**An- Najah National University**

**Faculty of Graduated Studies**

# "Selective Cyclooxygenase 2 inhibitors: synthesis and biological evaluation"

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# Dedication

***To the most affectionate father in the world who is supporting and encouraging me throughout my life.***

***To the greatest mother who is carrying my burdens, tiring on me, forgiving my mistakes and standing beside me until became what I am now.***

***To my Sisters and my brother.***

***To My grandparents and my big family.***

***To every friend, I knew stood beside me and supported me***

***I dedicate this work***

# Acknowledgement

First of all, I thank the holy god for giving me the ability, qualifications and the patience to accomplish this research and be proud of myself.

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My family, which doesn’t have any words to express my love, thanks and gratitude to them for their psychological and moral support throughout the years of my studies and work in my research. I hope you will be proud of me.

Hadeel Sawaftah

## الاقرار

**أنا الموقع أدناه موقع الرسالة التي تحمل العنوان:**

**“Selective Cyclooxygenase 2 inhibitors: synthesis and biological evaluation”**

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

## Declaration

The work provide 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:** **اسم الطالب:**

**Signature:** **التوقيع:**

**Date:** **التاريخ:**

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# List of abbreviations

| Symbol | Abbreviation |
|---|---|
| COX | Cyclooxygenase |
| COX-1 | Cyclooxygenase 1 |
| COX-2 | Cyclooxygenase 2 |
| PGs | Prostaglandins |
| GI | Gastrointestinal |
| CNS | Central nervous system |
| NSADs | Non-steroidal anti-inflammatory drugs |
| SAR | Structure activity relationship |
| $CDCl_3$ | Deuterochloroform |
| $CHCl_3$ | Chloroform |
| V-H | Velsmeier haack reagent |
| Cu | Copper |
| CuAAC | Copper-catalyzed Azide-Alkyne Cycloaddition |
| DCM | Dichloromethane |
| $POCl_3$ | Phosphorous oxy chloride |
| DMF | Dimethylformamide |
| DMSO | Dimethylsulfoxide |
| EDC | Ethylcarbodiimide hydrochloride |
| $Et_3N$ | Trimethylamine |
| $NaNO_2$ | Sodium nitrite |
| $NaN_3$ | Sodium azide |
| Hr/s | Hour or Hours |
| $H_2O$ | Water |
| DW | Distilled water |
| HCl | Hydrochloride |
| $IC_{50}$ | The half maximal Inhibitory concentration |
| µM | Micro molar |
| IS | Selectivity index |
| DIPEA | N,N-Diisopropylethylamine |
| TBTU | O-(Benzotriazol-1-yl)-N, N, N’,N’-tetramethyluronium tetrafluoroborate |
| DMAP | 4-Dimethylaminopyridine |
| Hz | Hertz |
| MHz | Mega Hertz |
| MeOH | Methanol |
| Min | Minutes |
| RT | Room Temperature |
| MW | Molecular weight |
| NIR | Near infrared |

| | |
|---|---|
| NMR | Nuclear Magnetic Resonance |
| HRMS | High resolution mass spectroscopy |
| $^{o}C$ | Degree Celsius |
| µl | Microliter |
| Mg | Milligram |
| G | Gram |
| pH | Power of hydrogen |
| TLC | Thin layer chromatography |
| UV-Vis | Ultraviolet-Visible |
| $\lambda_{max}$ | Lambda max |
| $R_f$ | Retention factor |
| Rpm | Round per minute |
| BC | Background |
| TA | Total activity |
| Blk | Blank |
| NSB | Nonspecific binding |
| S | Standard |
| C | Concentration |

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## Abstract

Derivatives of diaryl pyrazoles and triazoles were synthesized to produce series of selective COX-2 inhibitors. In order to synthesize the series of these derivatives, Vilsmeier-Haack reaction and click reaction were followed in the synthesis of pyrzoles and triazoles based derivatives respectively. All produced compounds were *In vitro* evaluated by inhibition studies on COX-1 and COX-2 isozymes. Eleven compounds were successfully synthesized. Five compounds were the most potent and selective on COX-2 isozyme with $IC_{50}$ values in 0.551–0.002 μM range. In diarylpyrazole derivatives best inhibition was showed by **compound 4b** with $IC_{50}$ = 0.017 μM as the *N*-aromatic rings was substituted with sulfonamide and the other aromatic ring was unsubstituted. However, **Compound 4d** showed the best selectivity onCOX-2 ($IC_{50}$ = 0.098 μM, SI = 54.847) as the *N*-aromatic ring was substituted with sulfonamide and the other aromatic ring was substituted with sulfone. In the diaryltriazole based derivatives, **compound 15a** was the most potent among the entire synthesized library including the reference compound (Celecoxib) with $IC_{50}$ = 0.002 μM and SI = 162.5 as the presence of spacer could facilitate binding with extra hydrophobic pocket of COX-2 enzyme. Compounds with a variety of substitutions on pyrazole and triazole

cores and different linker lengths are recommended to be synthesized in further studies to evaluate their COX-2 inhibitory activity and selectivity. Moreover, an *in vivo* anti-inflammatory and cardiotoxicity studies will be managed to support the obtained *in vitro* results.

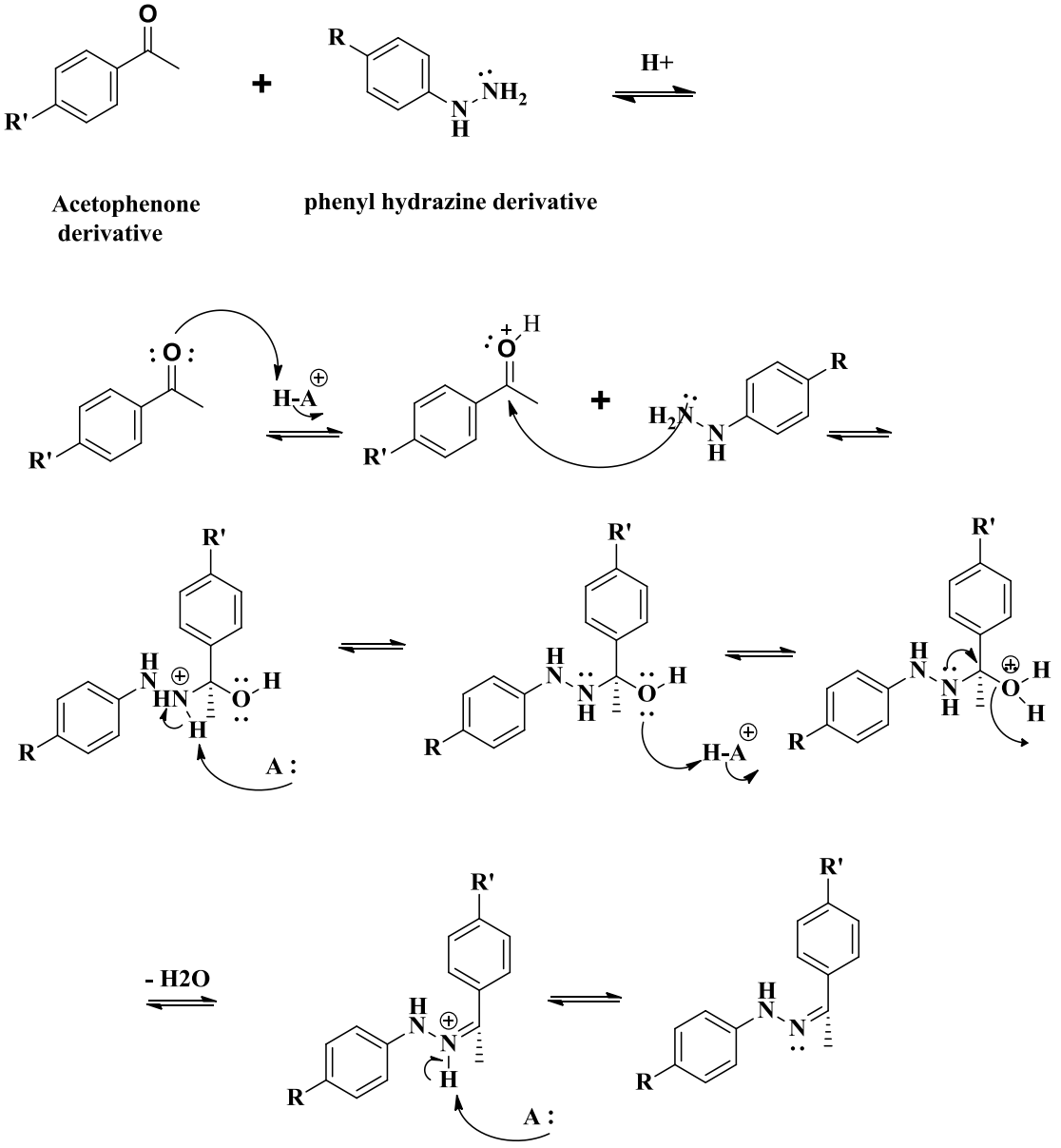
# Chapter One

## 1. Introduction

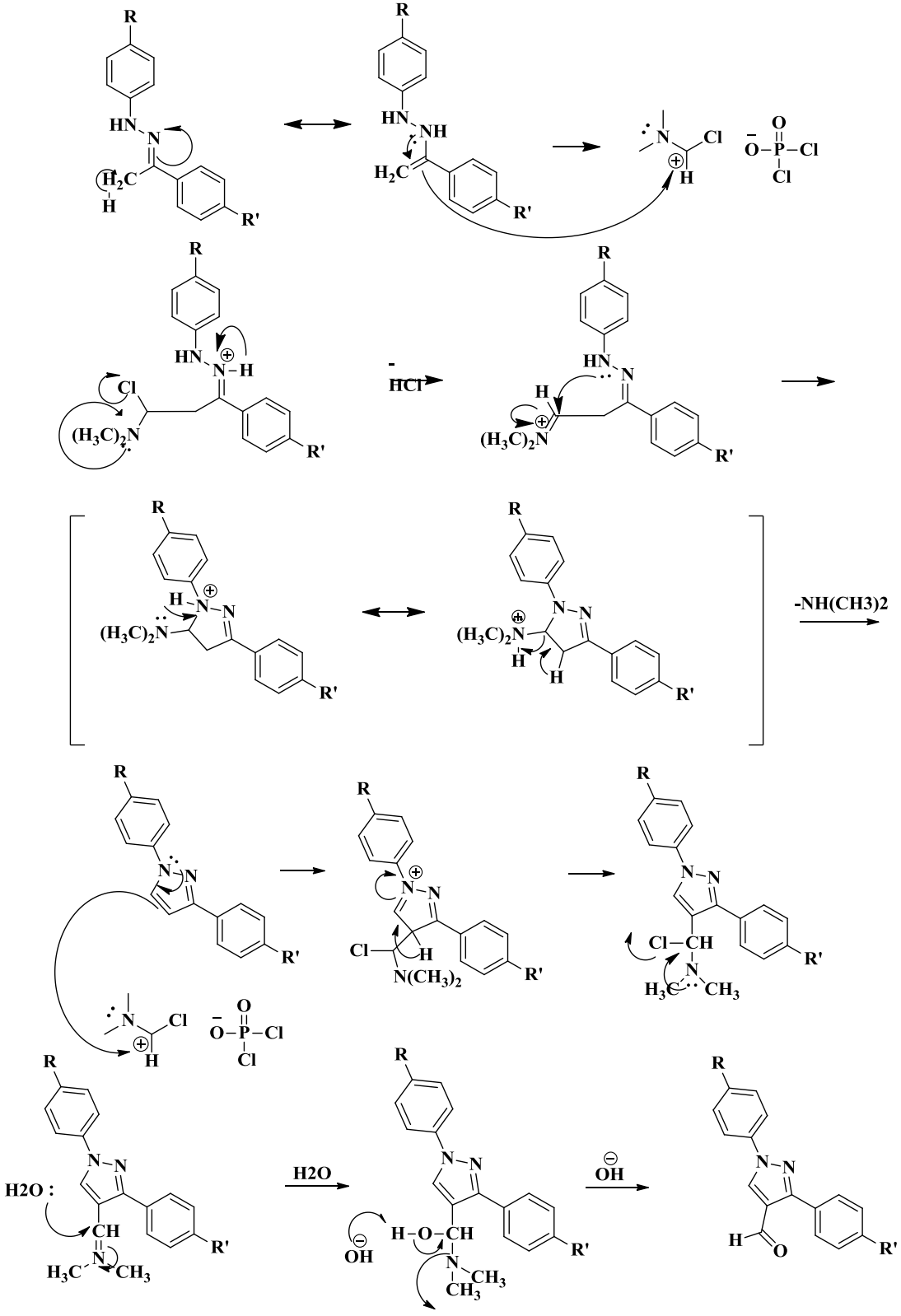
### 1.1 Pyrazoles:

