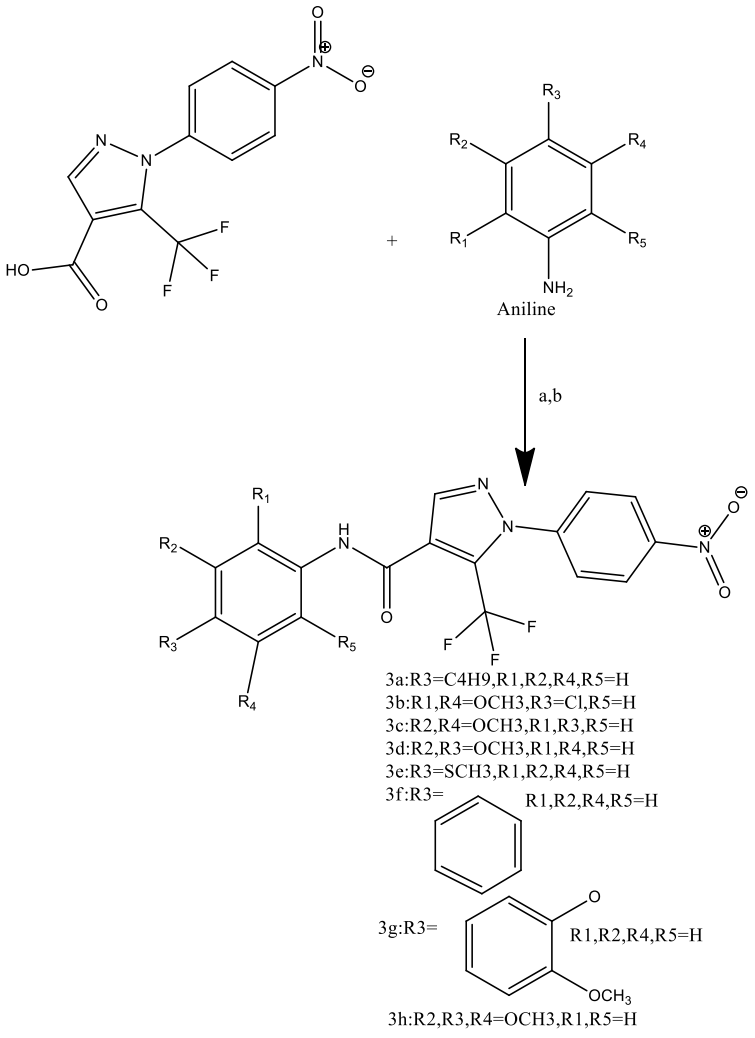
Chapter Three

Results

3.1 Chemistry

Scheme 1 shows that pyrazole-carboxamide (3a–3h) derivatives were made. 1-(4-nitrophenyl) 5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid was dissolved in 15 milliliters of DCM. After adding DMAP and EDCI, the mixture was agitated while an argon gas source was present. After 30 minutes, aniline was added, and the mixture was shaken for 48 hours. Next, 32% HCl was extracted using sodium sulfate anhydrous as an adsorbent, and the mixture was filtered. We used column chromatography to purify each and every synthetic product.……

Scheme 1: After stirring acid (1) with Aniline derivatives in (15 ml) of DCM for 48–72 hours, the addition of DMAP (a) as an acyl transfer agent and EDCI as an activator of the carboxyl group (b) in the presence of argon gas.

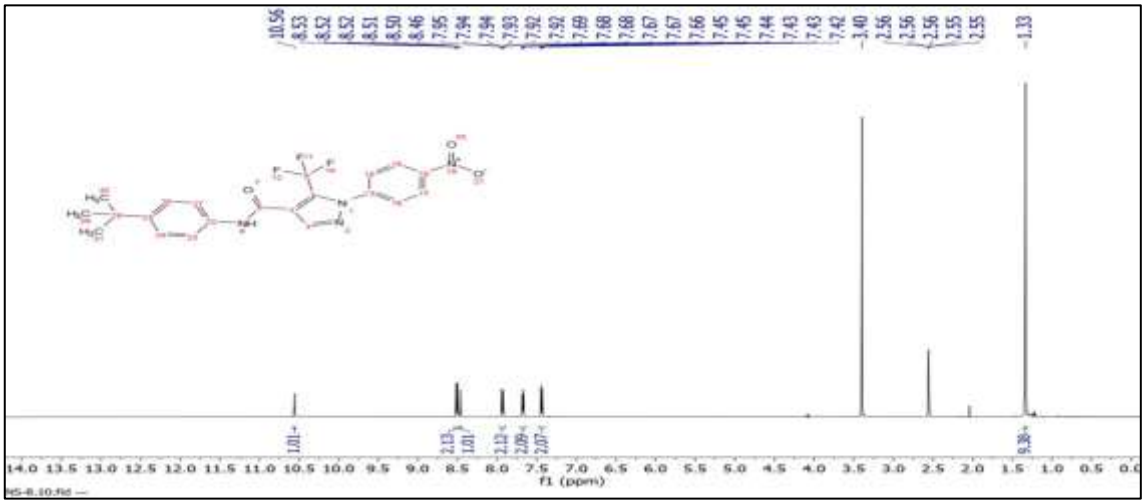


This scheme illustrates the steps involved in creating and analyzing derivatives from compound and aniline derivatives. The starting components are the acid (also referred to as acid) and the various substituted anilines (also referred to as aniline derivatives) that react with the acid. The aniline derivatives' substituents (such as R groups like -C_4H_9, -Cl, and - OCH_3) affect the final products, which can include 3a, 3b, and others. For 48–72 hours, so this process is conducted in dichloromethane (DCM). In the subsequent step, DMAP and EDCI are introduced in order to facilitate acylation inside an atmosphere of inert argon. The HRMS was used to verify the synthesized derivatives, which showed that the observed masses and estimated values of the products coincided, which is evidence that the synthesis was practical. For the chemicals that are being investigated, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy provides essential information regarding their structural details. The presence of an amide proton can be deduced from the acylation signature, a single signal that falls within the range of 8.26–10.41 ppm. In addition, the range of signals is exhibited in the aromatic area, which reflects the complexity of the aromatic systems present in the final product.

To be more specific, the pyrazole ring is responsible for the generation of unique signals that are characteristic of the protons on the ring structure in the range of 6–8 ppm. In addition, at approximately 2.73 ppm, a noticeable signal corresponding to the pyrazole rings -CH_3 group may emerge. This signal confirms the presence of the ring as well as its structure. NMR spectroscopy, which is a form of nuclear magnetic resonance based on carbon-13, can be utilized to get further information regarding the compounds. The presence of amide functionality is confirmed by a significant peak recorded at 170 ppm, which can be attributed to the carbonyl carbon of the amide. There is an inference that complex aromatic compounds are present based on the signals that were discovered between 160 and 90 ppm. These signals display a wide range of aromatic carbons. Furthermore, a signal that corresponds to the pyrazole ring's -CH2 group offers additional proof for the structure of the pyrazole ring which is provided by the signal.

Here, this is an example of 3a H-NMR, which amide appears at 10.5 ppm, 9H of 3(CH3) appears at 1.3 ppm, other parallel H protons give a signal between 7.5 and 8.5 ppm, and finally the solvent DMSO appears at approx. 3.5 ppm.

An example of H-NMR for 3a compound:



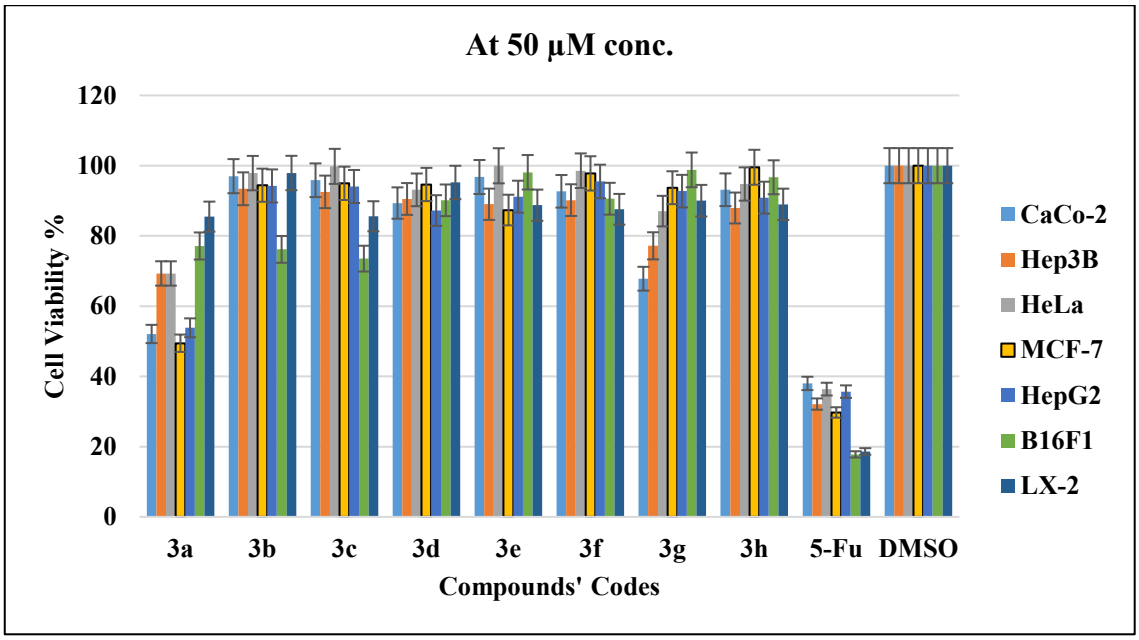
This schematic illustration effectively shows a synthetic pathway that leads to several derivatives of aniline. The characterization results (HRMS, ^{1}H NMR, and ^{13}C NMR) demonstrate the effective formation of the needed products and highlight significant spectrum features that uphold the structural integrity of the synthesized compounds. Understanding the chemical properties and potential applications of these substances hinges on this description. The present version preserves the background of the synthesis and characterization procedure while emphasizing the use of pyrazoles in NMR analysis.

3.2 *In Vitro* Evaluation of Cytotoxicity

Hek293t (normal kidney) and LX-2(normal liver) were employed in the MTS test to ascertain the pyrazole carboxamide derivatives' cytotoxic potential. This test used five different concentrations (300, 100, 50, 10, and 1 μM). According to the test, all compounds have low inhibition percentages on the Hek293t cell line all concentrations compared to 5-FU, despite that the concentrations used for testing the inhibition were tenfold of the concentrations used for testing the activity of the produced compounds on COX enzyme. The inhibition results are mentioned in Figure 3.1. All compounds showed slightly cytotoxic activity against evaluated normal cell lines. The cell viability percentage values of all lines were so close to the values of DSMO (no medical compounds), except compound 3a, which influences the cell viability of CaCo-2, HepG2, and Hep3B lines. The cytotoxicity results of all of their compounds are explained in Figure 3.1 compared to the positive control (5-FU).

Figure 3.1

Utilizing LX-2, B16F1, HepG2, MCF-7, HeLa, Hep3B, and CaCo-2 cells, the cytotoxicity results of the compounds were compared to the 5-FU positive control



Two serial concentrations (40 and 10 µM) of ketoprofen were tested, with the drug serving as a positive control. Table 2 displays the calculated 50% of the maximum inhibitory level (IC50) and selectivity (SI) for the prepared compounds.