Pyrazole derivatives had taken attention in various research fields due to their diverse biological activities and their significant rule in many disease conditions. They also showed an important activity against bacterial, viral, fungal and parasitic infections. Moreover, most of these derivatives showed analgesic, anesthetic, anti-inflammatory and anti-diabetic activities [37, 38]. There are many techniques were used for pyrazoles synthesis. A classical method is made by reacting α,β-unsaturated ketones (chalcone) derivatives obtained from aldol condensation reaction between aldehydes and ketones under basic conditions which undergo cyclization with hydrazine derivatives to produce different substituted pyrazoles [39, 40]. Another method of pyrazole synthesis obtained by vilsmeier-haack reaction. The reaction made by reacting hydrazones derived from aromatic or aliphatic methyl ketone derivatives to yield pyrazole-4-carboxaldehydes upon stimulation by vilsmeier-haack reagent (DMF/$POCl_3$). This method was reported by many previous studies [41, 42]. Hydrazone backbone became one of the most important component that can undergoes different condensation reactions for synthesizing different *N*-heterocycles containing compounds [43]. Series of hydrazone derivatives were synthesized by reacting substituted aldehydes or ketones with thiosemicarbazide and had been evaluated for anticonvulsant [44] and anti-tuberculosis activities [45]. Starting with hydrazine group

containing compounds, condensation will occur upon refluxing with ketone containing compounds like acetophenone derivatives in methanol with acetic acid glacial as acidic catalyst. **Scheme (1)** shows the mechanism of condensation reaction. Then formyl pyrazoles are formed upon hydrazone treatment with vilsmeier-haack reagent (V-H reagent) [42]. The mechanism of vilsmeier-haack reaction can be concluded in sequential steps: Initially, electrophilic attack on hydrazone by V-H reagent, the produced intermediate losses HCl molecule to provide a second intermediate. The N-H group in this intermediate initiates the cyclisation and forming a pyrazole intermediate by nucleophilic attack. A more stable derivative of pyrazole will form after losing $Me_2NH$ group. Another V-H reagent reacts with formed pyrazole in a process of subsequent electophilic substitutions ends up with imminum salt formation. The last product (4-formyl pyrazole) yield after the hydrolysis of imminum salt in a basic environment which eliminates $NHMe_2$ group and final cyclisation takes place to give the derivative of pyrazole [42]. **Scheme (2)** below shows the mechanism of pyrazole derivatives synthesis.




**Scheme (1):** mechanism of condensation reaction.



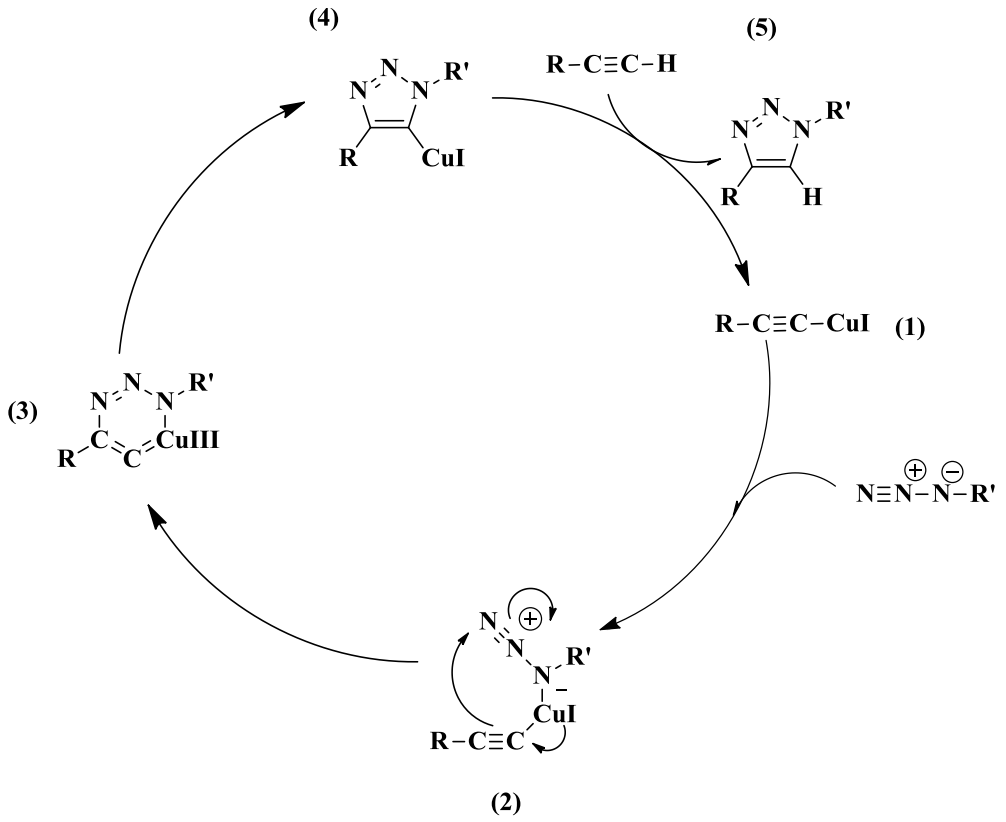

**Scheme (2):** mechanism of pyrazole derivatives synthesis.

## 1.2 Triazoles:

Triazoles are *N*-heterocylic derivatives which consider as important core in many applications. Biologically, it was reported about their antimicrobial, anti- HIV and other biological activities [46] .

Click chemistry was introduced in 2001 by Sharpless and co-workers. It comprises a series of nearly perfect chemical reactions that were involved in many synthetic approaches with a variety of starting materials and reagents. Moreover, the reaction is performed in mild conditions and high yield of product is expected. Click reaction forms irreversible bond between Carbon-heteroatom containing compounds that was inspired by the natural occurring proteins which connect amino acids building blocks by reversible amide links. In order to compensate the inability of controlling the reversible chemistry of carbonyl, the focus was conducted on using highly energetic reactant "spring-loaded" electrophiles like acetylenes and olefins and by controlling the outcomes kinetically [47]. Arthur Michael had reported the first synthetic triazole in 1893 came from the reaction between phenyl azide and diethyl acetylenedicarboxylate [48]. Huisgen cycloaddition is a 1, 3-dipolar cycloaddition reaction that specifically generates 1, 2, 3-triazoles from the reaction between alkynes (dipolarophile) and organic azides (1, 3-dipole). It was described in the period between the late $19^{th}$ to the early $20^{th}$ century after the investigation of the chemical mechanism and synthetic approaches which was established in 1963 by the German chemist Rolf Huisgen [49]. Huisgen's reaction could not proceed in the mild conditions.

So, copper (I)-catalyzed reaction was developed. This Allows performing the reaction readily in mild and physiological conditions. CuAAC is the acronym of The developed version refers to (Copper-catalyzed Azide-Alkyne Cycloaddition) [50]. Additionally, Non-catalyzed Huisgen's reaction gives both 1,5- and 1,4-isomers, whereas only 1,4-isomer could be produced from the catalyzed version [48] Azide-alkyne cycloaddition reaction catalyzed by Copper (Cu(I)) considered as a typical click reaction providing a high yield, steroselectivity and good regioselectivity [46]. A terminal alkyne, an Azide and a copper catalyst are the main components of reaction. Other reagents and reaction parameters are variable. The mechanism of reaction was explained by Sharpless and co-workers. It can be concluded in sequential steps: Copper (I) catalyst that can be generated in situ when $CuSO_4$ mixed with sodium ascorbate (NaAsc) reacts with the terminal alkyne to form a Copper (I) acetylide that initiate the reaction. Then a complex of the three components is formed after the incorporation of alkylated nitrogen (N1) of azide that links with Cu (I). The complex then transfer to metallocycle upon the first C-N bond formation that leads to increase in the ring contraction with changing the oxidation state of Cu to +3 instead of +1, cuprous triazolide forms after reduction of copper (III) to copper (I). The final triazole produces after protonation obtained from an alkyne molecule if no other source of proton is available. The cycle will be repeated upon forming of a new Copper (I) acetylide molecule [51]. **Scheme (3)** below shows the CuAAc reaction steps.




**Scheme (3):** CuAAc reaction mechanism**.**

## 1.3 Cyclooxygenase enzyme (COX):

Cyclooxygenase enzyme has two isoform of enzymes (COX-1 and COX-2) that catalyze the biosynthesis of prostaglandin $H_2$ and formation of reactive oxygen species from arachidonic acid which release from membrane phospholipids by the action of phospholipase $A_2$ [1]. Prostaglandin $H_2$ is the precursor for formation of other prostaglandins that act as immunomodulaters and inflammatory mediators specially prostaglandin $E_2$, exert their role by vasodilatation, pyretic effects and their proliferation ability [2, 3]. Thromboxane $A_2$, Prostaglandin $E_2$, D2, $I_2$ ( Prostacyclin) and $F_{2\alpha}$ are other mediators responsible for many biological responses [4].

**Figure(1)** shows the biochemistry of prostanoids and their pathway of formation [5].

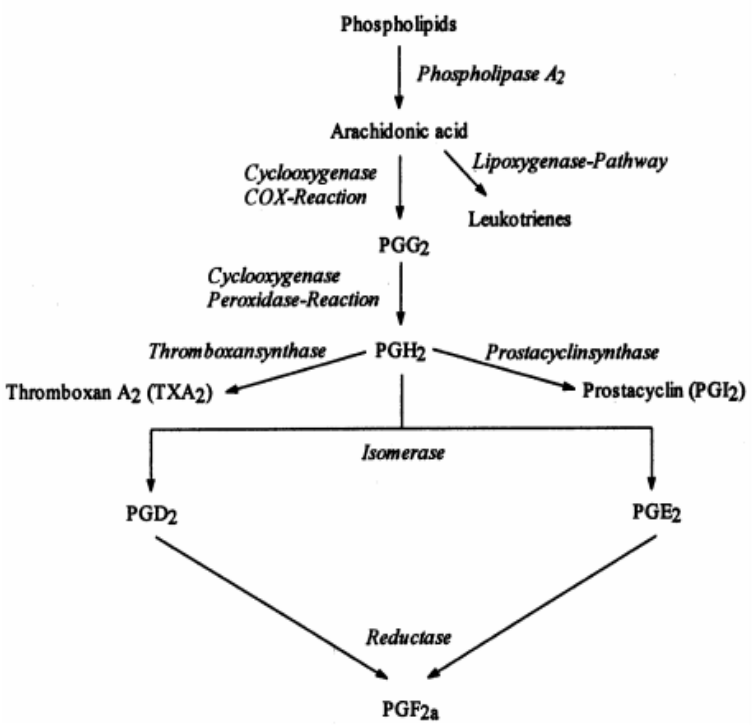



**Figure 1:** Prostanoids biosynthesis pathway.

The identification of COX enzyme as a target for non-steroidal anti-inflammatory drugs (NSAIDs) was in 1971 by Vane, in which their mechanism of inhibition was shown [6].

COX-1 and COX-2 isozymes are naturally produced from distinct genes where COX-1 is expressed in different cells within the body which has many important physiological functions in protection of gastric mucosa, platelets aggregation and maintain renal homeostasis, while COX-2 is mainly expressed upon stimulation by inflammatory mediators like cytokines [7, 8]. Oncogenes, growth factors and promoters of tumor induce COX-2 expression too. This demonstrates its role in both cell growth and

inflammation process [9-11]. Both COX isoenzymes are classified as membrane bound enzymes attach to endoplasmic reticulum. Ovine's COX-1 three dimensional structures was first defined in 1994 followed by the discovery of COX-2 in human and murine. COX enzyme is a homodimer that has three structural domains construct its monomer which are N-terminal (epidermal growth factor like), domain binds to membrane attach to phospholipids bilayer, and C-terminal domain which is the catalytic domain that contains the active site for COX substrate and inhibitors, in addition to heme containing active site for peroxidase enzyme. Both of these sites are interrelated in function and structure [12]. **Figure (2)** shows the domains of COX enzyme [13].

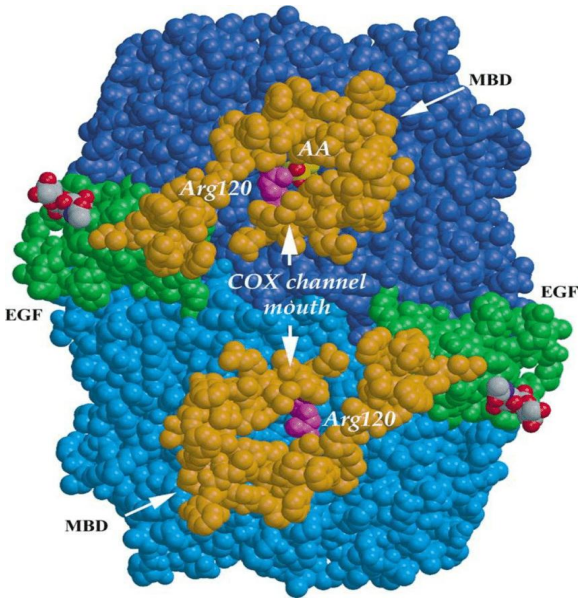



**Figure 2:** domains of COX enzyme.

COX isoforms are 67% identical in amino acid sequences. At the top of enzyme channel phenylalanine amino acid (Phe503) in COX-1 is replaced by less bulky Leucine (Leu503) in COX-2 to provide the later wider upper

space available for binding. However, the most substantial difference is the presence of Valine (Val523) in COX-2 instead of Isolusine (Ilu523) in COX-1 that allows COX-2 to expand to special side pocket with moving back the side chain of Phenylalanine (Phe518). Additional binding interaction can occur within COX-2. Moreover, the chemistry in this side pocket is differing in COX-2 due to presence of basic Arginine instead of Histidine in COX-1 that allows the former polar interactions. The overall available space in COX-2 binding region is 25% greater than the space of COX-1 [14, 15]. **Figure (3)** shows these structural differences [16].

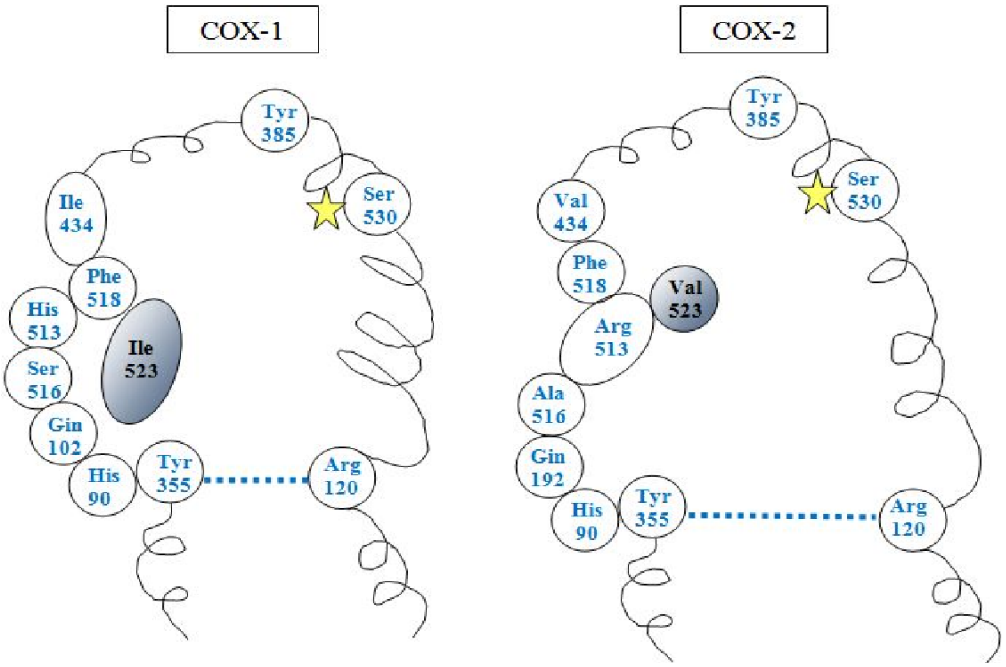



**Figure 3:** Structure of COX-1 and COX-2 isozymes.

## 1.4 Non-steroidal anti-inflammatory drugs (NSAIDs):

NSAIDs are structurally diverse groups of similarly acting compounds that have an effective role in relieving pain, inflammation and fever [17]. They have many biochemical activities in inflammation process. They suppress

the synthesis of prostaglandins and inflammatory mediators formation, control inflammatory proteases activity and stabilize liposomal membrane [18]. They are among the most prescribed and non-prescribed medications [19]. Therapeutically, NSAIDs are used in treatment of musculoskeletal, arthritis, osteoarthritis and other inflammatory disorders [20]. Traditional derivatives of NSAIDS are still used widely nowadays, but their side effects on gastrointestinal tract have limited their use. The mechanism of action which NSAIDs alleviate pain and their rule in inflammation process is made by inhibition of cyclooxygenase enzyme, thus inhibition prostaglandins synthesis. The inhibition of COX-1 caused unwanted side effect on GI tract [21, 22]. It was reported an increased gastrointestinal risk for osteoarthritis patients who receive NSAIDs as a current treatment [23].

Also NSAIDs showed side effects on cardiovascular system, increased cardiovascular events was noticed specially with heart failure patients [24].

There are many different ways to classify NSAIDs; one of them is according to their chemical structure. NSAIDs are categorizes to several subgroups that are:

- Aspirin and other salicylic acid derivatives, as diflunisal.
- Propionic acid derivatives, as ibuprofen, naproxen, ketoprofen.
- Acetic acid derivatives, as indomethacin, etodolac and sulindac.
- Oxicam (enolic acid) derivatives, as piroxicam and meloxicam.
- Fenamates or anthranalic acid derivatives, as mefenamic acid and meclofenamate.

Heteroaryl acetic acids, as diclofenac considered the most commonly available and used NSAID world widely [25].

According to inhibition mode of COX inhibitors. They are classified to irreversible inhibitors such as Aspirin, Reversible competitive and Time dependent inhibitors in which other drugs belong to.

Inhibitors structure must possess many features allow an optimal binding with enzyme active site. The main scaffold must contain an aromatic system with acidic group that can be ionized and form an ionic interaction with Arginine's guanidinium group. Extra bonding interactions and increase in potency when second aromatic group perpendicular to the first one added to the scaffold. Increase the distance between acidic and aromatic group by maximum two atoms will improve the potency. Also adding methyl group on the first carbon will produce more potent with a chiral center compound. It was found that S-isomers are the most potent isomers. Lastly, increasing the size of alkyl group leads to decrease in potency except when it incorporated into a heterocycle.

Salicylate derivatives inhibit COX enzyme by irreversible acetylation of Serine amino acid of enzyme. Therefore, carboxyl group is very important in activity and hydroxyl group must be in the ortho position to it. Any substitutions on hydroxyl or carboxyl will alter the potency and toxicity of compound. Many derivatives of aspirin had been synthesized to overcome its GI side effects such as Sodium, Magnesium and choline salisylate,

Benorylate, salsalate, Sodium thiosalisylate and Diflunisal. Benorylate, salsalate and diflunisal were the best in overcoming GI side effects.

Propionic acid derivatives comprise the largest number of aryl-acidic anti-inflammatory compounds. The most important features that these compounds should have: 1) the side chain of propionic acid, 2) Phenyl group with electronegative substitution (e.g: F, Cl) in Meta position to acid side chain, 3) Second Aryl hydrophobic group in non-planner configuration to the first one, this can be achieved by steric effect of the electronegative groups. Various derivatives with second hydrophobic group were synthesized, Naphthalene derivatives at C-4 is an example. A second hydrophobic group attaches to C-3 by ether or carbonyl group linkage was evaluated too [18]. There are many drugs belong to this class such as Ibuprofen which contains a secondary-butyl group instead of second aromatic substituent which reduce its potency. In contrast, Flurbiprofen was more potent and showed a good therapeutic effect due to the presence of non-coplanar second aromatic group with 3-fluro substituent. Naproxen and ketoprofen are examples of compounds possess naphthalene and carbonyl derivatives respectively.

Derivatives of aryl acetic acid had been synthesized and different scaffolds were evaluated. Indole derivative (Indomethcin) was synthesized in 1961. It complies with SAR of NSAIDs in many points. Acidic group, a second perpendicular group attaches to indole and separated by one carbon atom with its unique configuration that allows it to be in an active conformation.

In addition, rotation is restricted by amide group and 2-methyl substituent helps in producing the active form. Indene derivative was introduced after the CNS side effects that Indomethacin had showed. Sulindac was the developed pro-drug that has a fluro and methylsulfinyl which converts to sulfide active form. It showed a comparable analgesic effect but the potency was reduced to the half in reducing fever and in anti-inflammatory activity. On the other hand, Fewer GI and no CNS side effect were noticed compared to Indomethcin.

Fenamates containing compounds are anthranilic acid derivatives. The most potent derivatives were obtained when the second aromatic was substituted in 2’ and 3’ position, due to keeping the ring out of the plan. Mefenamic acid and Meclofenametes are the drugs belong to this class where the later showed the highest potency because of chlorine substitutions on the second ring [26, 27].

## 1.5 Selective cyclooxygenase II inhibitors:

The newer class of COX inhibitor; selective COX-2 drugs were associated with lower gastrointestinal side effects compared to traditional NSAIDs but there were a big concern about their cardiovascular adverse effects. It was reported that Rofecoxib was associated with five folds elevation in myocardial infarctions [22]. A prospective study with randomized controlled trials had compared between selective COX-2 inhibitors and traditional NSAIDs. It had concluded that selective COX-2 and high doses of diclofenac and ibuprofen were associated with moderate elevation of cardiovascular

events [28]. One of the explanations of cardiotoxicity associated with some selective COX-2 inhibitors is the imbalance between thromboxane A2 and prostacycline production. COX-1 is mainly expressed by platelets while COX-2 expressed by endothelial cells, selective inhibition of COX-2 leads to this imbalance [28].

Selective COX-2 agents were introduced to the market in 1999 and there are a limited number of these drugs in the market at this time [29]. Since release of these agents in the market, marked elevation of their use was notice. There was 35% to 61% increase in frequency of their use by 1999 to 2002. All these findings produce a challenge to synthesize innovative therapies with high benefit, selectivity and fewer side effects [30].

Many studied were made to establish selectivity on COX-2 over COX-1. Selective COX-2 inhibitors must have polar substitutions that can interact with (His90), (Arg513) and (Gln192) in the third region by hydrogen bond. Example of such polar substitutions are phenyl sulfonamide moiety, methyl sulfonamide and 3-sulfonylvinylbenzophenone substitutions on diaryl heterocyclic. Another approach of selectivity was made by adding extra lipophilic substitutions to interact with upper lipophilic space of the first region with (Leu503) by electrostatic or hydrophobic bonds such as 3',5'-bis-substitution on flurbiprofen [31, 32]. One of the most important studies aimed at synthesize COX-2 selective inhibitor had suggested quintessential elements must be present in COX-2 inhibitor for best potency and selectivity. The presence of cyclic ring either hetero or carbon ring with two adjacent

aryl substituent attached to it both with increasing the flexibility of the compound by lengthen the linker chain will provide noticeable improvement in potency and selectivity. More accessible compounds and stronger interactions with amino acids residues in the sub pocket of COX-2 enzyme will occur [33]. **Figure (4)** demonstrates the difference in selectivity between traditional NSAIDs and selective COX-2 inhibitors [4].