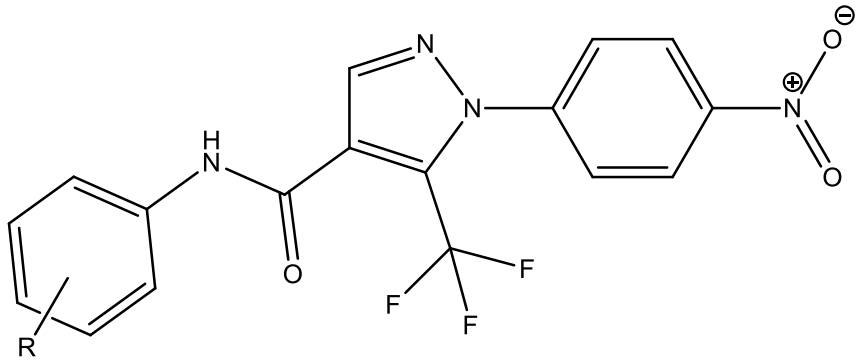


Table 3.1

Shows the calculated 50% of the maximum inhibitory level (IC50) and selectivity (SI) for the prepared compounds

			IC50 µM			% inh. at 50 µM	
Original code	new code	R	COX1	***COX2***	SI	LX-2	Hek293t
NS8	3a	4-tert-butyl	6.62±0.87	9.03±0.66	0.73	14.51±1.55	19.73±2.75
NS10	3b	4-chloro-2,5dimethoxy	0.46±0.25	3.82±1.36	0.12	2.08±0.25	20.14±1.65
NS11	3c	3,5-dimethoxy	1.74±1.02	5.78±0.47	0.30	14.05±2.88	7.79±0.81
NS12	3d	3,4-dimethoxy	5.59±2.14	4.92±2.29	1.14	4.78±1.04	7.40±0.69
NS13	3e	4-S-CH3	6.67±1.81	8.98±1.85	0.74	11.25±0.99	13.08±1.08
NS14	3f	4-aminobiphenyl	5.91±1.11	6.00±1.33	0.98	12.42±1.25	13.29±2.00
NS16	3g	4-(2-methoxyphenoxy)	4.45±2.07	2.65±1.55	1.68	9.97±2.04	11.97±1.68
NS17	3h	3,4,5-tri methoxy	4.51±0.45	4.82±0.99	0.93	10.99±0.15	10.60±1.89
+ ve control			0.035±0.015[a]	0.164±0.12[a]	0.21[a]	81.36±2.42[b]	73.05±2.35 [b]

[a]Ketoprofen, [b]5-FU, $pValue \leq 0.05$.

In the results table we can see that 3a with 4-tert-butyl branch, and 3e with (4-SCH3) branch have so close results for IC_{50} both against COX-1 and COX-2 and SI. They have the lowest activity with IC_{50} (6.62±0.87, 6.67±1.81) µM against COX-1 respectively and (9.03±0.66, 8.98±1.85) µM against COX-2 respectively, but they have good SI regarding reference drug.

The addition of chloro atom for 3b compound with (chloro- dimethoxy moiety), raises the activity against COX-1 compared to 3c and 3d compounds which have dimethoxy moiety by approximately four folds of 3c and ten folds of 3d. For COX-2 also 3b showed a slight significance than 3c and 3d which have IC_{50} of (3.82±1.36, 5.78±0.47, and 4.94±2.29) µM respectively. On the other hand, 3d has the highest SI in this comparison group (1.14).

Regarding SI 3g has the highest value of all synthesized compounds with (1.68) which is also higher than the reference drug, this happens because of the presence of methoxyphenoxy as a bulk group. Also, 3g is the most active synthesized compound against COX-2 with (2.65±1.55) µM value.

3f and 3h also have good SI with (0.98, and 0.93) respectively which means that the addition of 4-aminobiphenyl or trimethoxy raises the selectivity index compared to ketoprofen (0.21) SI.

Chapter Four

Discussion and Conclusion

4.1 Discussion

This study focused on the synthesis, characterization, and biological evaluation of eight novel pyrazole-carboxamide derivatives, and our goal is to employ a simple synthesis of a range of pyrazole-based COX-2 inhibitors targeting selective inhibition of COX-1 and COX-2 enzymes as part of an anti-inflammatory therapeutic approach. Non-steroidal anti-inflammatory drugs (NSAIDs) are known for their capacity to inhibit COX enzymes, thus alleviating inflammation, pain, and fever. These derivatives were strategically designed to enhance binding affinity to COX enzymes by including substituents that influence COX-1 and COX-2 selectivity and potency.

The synthesized compounds displayed diverse inhibitory profiles across COX-1 and COX-2. Compound 3b, containing a 4-chloro-2, 5-dimethoxy group, exhibited potent COX-1 inhibition with an IC50 of 0.46 ± 0.25 μM. This finding aligns with previous reports indicating chloro substituents enhance COX-1 binding (Shtamburg, et al., 2012). Compound 3b also displayed a low COX-2 IC50 (3.82 ± 1.36 μM), indicating effective inhibition of both COX isoforms. However, despite its high potency, 3b's selectivity index (SI) remained lower (0.12), suggesting a balanced inhibition without preferential COX-2 targeting. In contrast, compound 3g (4-(2-methoxyphenoxy) substituent) demonstrated the highest selectivity for COX-2 (SI = 1.68), outperforming ketoprofen, which has an SI of 0.21. The bulkier methoxyphenoxy substituent in 3g likely promotes this selectivity by fitting optimally within the COX-2 binding site, as reported by (Ch et al., 2017). Additionally, 3f (4-aminobiphenyl) and 3h (3,4, 5-trimethoxy) also showed favorable selectivity indices (0.98 and 0.93, respectively), further supporting the notion that bulkier substituents enhance COX-2 selectivity.

Two concentrations of ketoprofen (40 and 10 μM) were employed as a benchmark to compare COX inhibition and selectivity. Ketoprofen's COX-2 selectivity index (SI = 0.21) was surpassed by several synthesized compounds, particularly 3g, with an SI of 1.68. Compounds 3a (4-tert-butyl) and 3e (4-S-CH3) displayed higher IC50 values (6.62 ± 0.87 and 6.67 ± 1.81 μM, respectively) with relatively low selectivity indices. This result aligns with findings by Abraham (2017), suggesting that while tert-butyl and -

SCH3 groups provide stability, they do not significantly enhance selectivity or potency in COX inhibition.

Moreover, the presence of a dimethoxy moiety influenced COX-2 inhibition. Compound 3b, with a 4-chloro-2,5-dimethoxy group, showed superior activity against COX-1 and COX-2 compared to other dimethoxy analogs, such as 3c and 3d, indicating that the addition of chlorine significantly enhances COX-1 binding. The structural arrangement of methoxy groups also affected selectivity. Compound 3d, with methoxy groups at both meta and para positions, demonstrated a selectivity index of 1.14 for COX-2, in contrast to 3c, where methoxy groups are positioned at two meta locations (m, m). This trend aligns with the Bergin (2014) work, which associates methoxy substituents with increased affinity for COX-2 binding.

Structural validation of the synthesized derivatives was confirmed via FTIR, HRMS, and NMR analyses. The melting points of all compounds fell within a narrow range, suggesting high purity and reproducibility in synthesis. The HRMS spectra matched the expected molecular weights, and NMR analysis confirmed the successful functionalization of each compound in line with theoretical expectations.

The findings from this study reveal that introducing bulkier groups and electron-withdrawing substituents on pyrazole-carboxamide scaffolds effectively enhances COX-2 selectivity. Notably, compounds 3g, 3f, and 3h show promise as COX-2 selective inhibitors with potential therapeutic applications, demonstrating superior selectivity indices compared to ketoprofen. The structural diversity and favorable safety profile of these derivatives make them suitable candidates for further exploration as anti-inflammatory agents.

Future studies should include molecular docking analyses to better understand the binding dynamics within COX-2 and identify potential modifications to improve selectivity. Moreover, the selective cytotoxicity observed in compound 3a against cancer cell lines warrants further investigation into its potential dual role in anti-inflammatory and anticancer therapies.

In summary, the novel pyrazole-carboxamide derivatives synthesized in this study have established promising COX inhibitory activities and notable COX-2 selectivity,

particularly in compounds 3g and 3h. These findings contribute to the growing body of knowledge on COX inhibitors, paving the way for more effective and selective NSAID development.

4.2 Conclusion

The researcher summarizes the outcomes of the synthesis of several novel pyrazole analogs in this thesis. When it comes to inhibiting the COX-1 enzyme, synthetic analog 3b is the most effective. It exhibited strong anti-COX-2 activity as well. All compounds exhibited good selectivity towards COX-2, with the exception of 3b, which showed lower selectivity than the positive control. The analog with the highest selectivity ratio, 3g, was compared to ketoprofen.

Comparing the compounds to ketoprofen, all but 3b demonstrated superior selectivity on COX2. This discrepancy could be because the compounds' multi-methyl moieties have larger hydrophobic properties than Ketoprofen's phenyl moiety. In comparison to the 5-FU product, all compounds, at all test concentrations, have a minor cytotoxic effect on normal cell lines, such as LX2 and Hek293t. However, compared to the cytotoxic concentrations, the effect dose on the COX enzyme was at least ten times lower. But 3a showed some efficacy against some cancer cell lines, including CaCo-2 (colon cancer), HepG2 (liver cancer), and MCF-7 (breast cancer).

4.3 Limitations and Recommendations of the Study

In future research, it is advised to synthesize more analogs with a wider variety of substitutions and conduct docking studies to better understand the structure-activity relationship and improve COX2 selectivity.

List of Abbreviations

Abbreviation	Meaning
µl	Microliter
µM	Micro molar
Mg	Milligram
MH	Megahertz
Hz	Hertz
Ppm	Part per million
5-FU	5-fluorouracil
CC	Cytotoxic Concentration
COX	Cyclooxygenase
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMSO	Dimethyl sulfoxide
DMSO-d_6	Dimethyl sulfoxide-d_6
EDCI	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
EtOAc	Ethyl acetate
FDA	Food and drug administration
HCL32%	Hydrochloric acid 32%
HIV	Human immunodeficiency virus
TB	Tuberculosis
HRMS	High Resolution Mass Spectrometry
hrs.	Hour or Hours
IC_{50}	The half maximal Inhibitory concentration
mmole	Millimole
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
nM	Nano molar
NMR	Nuclear Magnetic Resonance
NSAIDS	Non steroid anti-inflammatory drugs
^{o}C	Celsius Degree
PG	Prostaglandin
PGC-1 alpha	Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha
PGG2	Prostaglandin G2
PGH2	Prostaglandin H2

Abbreviation	Meaning
PGI2	Prostacyclin
DNA	Deoxyribonucleic acid
DMEM	Dulbecco's modified eagle's medium
DPBS	Dulbecco's phosphate buffered saline
pH	Potential of hydrogen
R_f	Retention factor
Rpm	Round per minute
MIC	Minimum inhibitory concentration
RPMI-1640	Roswell Park Memorial Institute Medium
SI	Selectivity index
TLC	Thin layer chromatography
NMR	Nuclear magnatic resonance
HRMS	High resolution mass spectroscopy
FTIR	Fourier transform infrared spectroscopy

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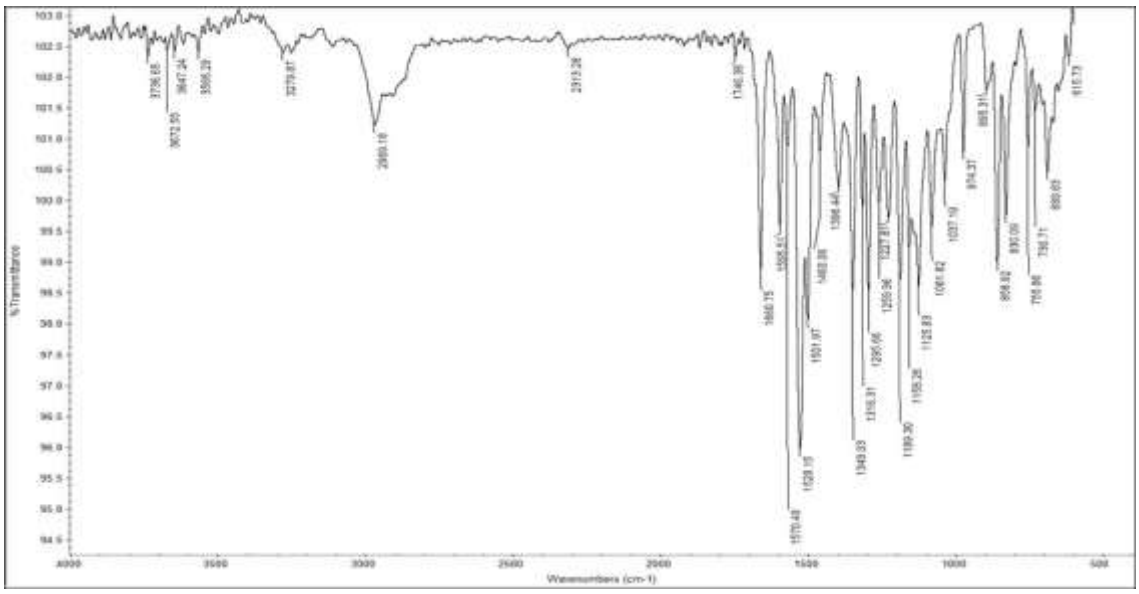
Appendices