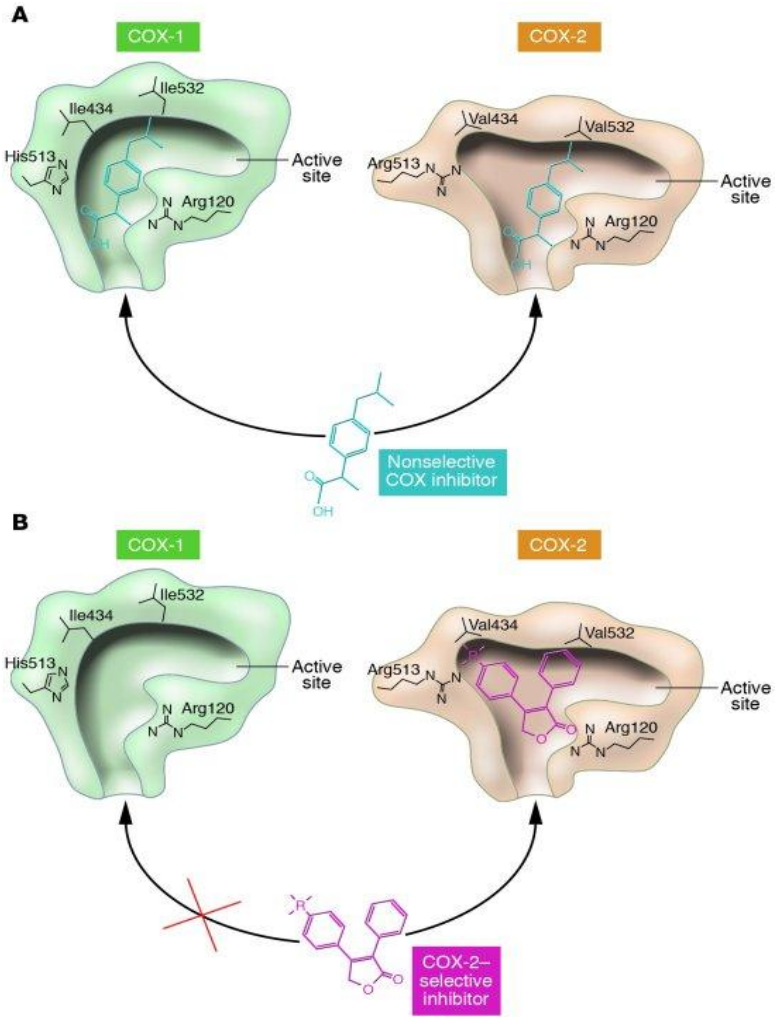



**Figure (4):** Difference in selectivity between traditional NSAIDs and selective COX-2 inhibitors.

## 1.6 Structure activity relationship (SAR) of COX-2 inhibitors:

COX-2 consists of three main regions: **(1)** first region located at the upper of the enzyme and formed by (Phe381), (Tyr385), (Leu384), (Trp387) and (Phe518), (Leu503), **(2)** Second region located at the lower part and formed by (Leu531), (Val116), (Val349), and (Leu359) and (Tyr355). **(3)** Third region located at the middle and formed by (Phe518), (Leu352), (Val523), (Gln192), (Arg513), (His90), (Ser353) and (Tyr355). According to the structure of many COX-2 inhibitors, hydrophobic interactions are the predominant bonds in the first region, hydrogen bonds with polar functional groups such as carboxylate and trifluromethyl in the second region and additional hydrogen bonds with polar oxygen or nitrogen containing functional groups in the third region [32]. **Figure (5)** shows the binding pocket of COX-2 enzyme [34]. Selective COX-2 inhibitors bind to COX-2 enzyme in different affinity that of NSAIDs. They do not form a salt bridge with the upper hydrophobic channel due to the absence of carboxylic group. They generally classified in to two groups; tricyclics composed of ring system of carbocylic or heterocyclic ring with 1, 2-diaryl substitutions with functional groups on one aryl ring like azido, sulfonamide, methansulfonamide, and methansulfonyl or tetrazole group. Coxibs are belonging to this group of compounds. The second group is acyclic compounds which composed of a cyclic center attached to two or three chains [31, 35]. Various sizes and types of rings were synthesized with bicyclic, tricyclic either fused or spiro ring system. In addition, four, five and

six membered rings have been considerably used as a central core for this class of compounds.

There are general features that COX-2 inhibitor should possess. Three spaced cycles scaffold is one of these features. Addition a substitution on C-3 central ring was effective in binding with less steric restrictions. One of previous studies had shown that most potent derivatives were those contain two fluorine atoms. It was found in a study used the pyrrole based esters as a scaffold that presence of two fluorine atoms in the same molecule had a positive effect on COX-2 inhibition. The presence of electron withdrawing group in the Para position is more preferable than corresponding electron donating group and among the used groups, $CF_3$ was the best followed by $NO_2 > F > CH_3$, $H > OCH_3$, $OCH_2CH_3$, and OH. Most previous studies were focused on substituted 1, 4- and 1, 5-diaryl and 1, 2, 3-triazoles with a pharmacophore having $SO_2CH_3$ on Para position. Also, It was demonstrated that the presence of $–SO_2CH_3$ had significantly increased the selectivity compared to $–SCH_3$ group [36].

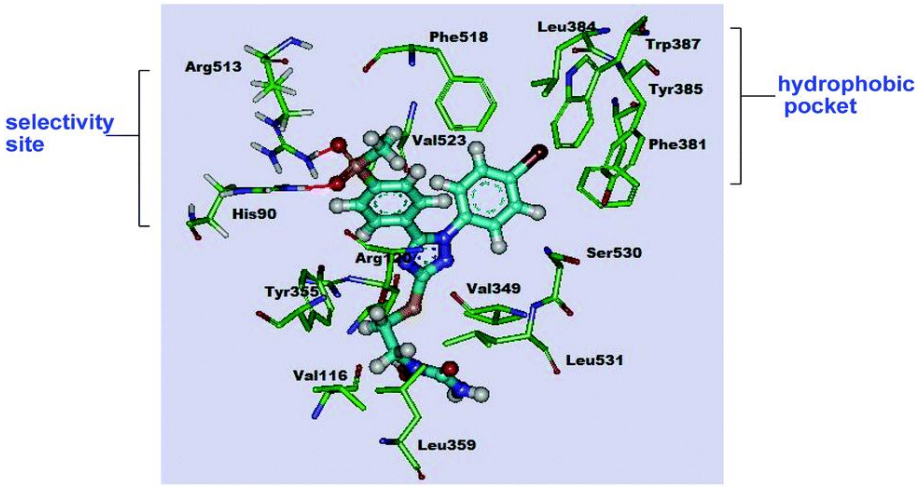



**Figure (5):** Binding bucket of COX-2 enzyme.

## 1.7 Literature review:

Many studies and researches were implemented to synthesize novel, potent and selective COX-2 inhibitors by using different methods and techniques. 1, 5-substituted tetrazole was used in producing several compounds. The scaffold was a tricyclic with central tetrazole core and two substituted heterocycles in which two series were produced. Sulfonamide and Methylsulfonyl substituted ring with the same third ring in both series attached with different functional groups to produce the final series of compounds. All produced inhibitors showed modest inhibition on COX-2 compared with Celecoxib with 6 and 7 µM $IC_{50\ values}$ for **4h** and **6h** compounds respectively. All other inhibitors showed $IC_{50}$ values of >100µM as shown in figure (6) [52].

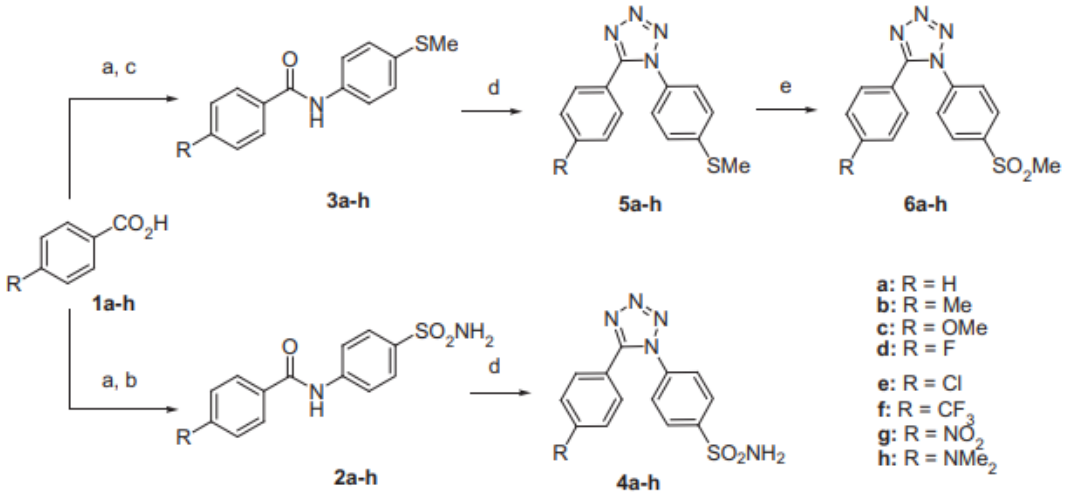



**Figure (6):** Tetrazole core inhibitors.

In another study, derivatives of tri aryl pyrazoline were synthesized by conserving sulfonyl or/ and sulfamoyl pharmacophore containing ring, changing the second ring and the substitutions on third ring. Compounds 13e**,** **13i, 13h** showed good activity and moderate selectivity on COX-2. Compound **13i** was the best which showed anti-inflammatory activity comparable with Celecoxib as shown in figure (7) [53].

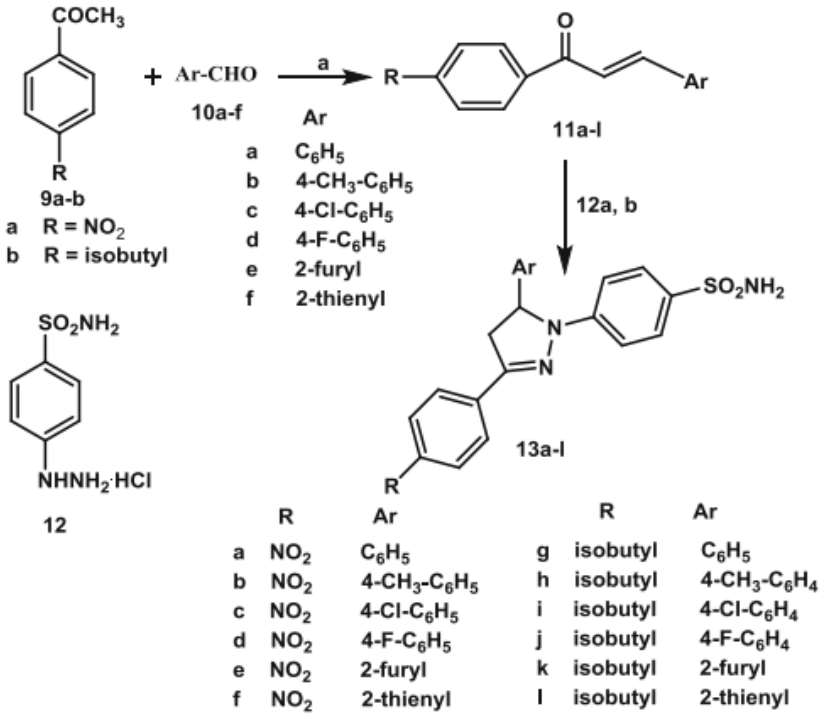



**Figure (7):** Pyrazoline core inhibitors.

A library of pyrazoline derivatives were synthesized in another previous study. A pyrazoline core with 1N substitution attach to diaryl in which one phenyl substituted with methyl sulfone group and the other with different groups. Compounds that contained groups of 4-methyl,4-methoxy, 2-phenyl methoxy and 4-Cl showed the most significant COX-2 selectivity as shown in figure (8) [39].