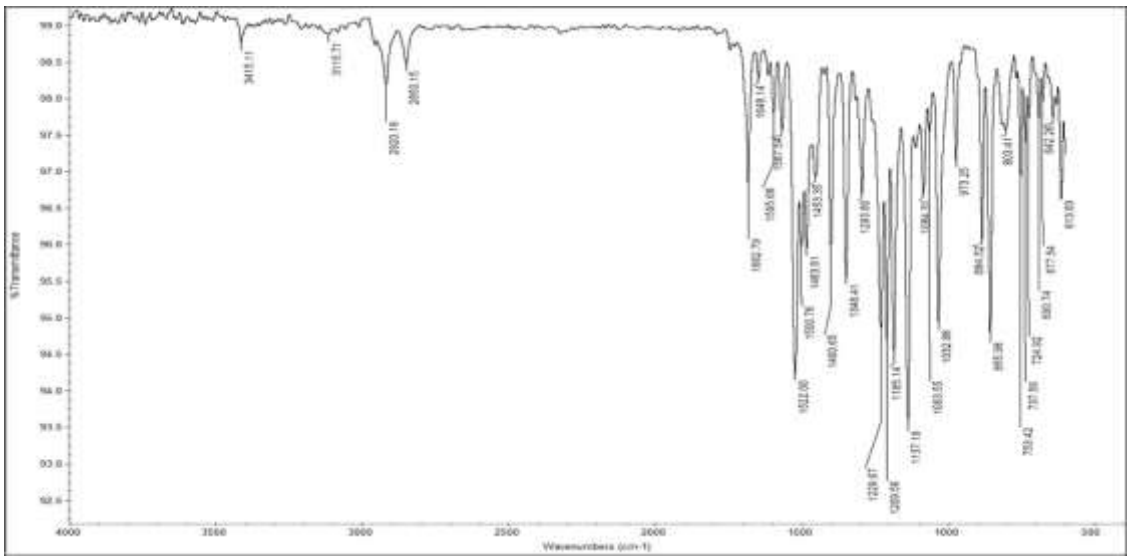
Appendix A

FT-IR for pyrazole-carboxamide derivatives compounds

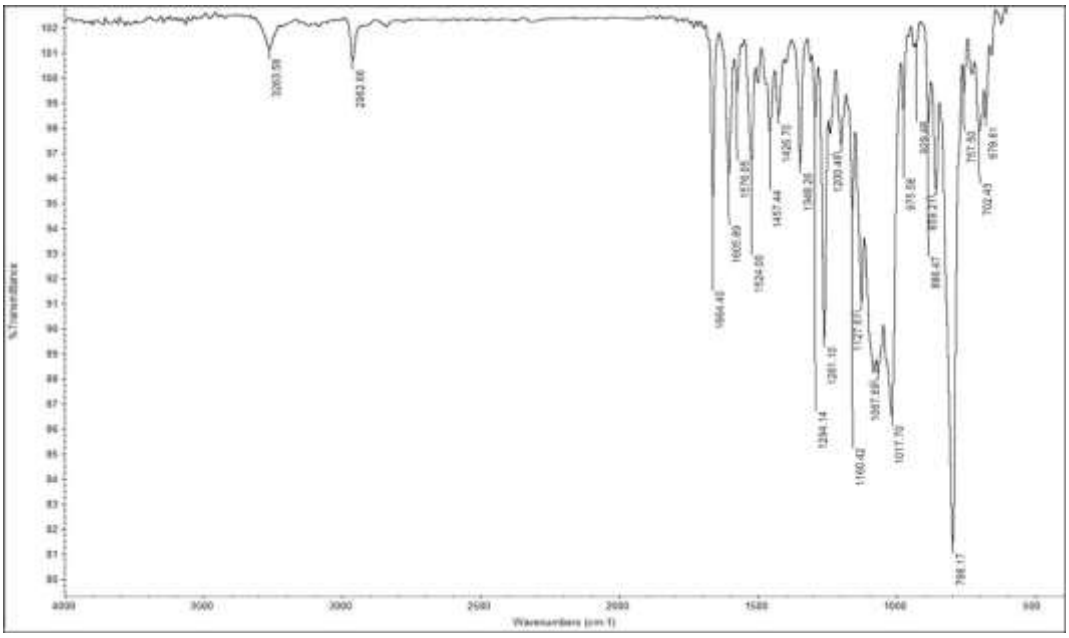
3a



3b



3c


%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
500
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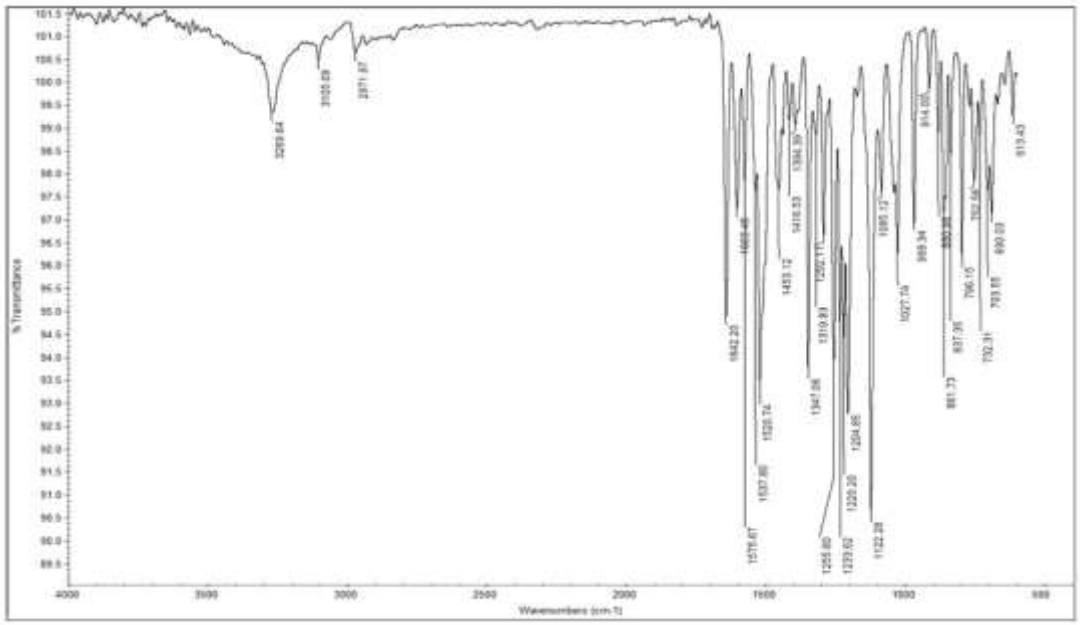
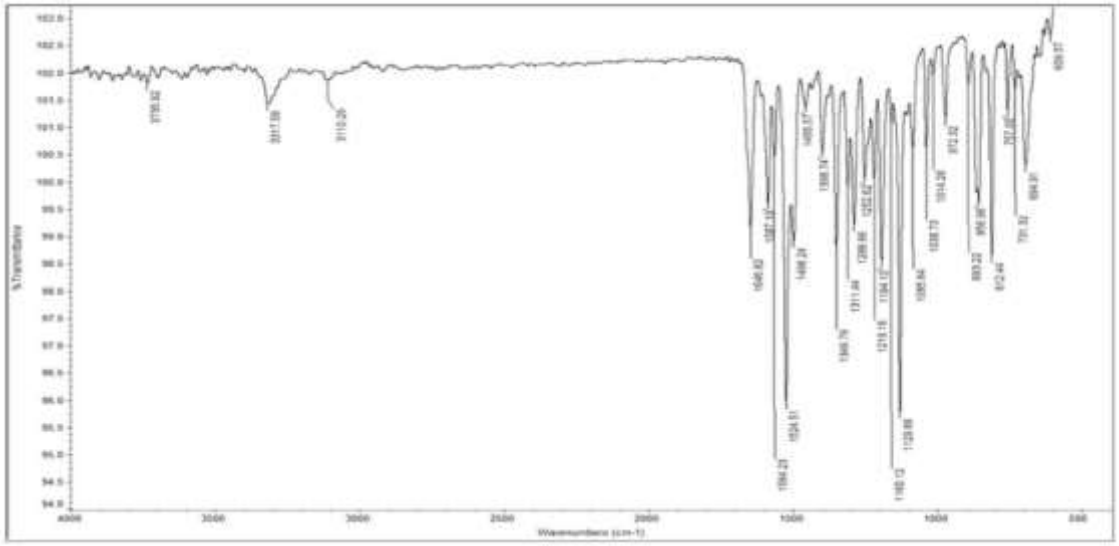
2942.66
1604.49
1570.65
1534.08
1457.44
1426.70
1366.26
1330.48
1281.10
1244.14
1160.42
1127.61
1087.69
1017.70
978.04
928.68
886.47
757.50
702.43
678.61
786.17

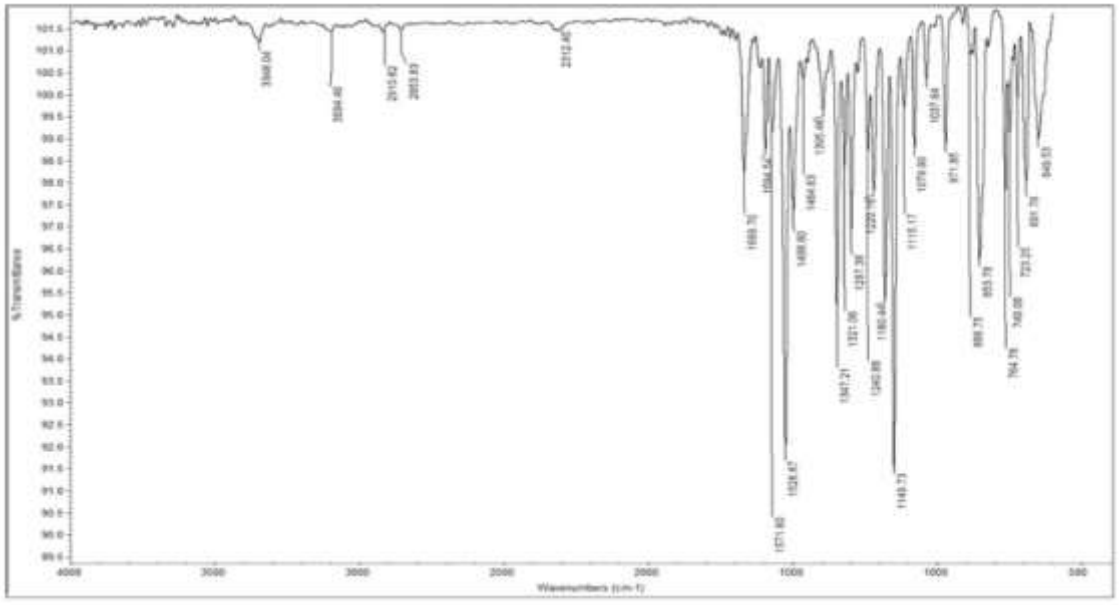
3d


% Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
500
3269.64
3105.09
2971.87
1642.25
1575.67
1537.80
1520.74
1453.12
1418.53
1394.39
1347.26
1319.93
1255.80
1233.52
1204.65
1122.28
1085.12
1027.79
969.34
881.33
837.95
796.15
732.31
703.65
680.03
619.43