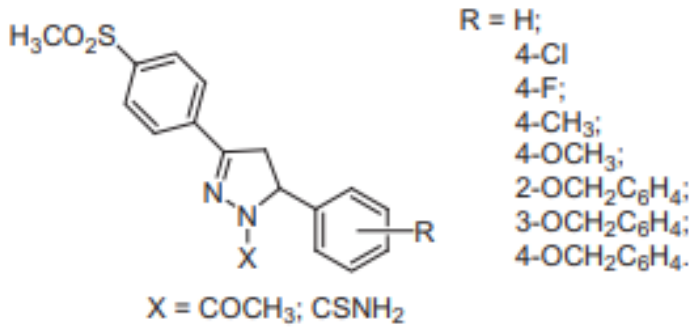



**Figure (8):** Pyrazoline-N1 substituted core inhibitors.

Compounds derived from benzimidazole were also have been synthesized in a recent study. They had contained benzimidazole attach to 1, 2 position of pyrazine ring connected with other two rings. Methylsulfonyl pharmacophore on one ring and different substitutions were on the other ring. The compound substituted with 4-methyl group showed the highest COX-2 inhibition with $IC_{50}$ of 0.07µM while the compound that was substituted with 3,4,5-trimethoxy group showed the best selectivity with SI of >909 as shown in figure (9) [54].

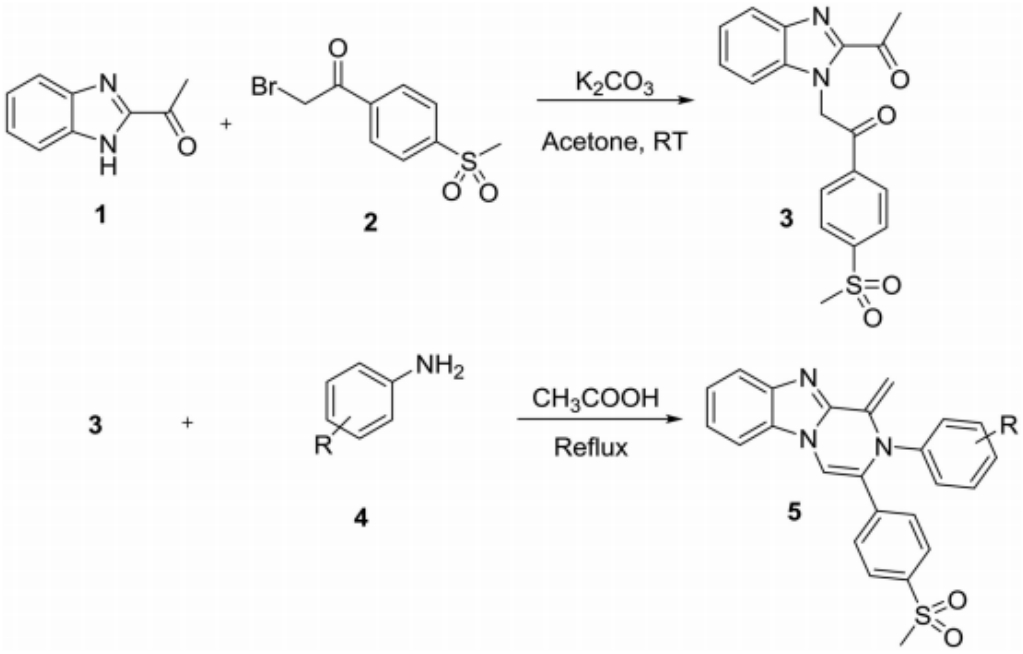



**Figure (9):** Benzimidazole core inhibitors.

Indole-N-acyl hydrazones derivatives were synthesized. Only 5-bromo-indole derivative (Compound **3a**) had showed a selectivity toward COX-2 but in less potency compared with Celecoxib as shown in figure (10) [55].

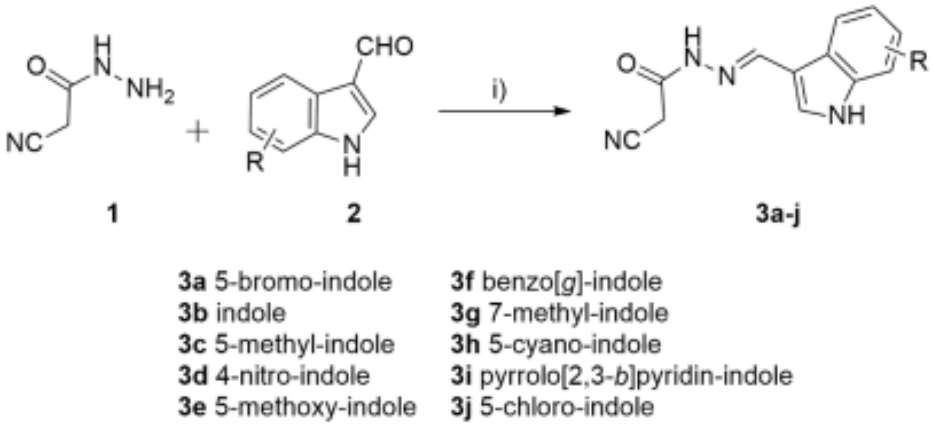
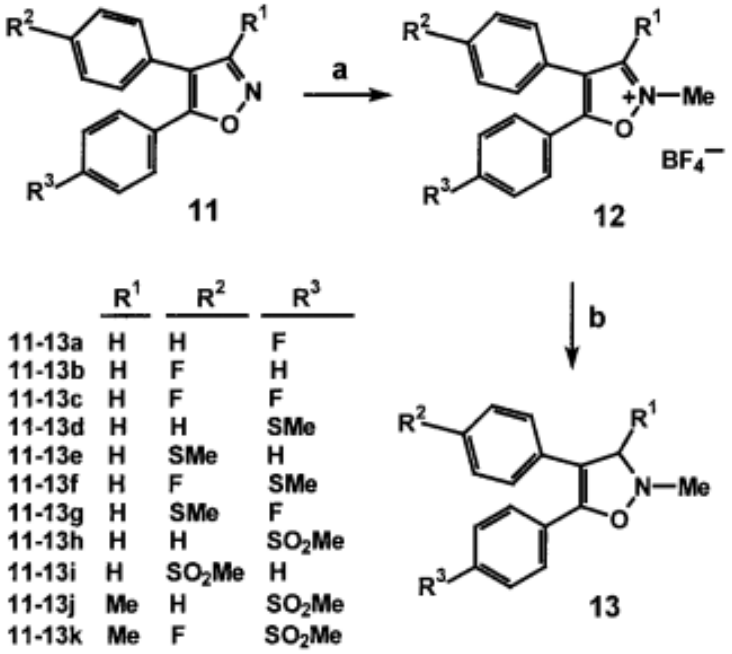



**Figure (10):** Indole core inhibitors.

Derivatives of 4, 5-Diphenyl-4-isoxazolines with different substitutions were synthesized. Two compounds that contained methyl sulfonyl pharmacophore were the most selective, The one that was substituted with 3-methy on izoxazole ring **13j** showed an excellent potency and selectivity on COX-2 with $IC_{50}$ of 0.004µM while the other that had an additional fluorine substitution on the Para position of phenyl ring **13k** showed a good selectivity but less potency than the later with $IC_{50}$ of 0.0316µM which indicate the crucial rule of 3-methyl and sulfonyl group in selectivity of inhibition as shown in figure (11) [56].




| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 11-13a | H | H | F |
| 11-13b | H | F | H |
| 11-13c | H | F | F |
| 11-13d | H | H | SMe |
| 11-13e | H | SMe | H |
| 11-13f | H | F | SMe |
| 11-13g | H | SMe | F |
| 11-13h | H | H | $SO_2Me$ |
| 11-13i | H | $SO_2Me$ | H |
| 11-13j | Me | H | $SO_2Me$ |
| 11-13k | Me | F | $SO_2Me$ |

**Figure (11):** Isoxazoline core inhibitors.

A series of compounds of triazole derivatives have been synthesized by using 1, 5-diaryl- 1, 2, 4-triazole as a scaffold. Most of synthesized compounds exhibited a potent anti-inflammatory activity but three compounds **(6b, 6c and 9c)** were the most potent. Compound **9c** was substituted with sulfonyl group on C-3 of triazole ring, Sulfonamide on one phenyl and chloride on the other one. The other two compounds **(6b, 6c)** were share the same structure of scaffold with methyl thio group attach to C-3 of triazole ring and sulfonamide substitution on one phenyl ring but differ in the substitution on other phenyl ring with F and Cl groups on each one respectively. Compound **6c** was the most potent and selective inhibitor with $IC_{50}$ and SI of 0.37µM and 0.018 respectively which was comparable to Celecoxib. This study indicates the importance of introducing sulfamoyl moiety on the Para position of phenyl group and Electron withdrawing group

on the other one in increasing the inhibition potency as shown in Figure (12) [57].

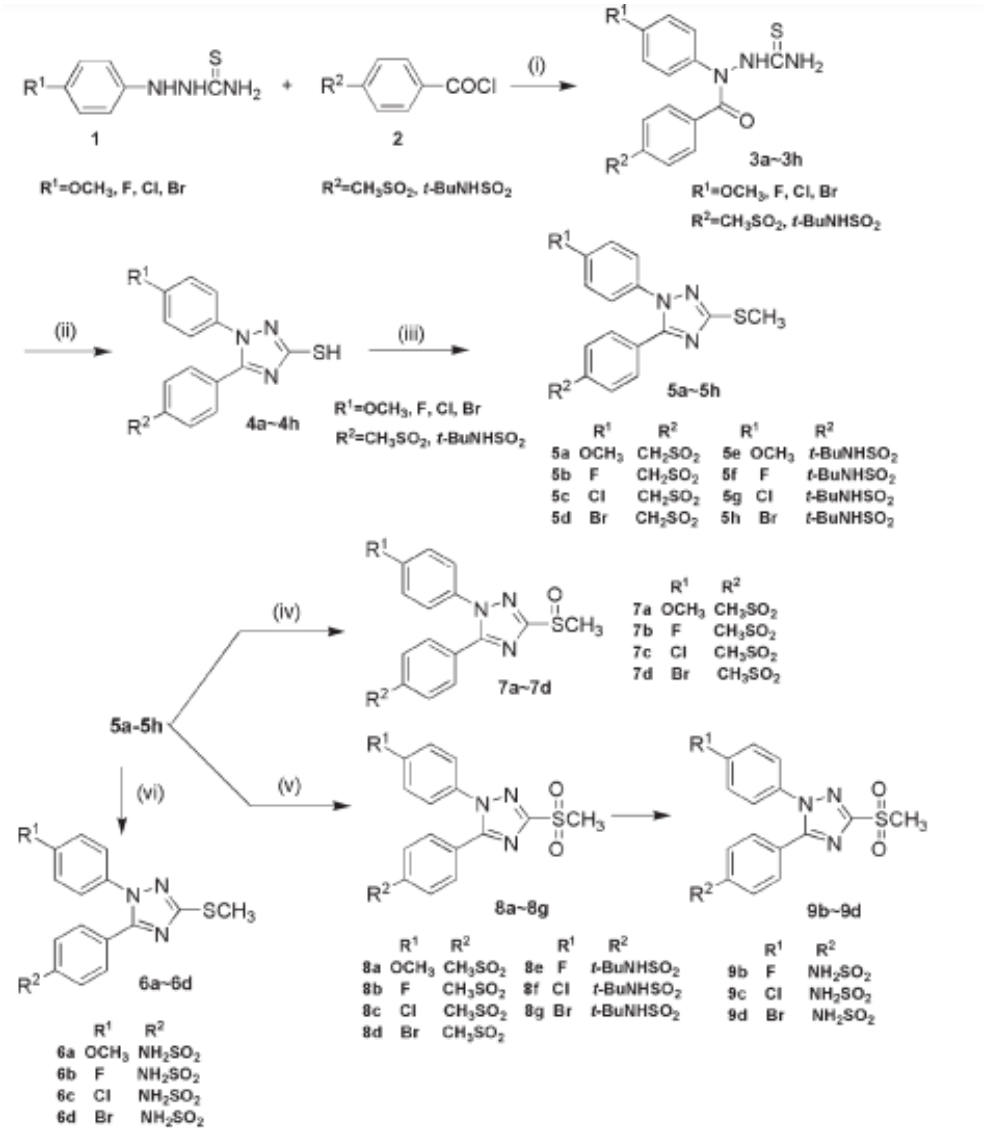
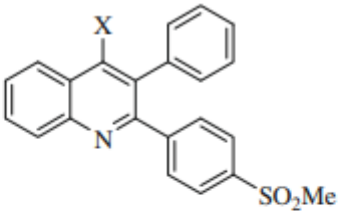