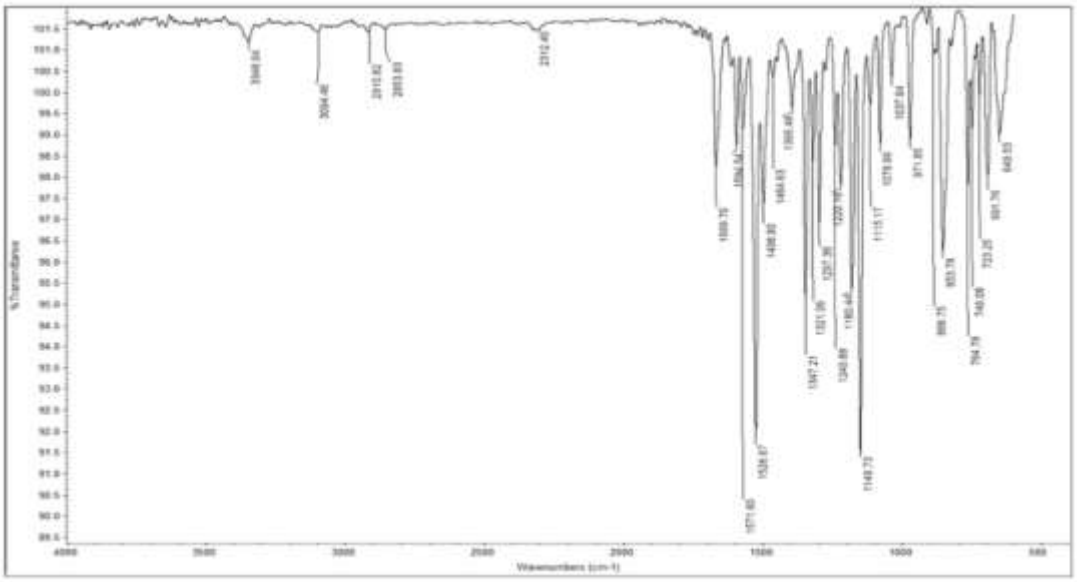
3e



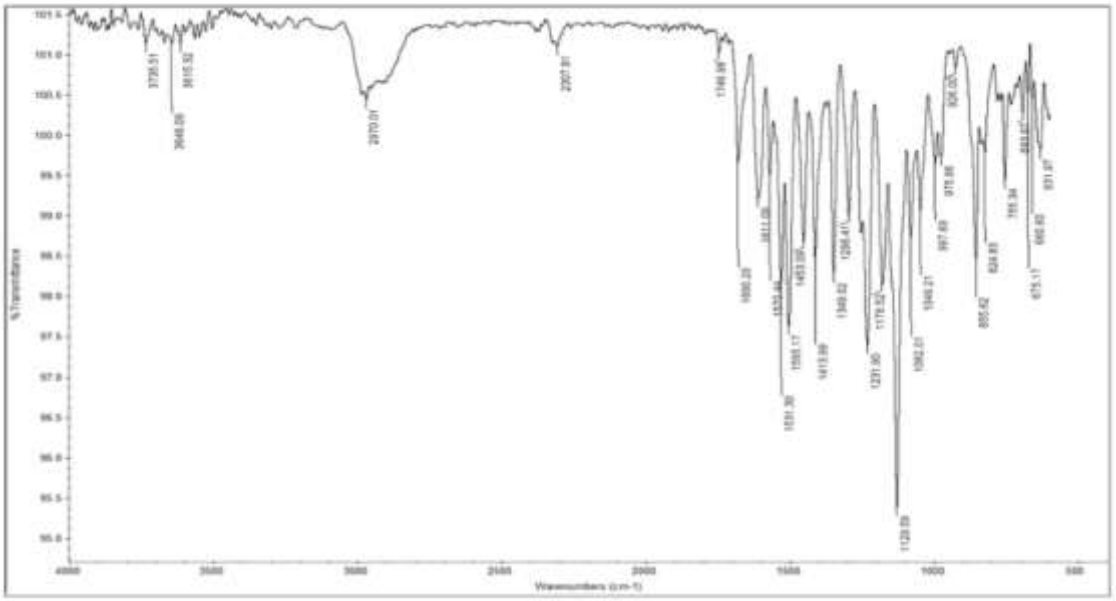
3f



3g



3h

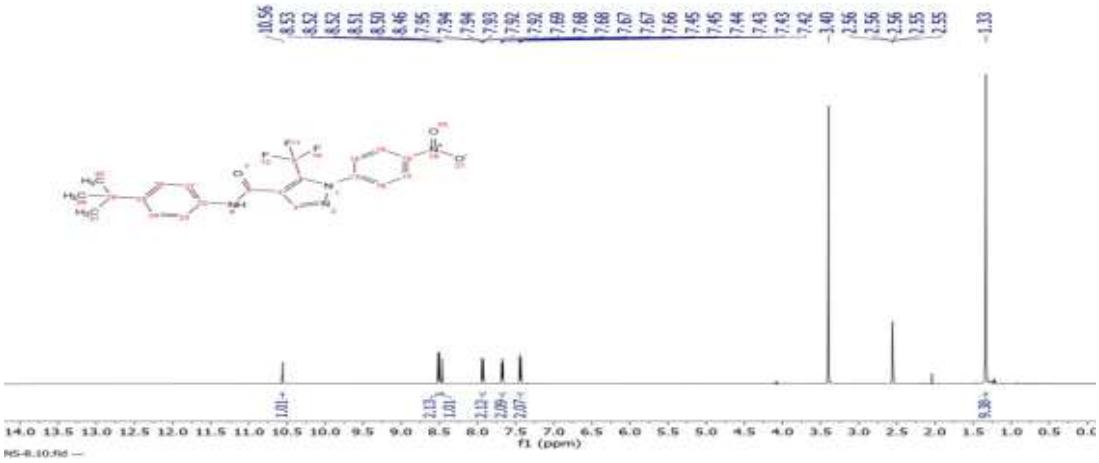


Appendix B

NMR spectrums (^{1}H-NMR and ^{13}C-NMR) for pyrazole-carboxamide derivatives compounds

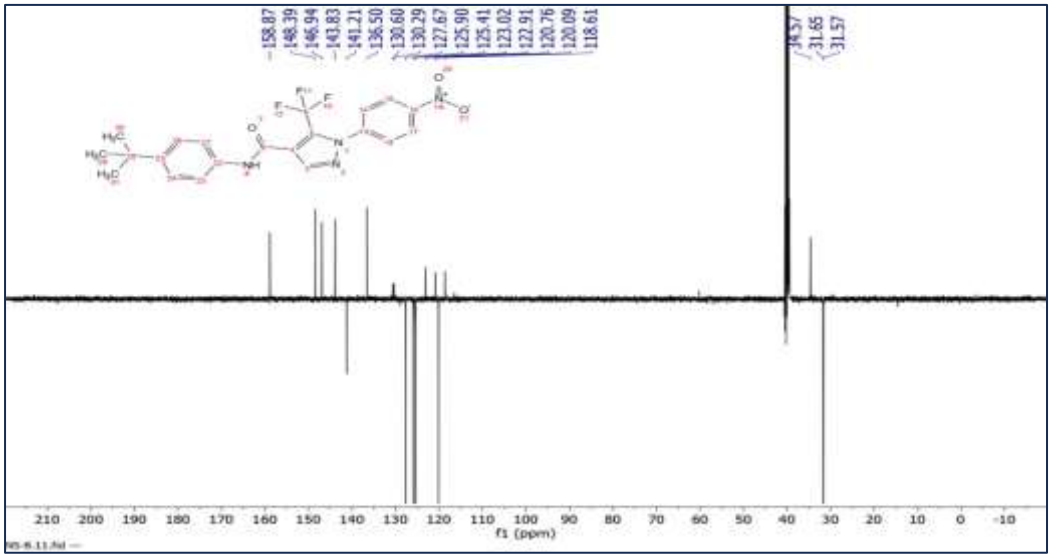
3a

H-NMR



3a

C-NMR



3b

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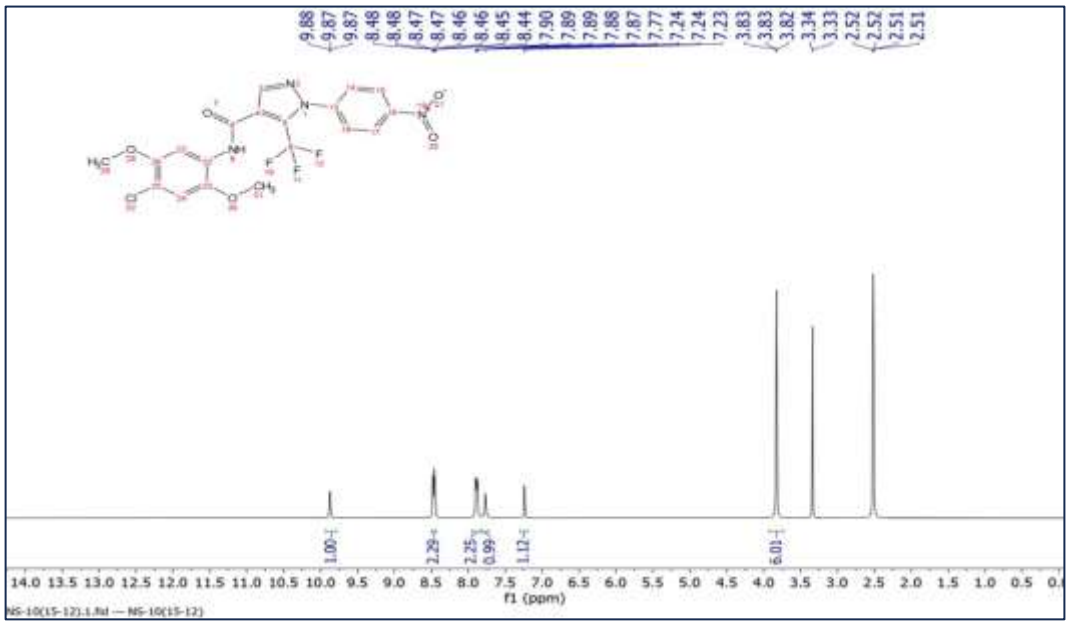

3b

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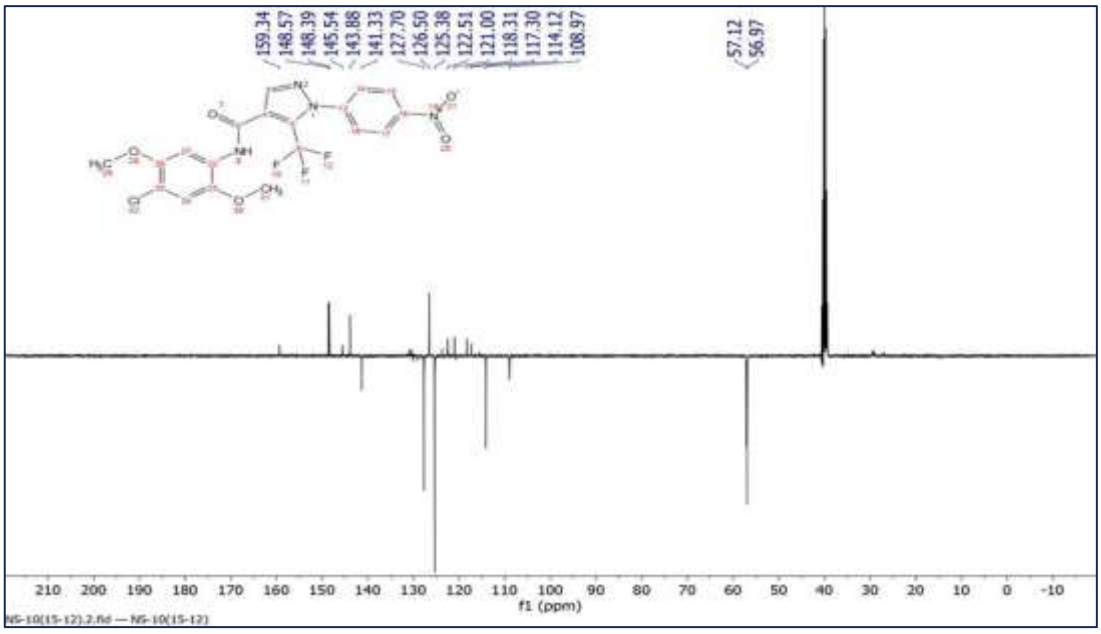