**Figure (12):** Triazole core inhibitors.

Derivatives of 2, 3-diarylquinoline were synthesized with methyl sulfonyl substitution on C-2 phenyl, Phenyl ring on C-3 and different substitutions on C-4. Compound **(8)** that was substituted with carboxylic acid on C-4 showed an excellent potency and selectivity on COX-2 with $IC_{50}$ value of 0.07µM and SI of 687.5 which was more selective than Celecoxib ($IC_{50}$ = 0.06 µM,

SI= 405). That indicates the importance of the size and nature of C-4 substitution on inhibition like carboxylic acid that interact with $Ser^{530}$ and the importance of Para methyl sulfonyl in selectivity by interacting with amino acids in the side pocket as shown in figure (13) [58].

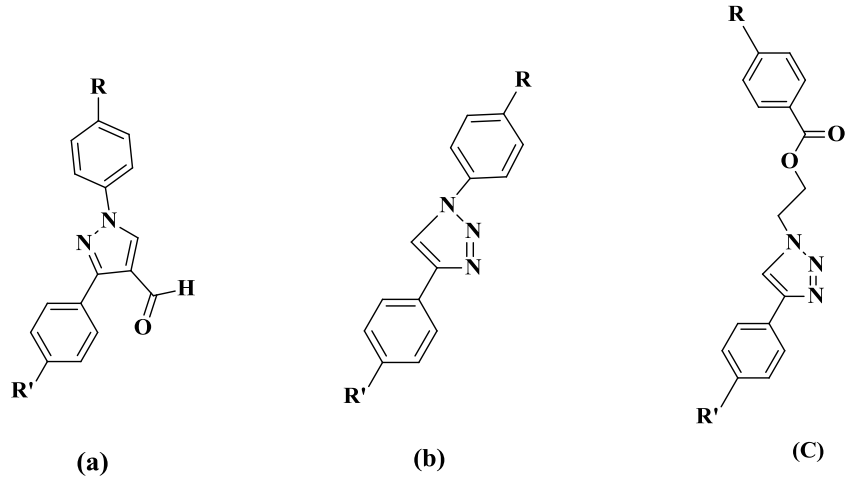



| Compound | X | $IC_{50}$[a] (μM) | | Selectivity index[b] (SI) |
|---|---|---|---|---|
| | | COX-1 | COX-2 | |
| **4** | H | 33.5 | 0.11 | 304.5 |
| **5** | Me | 13.3 | 0.11 | 121.3 |
| **6** | Ph | 10.2 | 0.13 | 78.5 |
| **7** | $NH_2$ | 14.4 | 0.08 | 180.0 |
| **8** | COOH | 48.1 | 0.07 | 687.1 |
| Celecoxib | – | 24.3 | 0.06 | 405 |

**Figure (13):** Quinoline core inhibitors.

## 1.8 Aims of the study

The aim of our study is to synthesize a library of selective COX-2 derivatives, characterize and test them to find potent and selective derivatives on COX-2 enzyme. In order to produce our library, three scaffolds were used and synthesized based on pyrazoles and triazoles with and without linker as shown in scheme 4.




**Scheme (4):** Scaffolds of selective COX-2 library.

## 1.9 Objectives

1. To synthesize library of selective COX-2 derivatives with varies substitutions.
2. To characterize the derivatives of established library by Infrared, Mass Spectroscopy and Nuclear Magnetic Resonance.
3. In vitro testing on COX-1 and COX-2 enzymes screening kits and determine the inhibitory concentration ($IC_{50}$) of the synthesized compounds.

# Chapter Two

## 2. Methodology

### 2.1 Reagents and materials

All reagents were obtained commercially and used without further purification. L-ascorbic acid sodium salt (catalog # A17759), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (catalog # A10807), trifluoroacetic acid (TFA) (catalog # A12198), 4'-bromoacetophenone (catalog # 10203851), 4-sulfonamidophenylhydrazine hydrochloride (catalog # 10211549), 4-acetylbenzene sulfonamide (catalog # 10203019), 4-bromobenzene sulfonamide (catalog # 10123953), 4-beomophenylmethyl sulfone (catalog # 10158882), 4-(methylsulfonyl) aniline hydrochloride (catalog # 10129542) were purchased from Alfa Aesar company (England). Diisopropylethylamine (DIPEA), *N, N, N',N'*-tetramethyluronium tetrafluoroborate (TBTU), 4-(methylsulfonyl) benzoic acid (catalog # 03822KEV), 4-sulfamoyl benzoic acid (catalog #MKCC6147), 4'-(methylsulfonyl) acetophenone (catalog # BCBS4132V), were provided by Sigma-Aldrich (Germany). 4-bromophenyl hydrazinium chloride (catalog # 177165) was purchased from Merck-sehuehard Company (Germany).

Sodium azide (catalog # 0E30428) was purchased from RiedeldeHaën Company (Germany). Toluene-4-sulfonylchloride (catalog # 1234411), anhydrous copper sulfate (catalog # 451657)**.** Acetone, ethanol (EtOH),

methanol (MeOH) and dichloromethane (DCM) were purchased from (C.S. Company, Haifa). Chloroform ($CHCl_3$) (catalog # 67-66-3), triethylamine ($Et_3N$) (catalog # 40502L05) and diethyl ether (catalog # 38132) were purchased from (Merck Millipore) and tetrahydrofuran (THF) solvent (catalog # 487308) was purchased from (Carlo Erba Company, MI. Italy). N, N-Dimethylformamide (DMF) (catalog # 55145) was purchased from (Frutarom Laboratory Chemicals). Sodium chloride, sodium hydroxide were purchased from (C.S. Company, Haifa), COX (human) Inhibitor Screening Assay Kit (Item # 701230).

## 2.2 Instrumentation

For Flash Chromatography, silica gel (Merck, 230-400 mesh) was used. Columns were eluted with positive air pressure. For evaporation of solvents, Rota Vapor (Heidolph) was used. NMR analysis was measured by Bruker Avance 500 spectrometer at Jordan University. Accumax Variable micropipette, UK was used for pipetting. Unilab microplate reader 6000was used to read the plate, USA. High-resolution mass spectra data (HRMS) were collected using a Shimadzu LCMS-IT-TOF using ESI (+) method at Doping and Narcotics Analysis Laboratory of the faculty of pharmacy, Anadolu University-Turkey.

## General procedure of performing column chromatography

Column was selected depending on sample size, Column preparation then made by adding silica gel on cotton capped column following with

dissolving the silica by mobile phase that considered after optimization by TLC. Sample dissolved in mobile phase then added on silica. Tubes collection finally made after elution for analysis.

## 2.3 Synthesis and characterization of the products

All the synthetic procedures and testing on enzyme screening Kit were conducted at An-Najah National University laboratories. NMR measurements were conducted at the University of Jordan and HRMS was conducted at Anadolu University-Turkey.

## 2.4 General synthetic procedures

### 2.4.1 General Synthesis and characterization of hydrazone derivatives

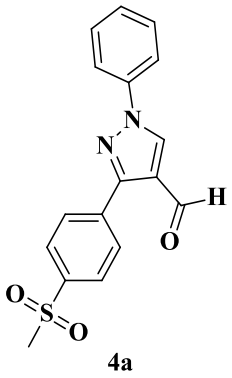
To a mixture of acetophenone and phenyl hydrazine derivatives in 20ml ethanol (EtOH). Amount of acetic acid glacial was directly added. The reaction was refluxed over night at 70ºC. Ethanol was removed by rotary evaporator under reduced pressure and the yielded solid product was used without further purification.

### 2.4.2 General Synthesis of pyrazole derivatives

Vilsmeier- haack reagent was synthesized. Dimethyformamide (DMF) was stirred under 0ºC, and then phosphorus oxychloride ($POCl_3$) was added drop wise. Hydrazone in 1 ml DMF was drop wisely added on the reagent. The reaction was refluxed for (5-6 hrs) at 70ºC. The reaction then was poured on cold distilled water (DW) mixed with concentrated sodium bicarbonate

solution. The solution then was filtered by suction filtration. The precipitate was taken and purified by flash chromatography.

### 2.4.2.1 Synthesis of (compound 4a)



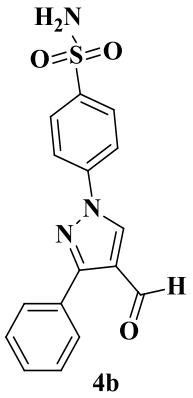

**4a**

To obtain **compound 3a**

4'-(methyl sulfonyl) acetophenone (198.2 mg, 1 mmol) with Phenyl hydrazine (-113 µl, 1.15 mmol), glacial acetic acid (86 µl, 1.5mmol). A pure orange product was obtained (Yield 97%, 280mg, and 0.97 mmol). **$R_f$:** 0.3 (Hexane: EtOAc 1:1)

Hydrazone **3a** (0.3 g, 0.92 mmol), $POCl_3$ (1.5 ml, 15.8 mmol), DMF (813 µl, 10.50 mmol). A pure off-white product was obtained (Yield 80%, 240 mg, 0.73 mmol). **$R_f$:** 0.3 (Hexane-: EtOAc 1:1).