3c

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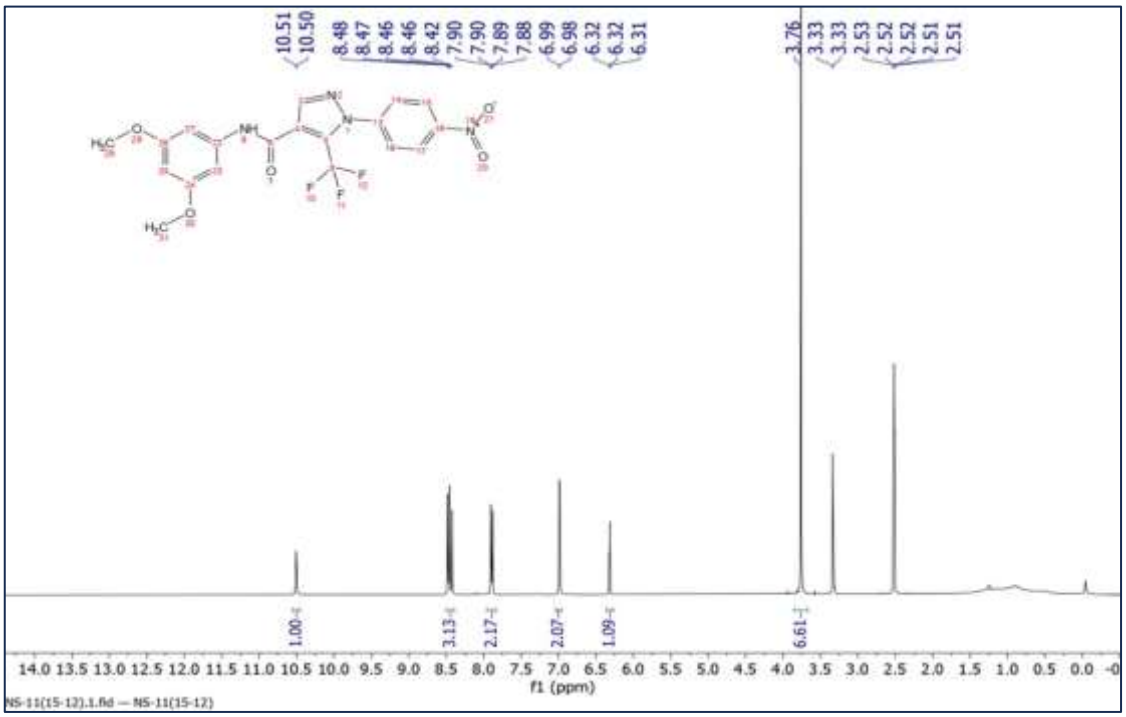
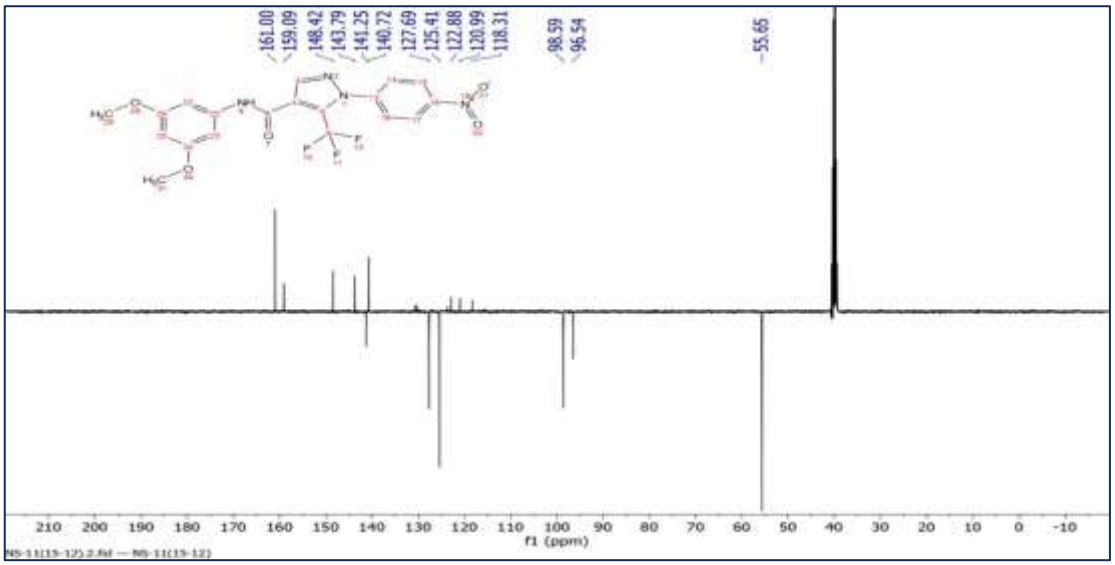