### 2.4.2.2 Synthesis of (compound 4b)



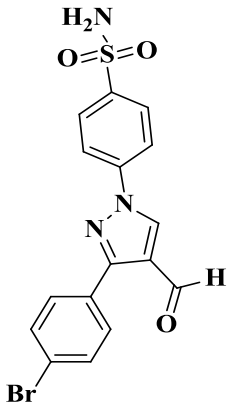

**4b**

To obtain **compound 3b**

Acetophenone (272.1µl, 2.33 mmol) with 4-sulfonamide phenyl hydrazine HCl (600 mg, 2.68 mmol), glacial acetic acid (116 µl, 2.027 mmol). A pure orange product was obtained (Yield 96%, 650 mg, and 2.25 mmol). **$R_f$:** 0.3 (Hexane: EtOAc 1:1)

Hydrazone **3b** (1 g, 3.456 mmol), $POCl_3$ (2774 µl, 29.625 mmol), DMF (1525 µl, 19.748 mmol). A pure off-white product was obtained (Yield 94%, 1070mg, 3.27 mmol). **$R_f$:** 0.3 (Hexane: EtOAc 1:1)

### 2.4.2.3 Synthesis of (compound 4c)



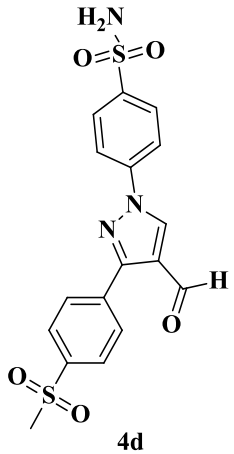

**4c**

To obtain **compound 3c**

4-bromoacetophenone (464 mg, 2.33 mmol) with 4-sulfonamide phenyl hydrazine HCl (600 mg, 2.68 mmol), glacial acetic acid (116 µl, 2.027 mmol). A pure red product was obtained (Yield 85%, 730mg, 1.9 mmol). **$R_f$:** 0.6 (Hexane: EtOAc 1:2)

Hydrazone **3c** (0.6 g, 1.6 mmol), $POCl_3$ (1282 µl, 13.7 mmol), DMF (708 µl, 9.14 mmol). A pure off-white product was obtained (Yield 92%, 600 mg, 1.48 mmol). **$R_f$:** 0.6 (Hexane: EtOAc 1:2)

### 2.4.2.4 Synthesis of (compound 4d)



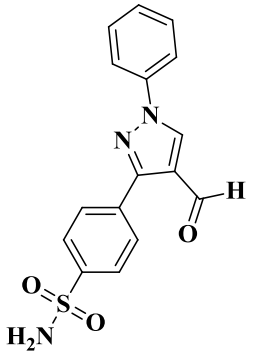

**4d**

To obtain **compound 3d**

4’-(methyl sulfonyl) acetophenone (308 mg, 1.55 mmol) with 4-sulfonamide phenyl hydrazine HCl (400 mg, 1.79 mmol), glacial acetic acid (133 µl, 2.33 mmol). A pure orange product was obtained. (Yield 88%, 500 mg, 1.36 mmol). **$R_f$:** 0.6 (Hexane: EtOAc 1:2)

Hydrazone 3d (0.6 mg, 1.48 mmol), $POCl_3$ (1156 µl, 12.68 mmol), DMF (655 µl, 8.46 mmol). A pure off-white product was obtained (Yield 43%, 260 mg, 0.64 mmol). **$R_f$:** 0.6 (Hexane: EtOAc 1:2)

### 2.4.2.5 Synthesis of (compound 4e)



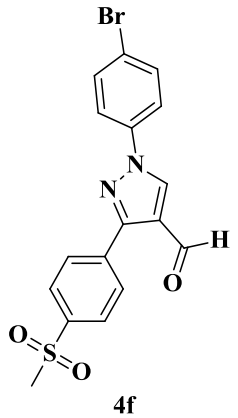

**4e**

To obtain **compound 3e**

4-acetyl benzene sulfonamide (400 mg, 2 mmol) with phenyl hydrazine (226 µl, 2.3 mmol), glacial acetic acid (172 µl, 3 mmol). A pure red product was obtained. (Yield 95%, 550 mg, 1.9 mmol). **$R_f$:** 0.5 (Hexane: EtOAc 1:1)

Hydrazone **3e** (0.5 g, 1.9 mmol), $POCl_3$ (1523 µl, 16.3 mmol), DMF (841 µl, 10.86 mmol). A pure off-white product was obtained (Yield 32%, 200mg, 0.61 mmol). **$R_f$:** 0.5 (Hexane: EtOAc 1:1)

### 2.4.2.6 Synthesis of (compound 4f)



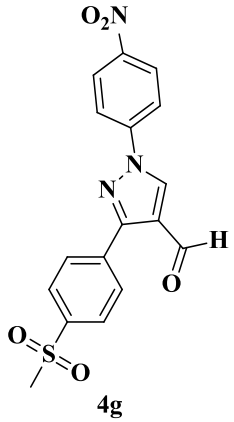

**4f**

To obtain **compound 3f**

4’-(methyl sulfonyl) acetophenone (400 mg, 2 mmol) with 4-bromo phenyl hydrazinum chloride (514 mg, 2.3 mmol), glacial acetic acid (172 µl, 3 mmol). A pure brown product was obtained (Yield 91%, 670 mg, 1.8 mmol). **$R_f$:** 0.3 (Hexane: EtOAc 1:1)

Hydrazone **3f** (0.6 g, 1.6 mmol), $POCl_3$ (1309 µl, 14 mmol), DMF (720 µl, 9.33 mmol). A pure yellow product was obtained (Yield 55%, 360 mg, 0.88 mmol). **$R_f$:** 0.3 (Hexane: EtOAc 1:1)

### 2.4.2.7 Synthesis of (compound 4g)




4g

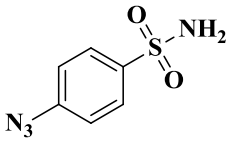
To obtain **compound 3g**

4’-(methyl sulfonyl) acetophenone (400 mg, 2 mmol) with 4-Nitro phenyl hydrazine (352 mg, 2.3 mmol), glacial acetic acid (172 µl, 3 mmol). A pure orange product was obtained (Yield 88%, 590 mg, 1.76 mmol). **$R_f$:** 0.3 (Hexane: EtOAc 1:1)

Hydrazone **3g** (0.6 mg, 1.8 mmol), $POCl_3$ (1445 µl, 15.4 mmol), DMF (794 µl, 10.28 mmol). A pure brown product was obtained (Yield 115%, 770 mg, 2.1 mmol). **$R_f$:** 0.3 (Hexane: EtOAc 1:1)

### 2.4.3 General Synthesis and characterization of Triazoles without linker

#### 2.4.3.1 Synthesis of (compound 6a)

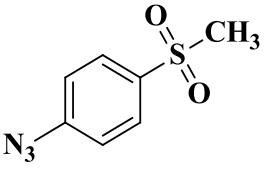


**6a**

Sulfanilamide **5a** (0.5 g, 2.9 mmol) was dissolved in 4M HCl (5 ml) at 0 ºC, 5M sodium nitrite ($NaNO_{2)}$ (240 mg, 3.48 mmol) solution was added drop wise and stirred for about 10 min. Then 5M solution of sodium azide ($NaN_3$) (283 mg, 4.35 mmol) was added to reaction drop wise. The reaction was stirred for 30 min at RT. A solid product was produced after evaporization and was used as its. (Yield 85.25%, 490mg, 2.47 mmol).

Compound **6a** was previously prescribed as [59, 60].

#### 2.4.3.2 Synthesis of (compound 6b)



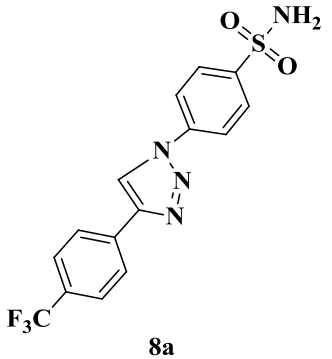
**6b**

4-(methyl sulfonyl) aniline HCl **5b** (0.5 g, 2.41 mmol) was dissolved in 4M HCl (5ml) at 0 ºC, 5M Sodium nitrite ($NaNO_2$) (199.41 mg, 2.89 mmol) solution was added on reaction drop wise and left on stirrer for about 10 min. Then 5M solution of sodium azide ($NaN_3$) (234mg, 3.6 mmol) was added to reaction drop wise. The reaction was left on stirrer for 30 min at RT. A solid

product was produced after evaporization and was used as its. (Yield 88%, 420mg, 2.13 mmol).

Compound **6b** was previously prescribed as [61].

### 2.4.3.3 Synthesis of (compound 8a)

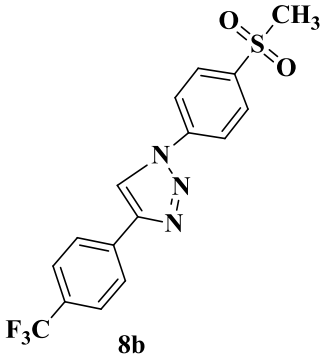



**8a**

In empty and clean RBF, A mixture of 4-azidobenzenesulfonamide 6a (150mg, 0.76 mmol), 4-ethynyl-α, α, α-triflurotoluene **7** (149µl, 0.912 mmol) and 4 ml DCM was added on stirrer. Sodium ascorbate (150.56mg, 0.76 mmol) was mixed and dissolved with 1.5 ml DW, Then $CuSO_4$ anhydrous (121.3mg, 0.76 mmol) was added to it. The later mixture was added on RBF with 2.5ml DW and the reaction was left on stirrer over night. The reaction washed with 50ml DW after dilution with 70ml DCM. Then reduced pressure was used to remove the solvent and flash chromatography on silica gel was used to purify the remaining crude, eluting with Hexane: EtOAc (2:1). A pure white solid product was obtained. (Yield 36%, 100 mg, 0.27 mmol).

**$R_f$:** 0.3 (Hexane/EtOAc 2:1).

### 2.4.3.4 Synthesis of (compound 8b)



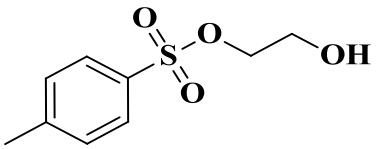

**8b**

In empty and clean RBF, A mixture of 1-azido-4(methyl sulfonyl) benzene **6b** (150mg, 0.76 mmol), 4-ethynyl-α, α, α-triflurotoluene **7** (149µM, 0.9 mmol) and 4 ml DCM was added on stirrer. Sodium ascorbate (150.6mg, 0.76 mmol) was dissolved in 1.5 ml distilled water, Then $CuSO_4$ anhydrous (121.3mg, 0.76 mmol) was added to it. The later mixture was added on RBF with 2.5ml DW and the reaction was left on stirrer over night. The reaction washed with 50ml DW after dilution with 70ml DCM. Then reduced pressure was used to remove the solvent and flash chromatography on silica gel was used to purify the remaining crude, eluting with Hexane: EtOAc (3:2). A pure white solid product was obtained. (Yield 88%, 420mg, 2.13 mmol).

**$R_f$:** 0.4(Hexane/EtOAc 3:2).

### 2.4.4 Synthesis of triazoles with linker

#### 2.4.4.1 Synthesis of (compound 11)

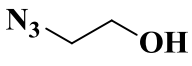


**11**

In empty and clean RBF, A mixture of ethylene glycol **9** (5ml, 89.72 mmol) and triethylene amine (6.25ml, 44.86 mmol) was added on stirrer with 6ml DCM for 30 min. Tosyl chloride **10** (8.55g, 44.80 mmol) was drop wisely added for about 30 min. The reaction was left on stirrer over night. The reaction then was washed with 1M HCl (50ml) after dilution with 70ml DCM. The solvent was removed by rotary evaporator and semisolid product was obtained. Flash chromatography was used for purification, eluting with Hexane: EtOAc (1:1). An oily pale yellow product was yielded. (Yield 8.5%, 1.65g, 7.63 mmol)

**$R_f$:** 0.4(Hexane/EtOAc 1:1).

#### 2.4.4.2 Synthesis of (compound 12)



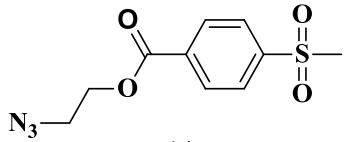
**12**

In empty and clean RBF, Monotosylated ethylene glycol (1g, 4.2 mmol) was added with 5ml ethanol on stirrer. Then $NaN_3$ (0.3g, 4.65 mmol) was added. The reaction was left on reflux over night on 70ºC. The reaction was diluted with amount of diethylether and poured on amount of silica to remove the

excess of $NaN_3$. A pale yellow oily product was produced after evaporization. (Yield 96%, 350 mg, 4.02 mmol).

**$R_f$:** 0.6 (DCM/MeOH 20:1).

### 2.4.4.3 Synthesis of (compound 14a)

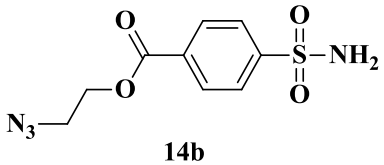


**14a**

In empty and clean RBF, A mixture of 4-(Methylsulfonyl) benzoicacid **13a** (150mg, 0.75 mmol), 2-azidoethanol **12** (98mg, 1.125 mmol), EDC (287.55mg, 1.5 mmol), DEMAP (138.04mg, 1.125 mmol) was added on stirrer. Then vacuum and argon was made three times. 6ml of DCM was added to the previous mixture. The reaction was left on stirrer overnight. The reaction was washed with 50ml 1M HCl after dilution with 70ml DCM and. Then reduced pressure was used to remove the solvent and flash chromatography on silica gel was used in purification of remaining crude, eluting with Hexane: EtOAc (1:1). A pure white solid product was obtained. (Yield 69%, 140 mg, 0.52 mmol).