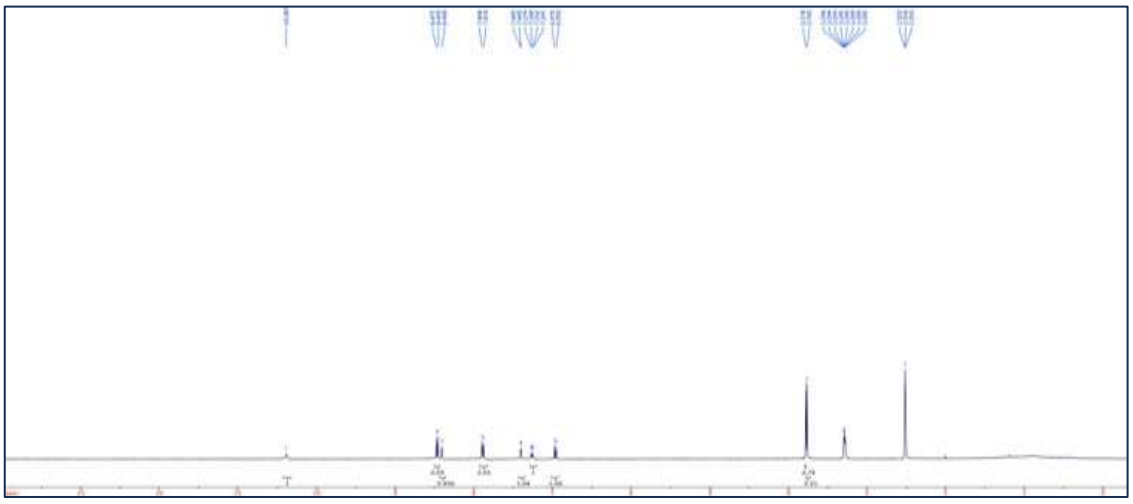
3c

C-NMR



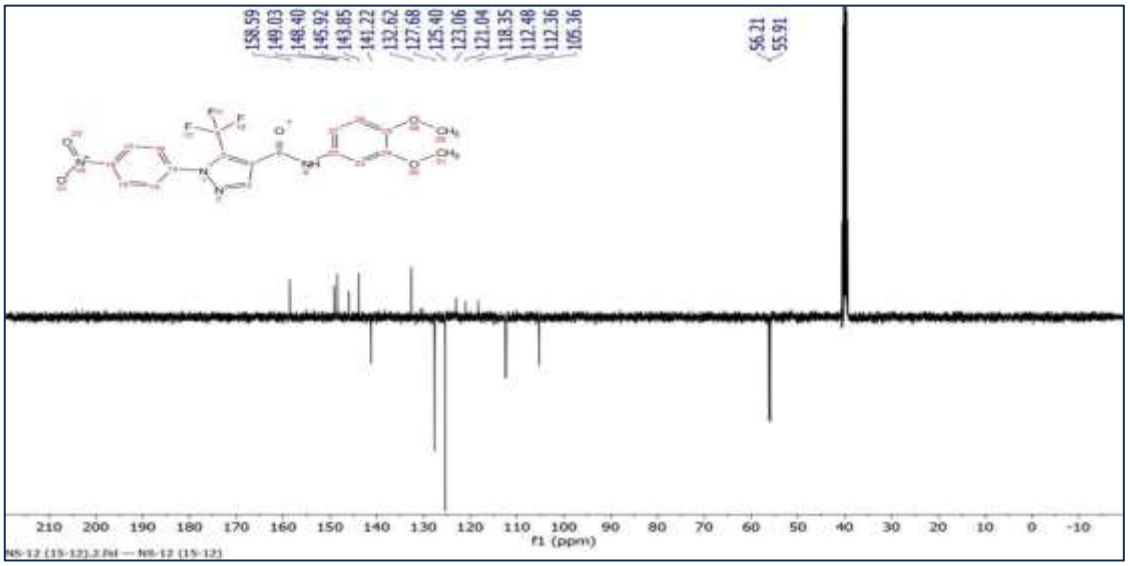
3d

H-NMR



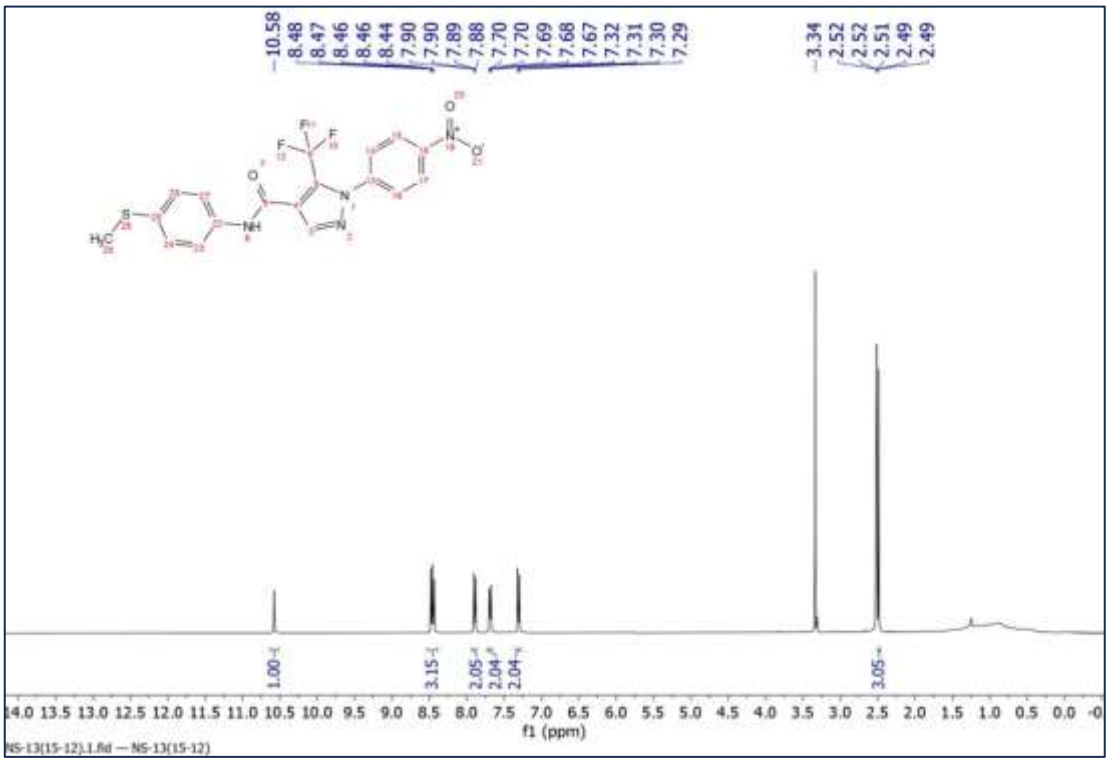
3d

C-NMR



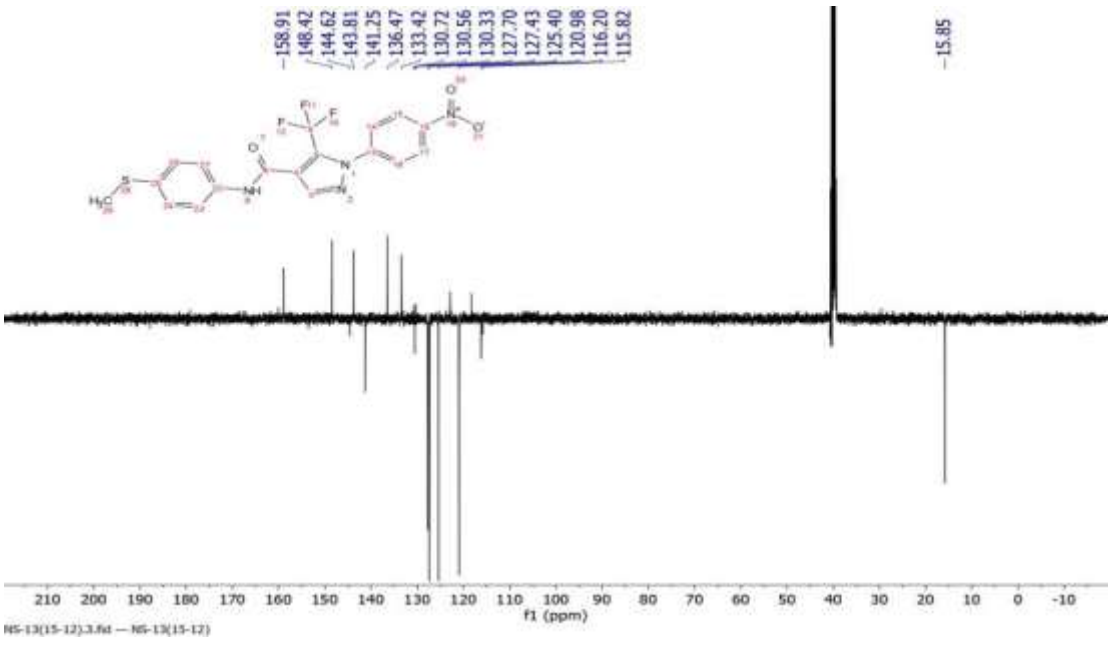
3e

H-NMR



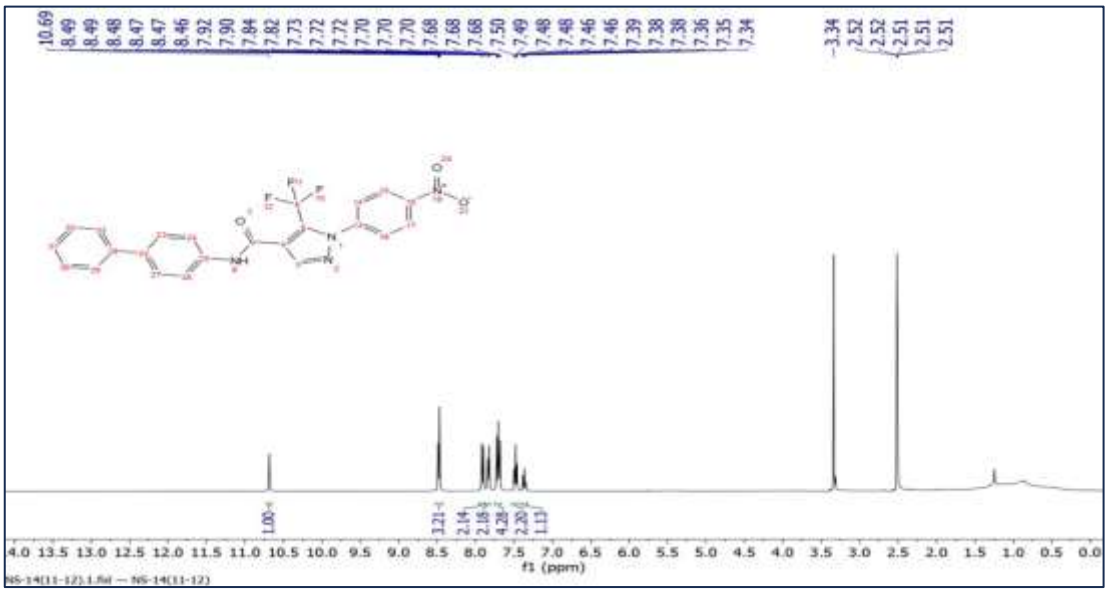
3e

C-NMR



3f

H-NMR



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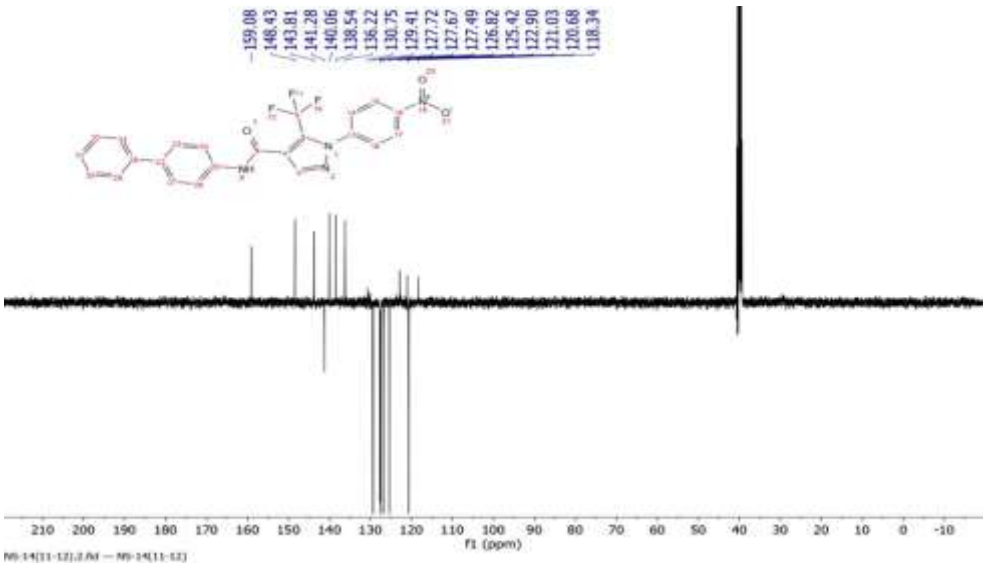
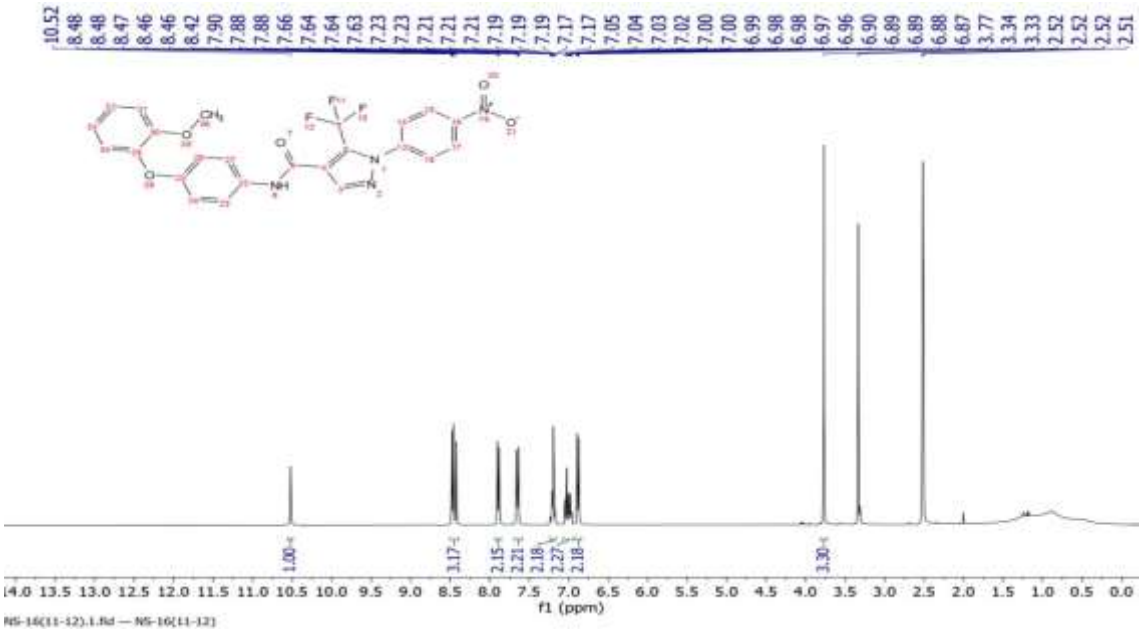
C-NMR



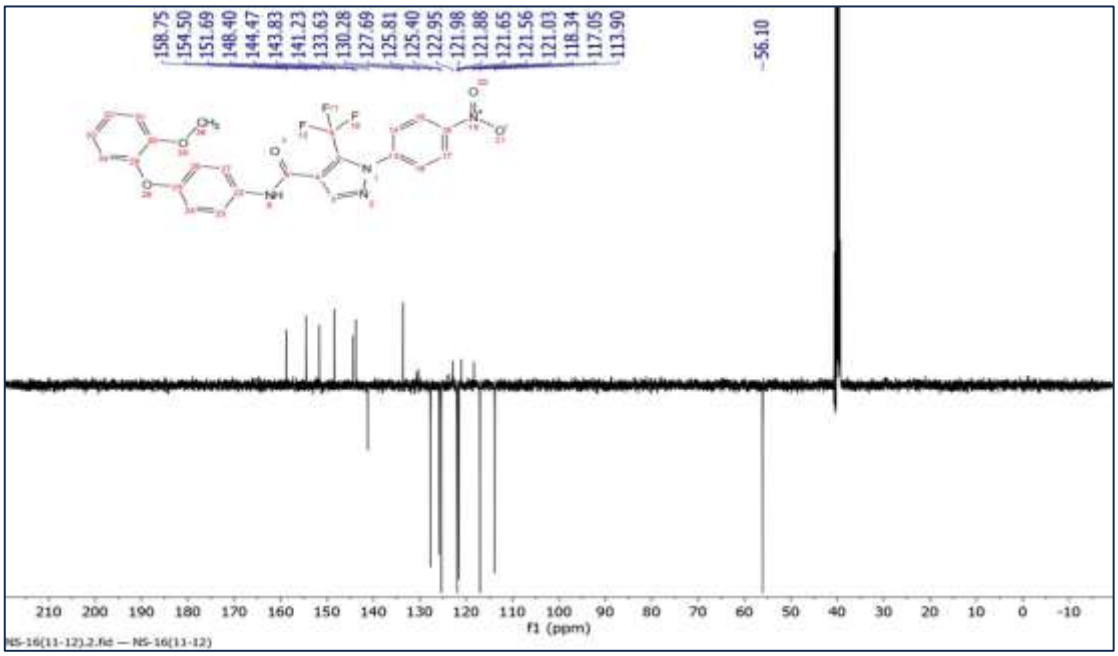
3g

H-NMR



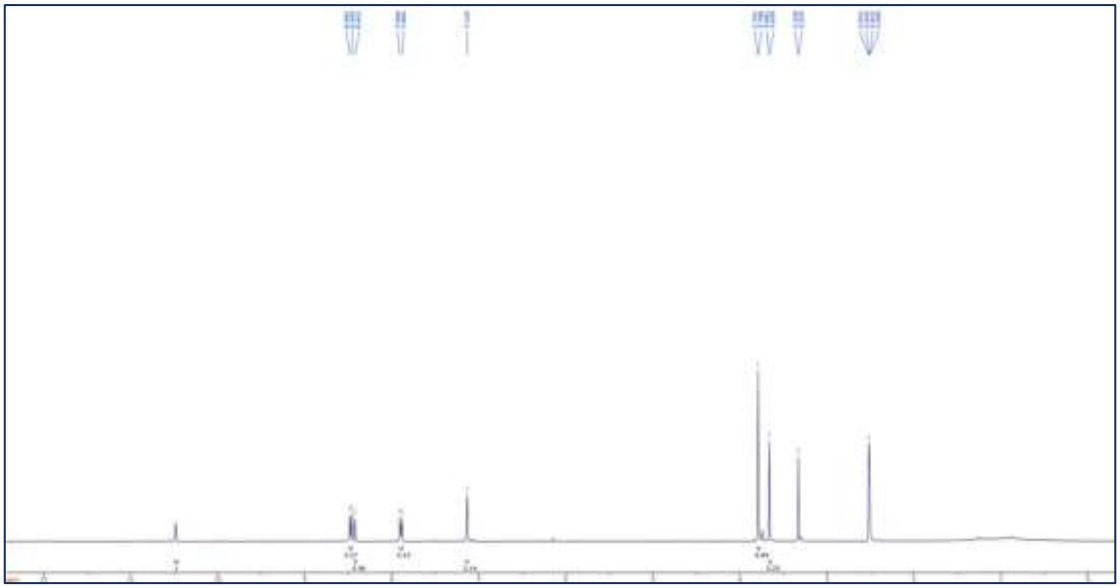
3g

C-NMR



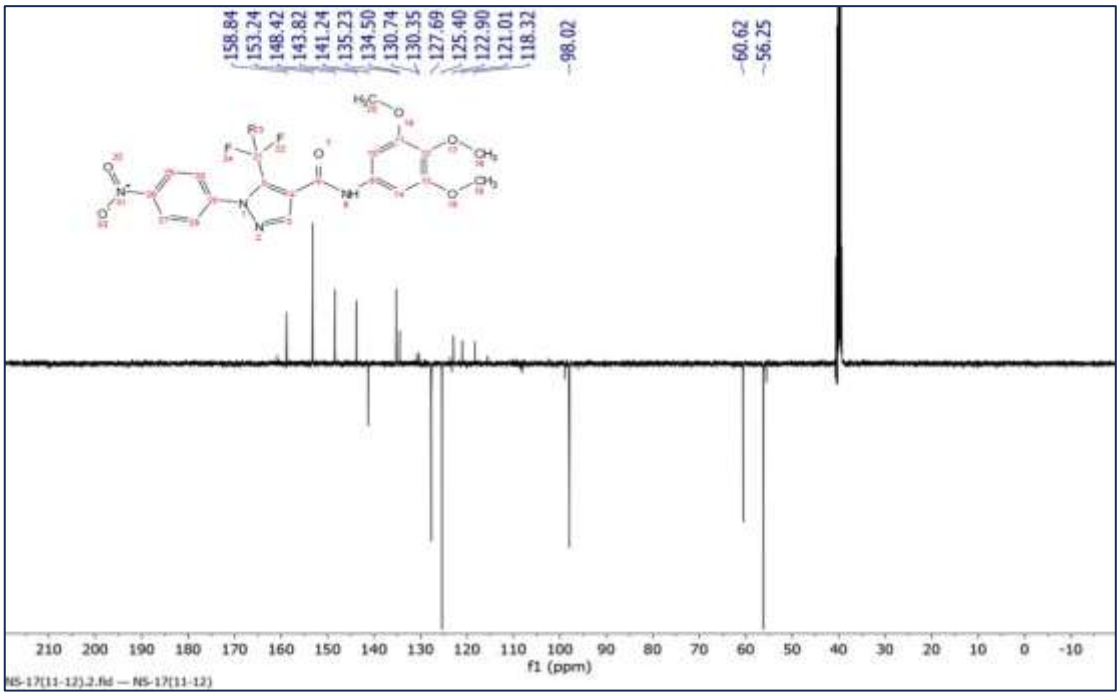
3h

H-NMR



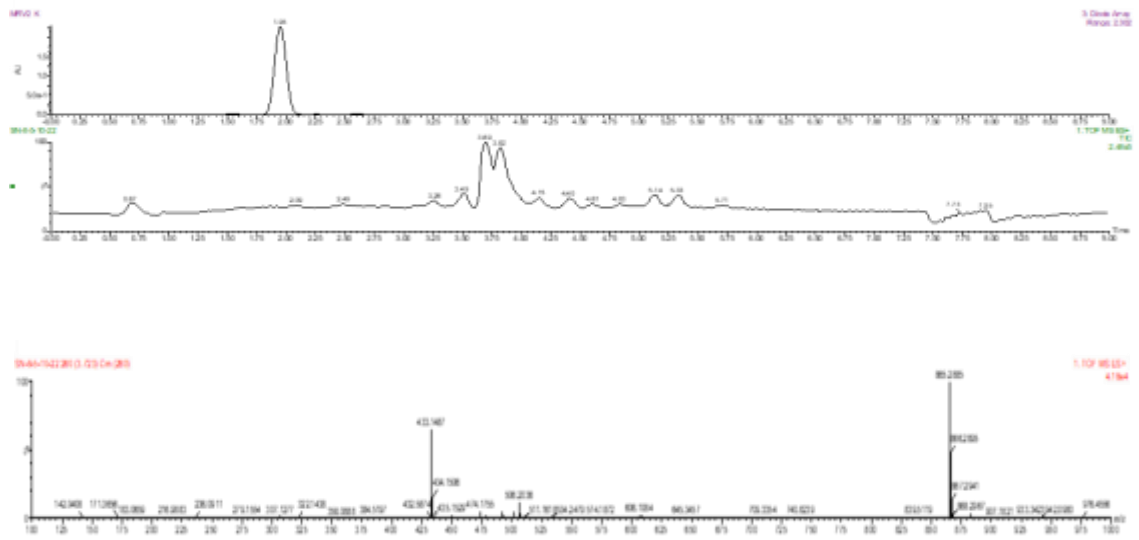
3h

C-NMR

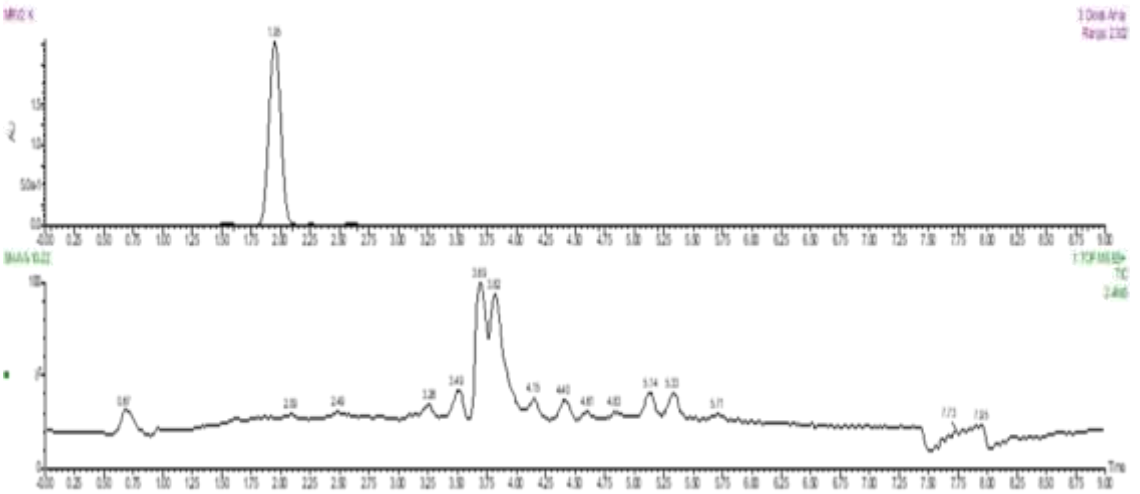

158.84
153.24
148.42
143.82
141.24
135.23
134.50
130.74
130.35
127.69
125.40
122.90
121.01
118.32
98.02
60.62
56.25
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
NS-17(11-12).2.fid — NS-17(11-12)

Appendix C
Mass spectroscopy HRMS

3a



3b



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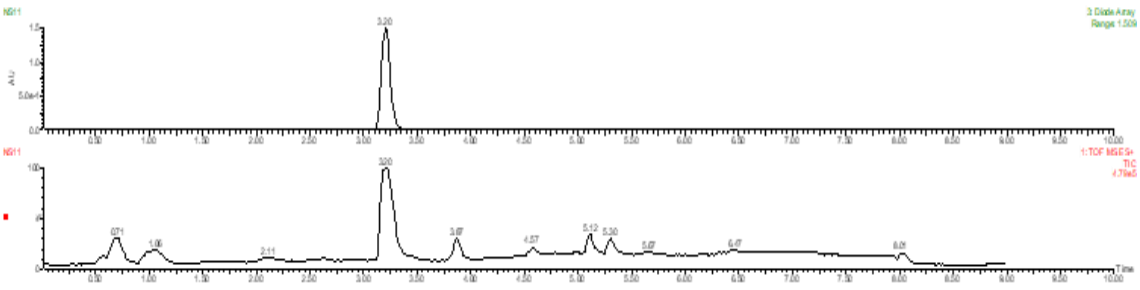

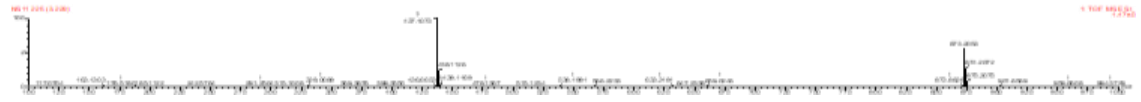

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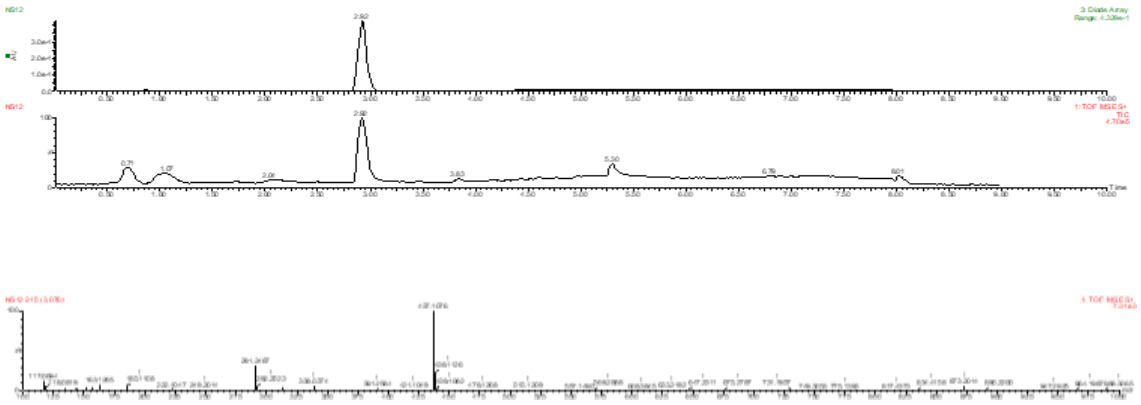
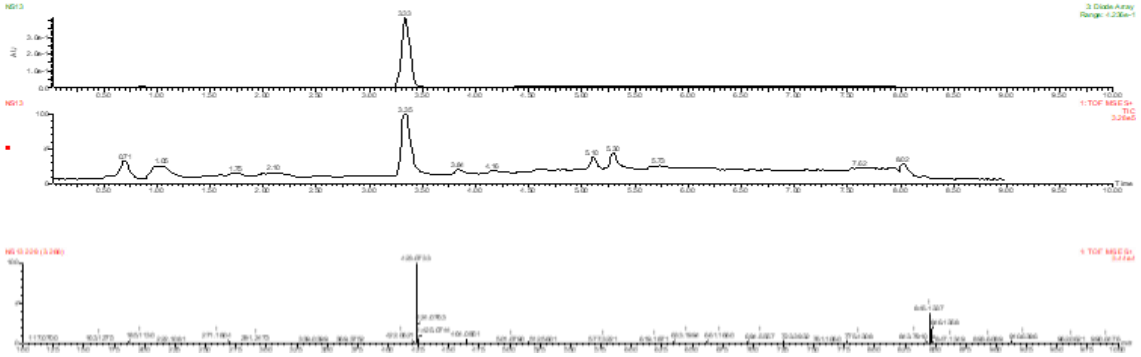
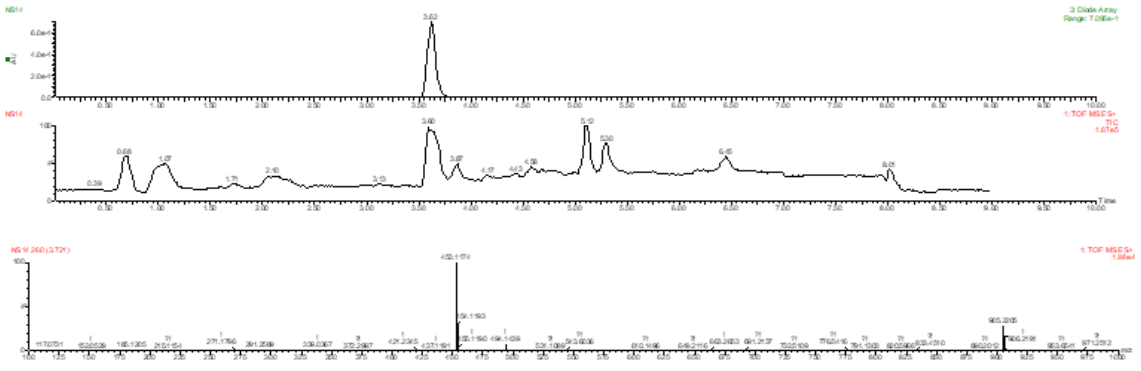


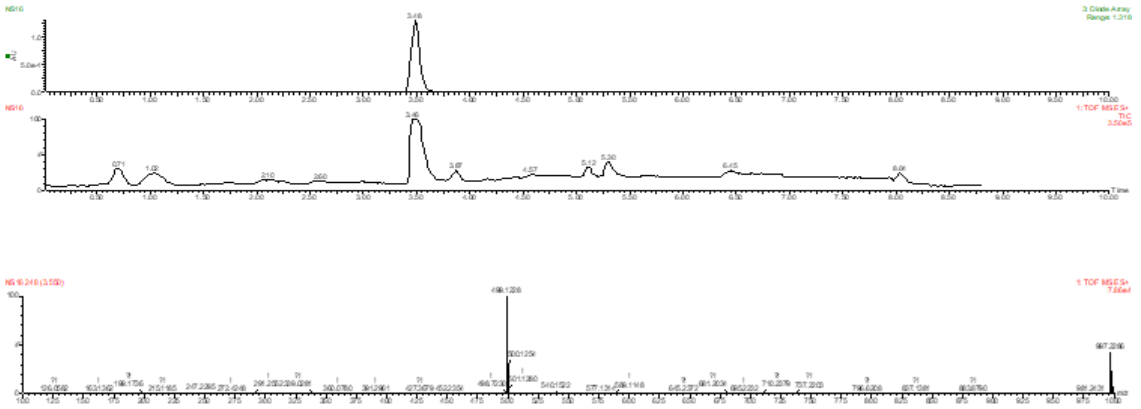
3e



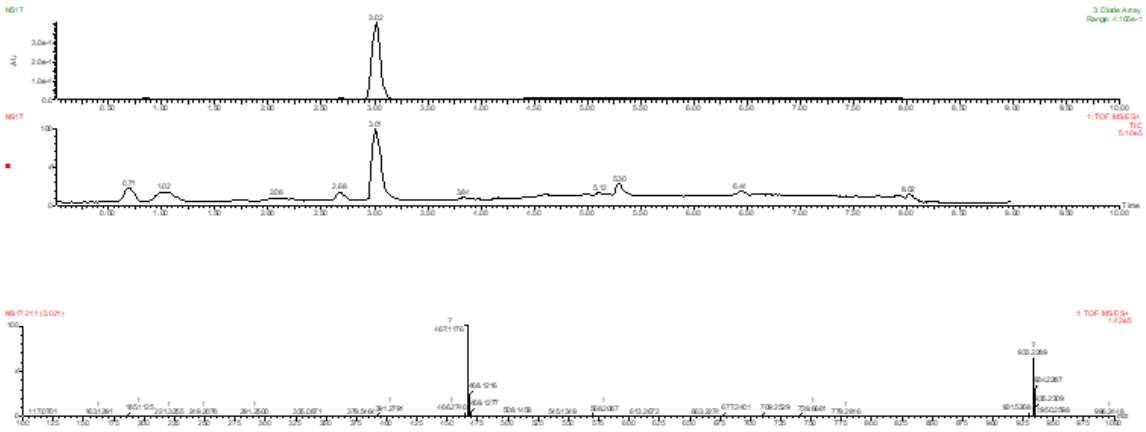
3f



3g

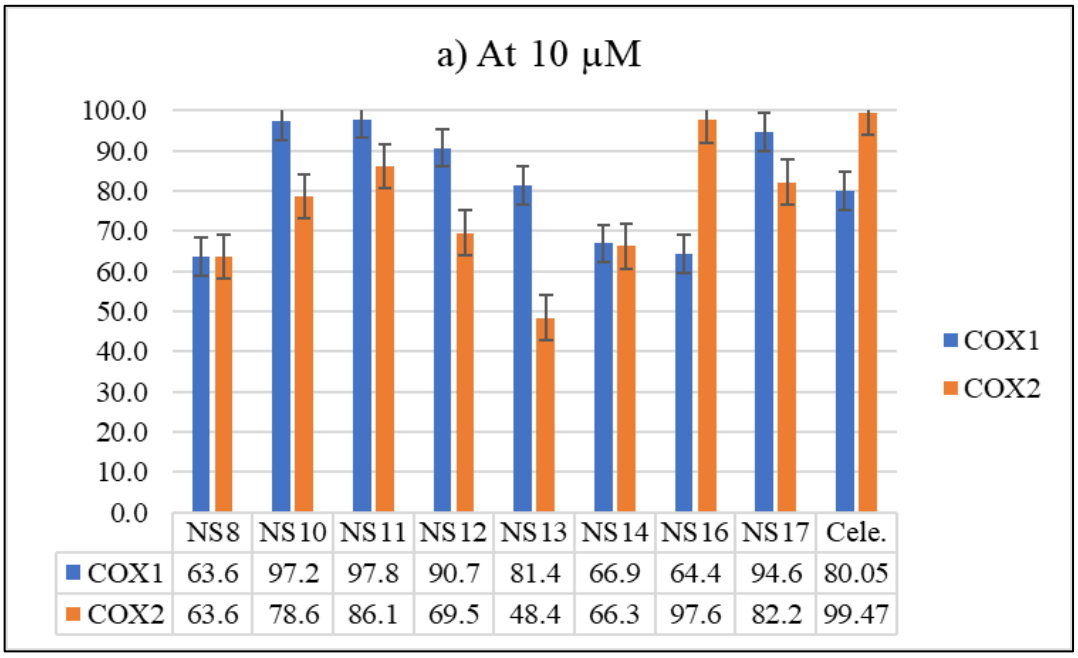


3h

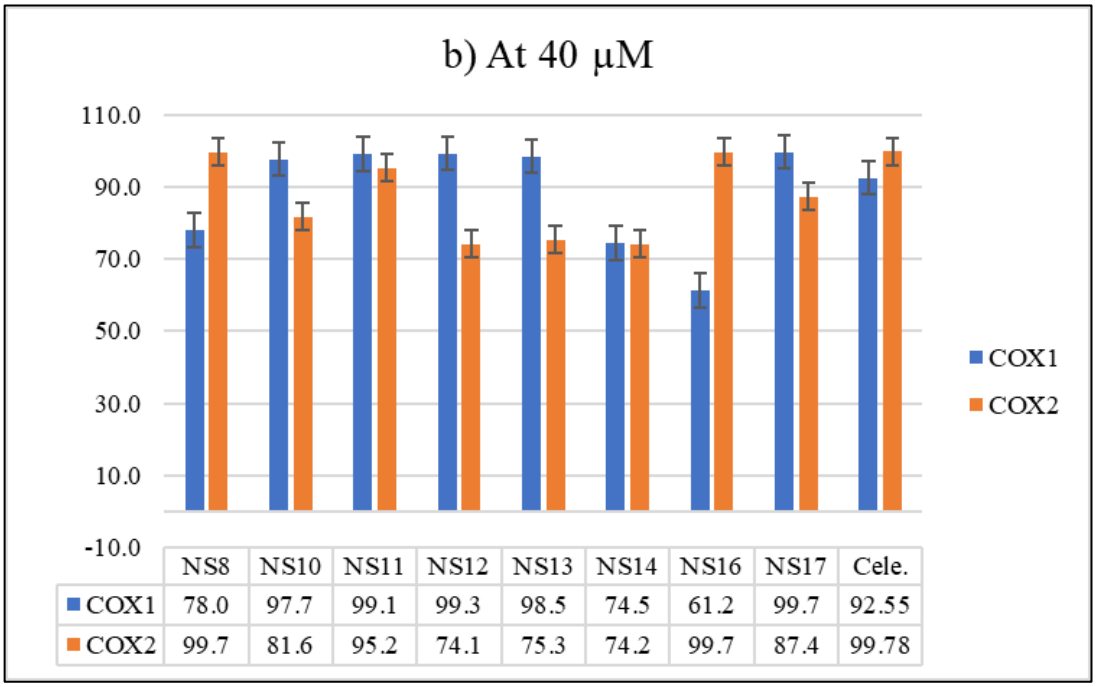


Appendix D

Estimation for effectiveness of pyrazole-carboxamide derivatives compounds on COX1, COX2-enzymes using two concentrations (01µM and 40µM)



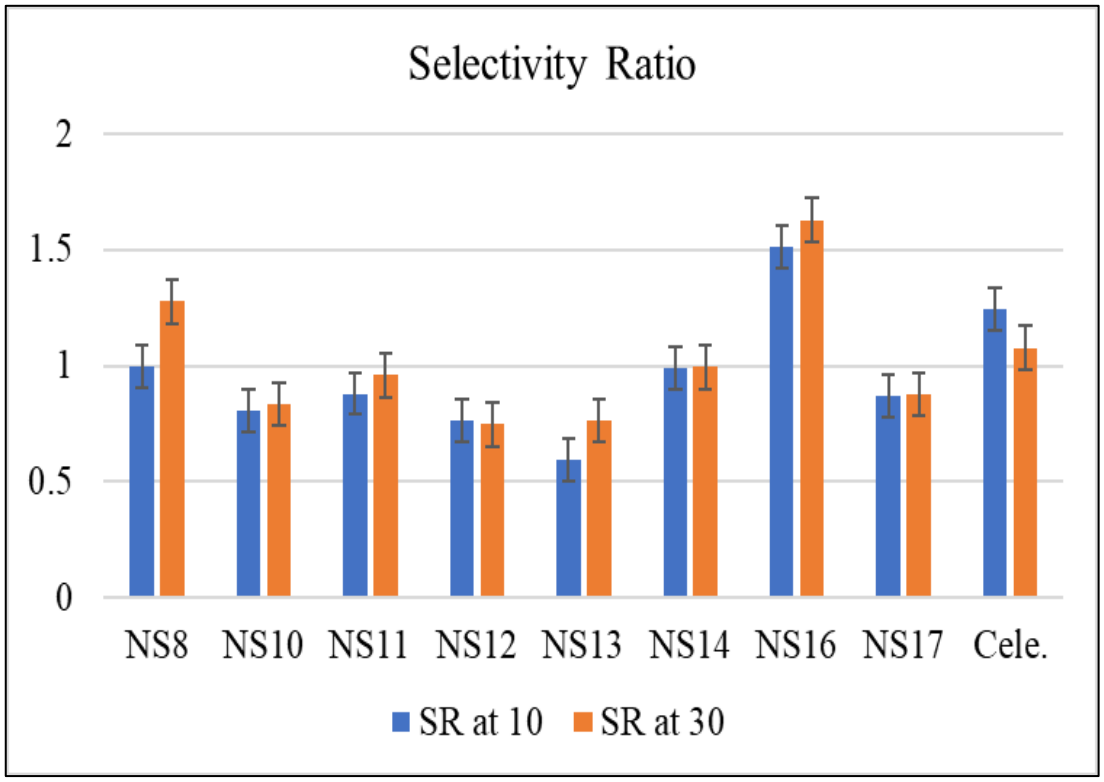
	NS8	NS10	NS11	NS12	NS13	NS14	NS16	NS17	Cele.
COX1	63.6	97.2	97.8	90.7	81.4	66.9	64.4	94.6	80.05
COX2	63.6	78.6	86.1	69.5	48.4	66.3	97.6	82.2	99.47



	NS8	NS10	NS11	NS12	NS13	NS14	NS16	NS17	Cele.
COX1	78.0	97.7	99.1	99.3	98.5	74.5	61.2	99.7	92.55
COX2	99.7	81.6	95.2	74.1	75.3	74.2	99.7	87.4	99.78

Appendix E

Estimation of selectivity index for our pyrazole-carboxamide derivatives using two concentrations of each compound (10 µM, 30µM)



جامعة النجاح الوطنية

كلية الدارسات العليا

تصميم وتوليف وتقييم بيولوجي
لمشتقات بايرازول–كاربوكساميد كمثبطات كوكس

إعداد

نسرين ناجح شويكي

إشراف

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الصيدلانية، من كلية الدارسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

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تصميم وتوليف وتقييم بيولوجي لمشتقات بايرازول-كاربوكساميد كمثبطات كوكس

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الملخص

العقاقير غير الستيرويدية المضادة للالتهابات (NSAIDs) هي أكثر العقاقير استخداما لتخفيف التورم وتقليل الألم وعدم الراحة وخفض درجات الحرارة المرتفعة. إنها مانع منافس لإنزيم الأكسدة الحلقية (COX). في أطروحة الماجستير هذه، تم تصنيع ثماني مشتقات بايرزول جديدة مع مشتقات الأنيلين وتحديدها وتقييمها من حيث انتقائها وفعاليتها تجاه COX-1 وCOX-2 باستخدام مجموعة اختبار تثبيط كوكس في المختبر. تم استخدام مقايسة MTS لتقدير السمية الخلوية لمركبات بايرزول المصنعة ضد النجم الكبدي البشري (LX-2) بالإضافة إلى خط الخلية الطبيعي (Hek293T). تم تحديد جميع المركبات الجديدة المركبة باستخدام تقنيات FTIR و HRMS و ^{1}H-NMR1 و ^{13}C-NMR. أظهرت النتائج أن المركب الأكثر فعالية ضد إنزيم COX-1 كان b3 مع IC_{50} = 0.46±0.25 ميكرومولار، كما أظهر نشاطا قويا ضد COX-2 مع IC_{50} = 3.82±1.36 ميكرومولار مع نسبة انتقائية = (0.12). من ناحية أخرى كانت أعلى نسبة انتقائية لمركب g3 مقابل COX2 مع IC_{50} =2.65±1.55 ميكرومولار فيما يتعلق بنسبة الكيتوبروفين (0.21) و IC_{50} ضد ميكرومتر 0.12±0.164 = COX−2، كما أنه أظهر أيضا أعلى مؤشر انتقائية اتجاه COX-2 (1.68). المركب 3d لديه انتقائية جيدة اتجاه COX2 (1.14) م 2.29±4.92= IC_{50} ميكرومولار .بالنسبة إلى HRMS كانت النتائج جميعها منطقية حيث أنه تم التحقق من كتل المركبات وكانت مناسبة للكتل المحسوبة.كما بينت نتائج NMR تصنيع ناجح للمركبات متناسقة مع مخرجات البحث. جميع المركبات أظهرت نشاطًا سامًا للخلايا ضئيلًا ضد خطوط الخلايا الطبيعية التي تم تقييمها، كما أظهر

المركب 3a بعض الفعالية اتجاه خطوط الخلايا السرطانية CaCO-2, MCF-7, Hep-G2.أخيرا,نوصي بعمل دراسات الإلتحام للمركبات المصنعة بهدف الفهم الصحيح لدراسة SAR وتحسين الإنتقائية اتجاه COX2.

الكلمات المفتاحية: إنزيم سيكولوكسيجيناز، مضادات الالتهاب غير الستيرويدية، مضادات الالتهاب، مثبطات كوكس، بايرزول–كاربوكساميد، غير متجانس.