**$R_f$:** 0.4 (Hexane/EtOAc 1:1).

### 2.4.4.4 Synthesis of (compound 14b)

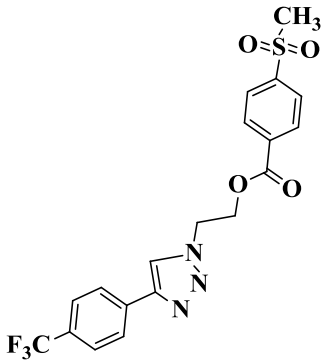



**14b**

In empty and clean RBF, A mixture of 4-sulfamoylbenzoicacid **13b** (150mg, 0.75 mmol), 2-azidoethanol **12** (97.4mg, 1.12 mmol), EDC (287.55mg, 1.5 mmol), DEMAP (138.04mg, 1.125 mmol) was added on stirrer. Then vacuum and argon was made three times. 6ml of DCM was added to the previous mixture. The reaction was left on stirrer overnight. The reaction was washed with 50ml 1M HCl after dilution with 70ml DCM. Reduced pressure was used to remove the solvent and flash chromatography on silica gel was used for purification of remaining crude was purified by, eluting with Diethylether: MeOH (9:1). A pure white solid product was obtained. (Yield 49%, 100 mg, 0.37 mmol).

**$R_f$:** 0.4 (Diethyl ether: MeOH 9:1)

### 2.4.4.5 Synthesis of (compound 15a)

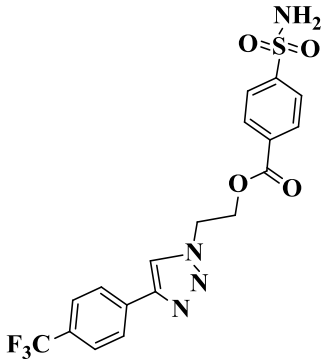



**15a**

In empty and clean RBF, A mixture of 2-azidoethyl 4-(methylsulfonyl) benzoate **14a** (240mg, 0.89 mmol), 4-ethynyl-α, α, α-triflurotoluene **7** (174.5µM, 1.1 mmol) and 4 ml DCM was added on stirrer. Sodium ascorbate (176.3mg, 0.89 mmol) was dissolved in 1.5 ml distilled water, Then $CuSO_4$ anhydrous (142mg, 0.89 mmol) was added to it. The later mixture was added on RBF with 2.5ml DW and the reaction was left on stirrer over night. The reaction was washed with 50ml DW after dilution with 70ml DCM. Reduced pressure was used to remove the solvent and flash chromatography on silica gel was used in purification of remaining crude was purified by, eluting with DCM: MeOH (20:1). A pure white solid product was obtained. (Yield 36%, 140 mg, 0.32 mmol).

**$R_f$:** 0.4 (DCM/MeOH 20:1).

### 2.4.4.6 Synthesis of (compound 15b)



**15b**

In empty and clean RBF, A mixture of 2-azidoethyl 4-sulfamoylbenzoate **14b** (280mg, 1.04 mmol), 4-ethynyl-α, α, α-triflurotoluene **7** (203.57µl, 1.24 mmol) and 4 ml DCM was added on stirrer. Sodium ascorbate (206mg, 1.04 mmol) was dissolved in 1.5 ml distilled water, Then $CuSO_4$ anhydrous (166

mg, 1.04 mmol) was added to it. The later mixture was added on RBF with 2.5ml DW and the reaction was left on stirrer over night. The reaction washed with 50ml DW after dilution with 70ml DCM. Reduced pressure was used to remove the solvent and flash chromatography on silica gel was used in purification of remaining crude, eluting with Hexane: EtOAc (1:1). A pure white solid product was obtained. (Yield 48%, 220 mg, 0.5 mmol).

**$R_f$:** 0.4 (Hexane/EtOAc 1:1).

### 2.4.5 In vitro test on COX-1 and COX-2 enzymes screening kits

The COX-1 and COX-2 inhibitory activity of our synthesized compounds as well as the Celecoxib as positive control were tested on the COX (human) Inhibitor Screening Assay Kit (supplied by Cayman chemicals (catalog no. 701230), Ann Arbor, MI, USA). The preparation of the reagents and the testing procedure were performed according to the manufacturer recommendations. In brief, various concentrations of the inhibitors and Celecoxib (concentration range 100 µM- 0.001 µM) dissolved in a minimum quantity of dimethylsulfoxide (DMSO) were incubated with a mixture of COX-1 or COX-2 enzyme, Heme in the diluted reaction buffer. The reaction was initiated by adding 50 µl of Arachidonic acid followed by incubation at 37 $^{0}$ C for exactly 30 seconds. Then reaction was stopped by adding 30 µl of stannous chloride solution to each reaction tube followed by incubation for 5 min at room temperature. The produced PGF2a in the samples by COX reactions was quantified via enzyme-linked immunosorbent assay (ELISA). The 96-well plate was covered with plastic film and incubated for 18 hr at

room temperature on an orbital shaker. After incubation, the plate was rinsed five times with the washed buffer followed by the addition of Ellman's reagent (200 µl) and incubated for about 60-90 mins at room temperature until the absorbance of Bo well is in the range 0.3-0.8 at 405 nm. The plate was then read by an ELISA plate reader Unilab microplate reader 6000. The inhibitory percentage was measured for the different tested concentrations against the control. The $IC_{50}$ was calculated from the concentration inhibition response curve and the selectivity index (SI) was calculated by dividing the $IC_{50}$ COX-1 on the $IC_{50}$ COX-2. Celecoxib was used as positive standard drug in the study.

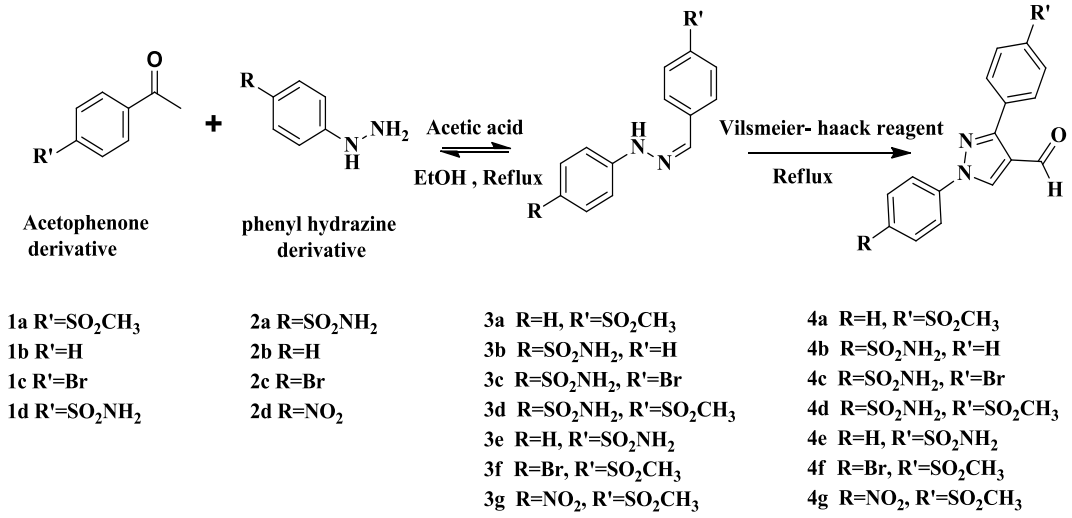
# Chapter Three

## 3. Results and discussion

In this thesis, we aim to synthesize new COX-2 inhibitors based on pyrazole or triazole nucleus. Since there are limited number of clinically approved COX 2 selective inhibitors with a high cost of synthesis. There is an emergence needs to synthesize new, effective, selective, cheap and safe COX-2 inhibitors. Therefore, herein we aim to develop effective COX-2 inhibitors with simple, cheaper and high yield synthesis based on pyrazole or triazole nucleus.

## 3.1 Synthesis and characterization of pyrazole derivatives

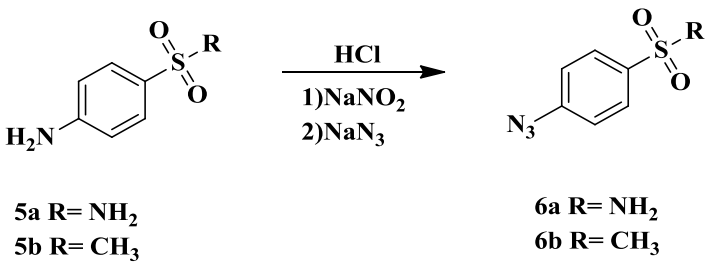
In order to synthesize pyrazole derivatives, first step was the synthesis of hydrazones **Compounds (3a-3g)** by reacting compounds of acetophenone derivatives with different phenyl hydrazine derivatives in ethanol (EtOH) using acetic acid glacial as acid catalyst. Hydrazones then were reacted with Vilsmeier- haack reagent which produced by reacting Dimethyformamide (DMF) with phosphorus oxychloride ($POCl_3$). Different pyrazoles were produced from this reaction **Compounds (4a-4g)**. **Scheme (5)** below shows hydrazones and pyrazoles synthesis. All synthesized pyrazoles were purified by flash chromatography. The structures of these compounds were confirmed by high-resolution mass spectrometry (HRMS), $^{13}C$ NMR and $^{1}H$ NMR spectral data. (See appendix)




**Scheme (5):** Synthesis of compounds (3a-4g).

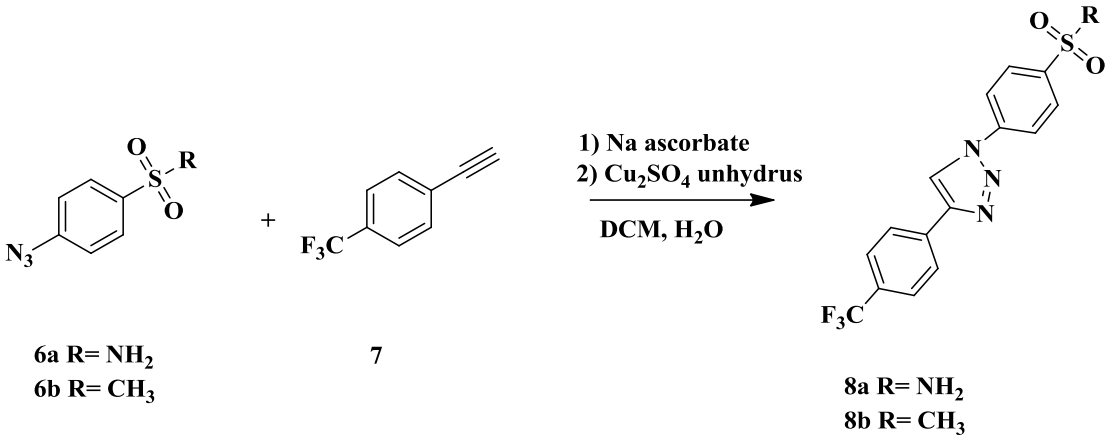
## 3.2 Synthesis and characterization of triazoles without linker

In order to synthesize triazole derivatives without linker **compounds (8a and 8b)**. First, the synthesis of the azide derivatives was synthesized by converting the amino group of Sulfanilamide or 4-(methyl sulfonyl) aniline to azide. This conversion was achieved by reacting the amino group with HCl, $NaNO_2$ and $NaN_3$ to synthesize **compounds 6a** or **6b**, respectively as shown in **scheme (6)**.




**Scheme (6):** Synthesis of compounds (6a, 6b).

After that, **compounds 6b** or **6b** was reacted with ethylene-triflurotoluene **(7)** through click reaction using sodium ascorbate as a reducing agent and $CuSO_4$ anhydrous in DCM/$H_2O$ (1:1) as shown in **schemes (7)**.

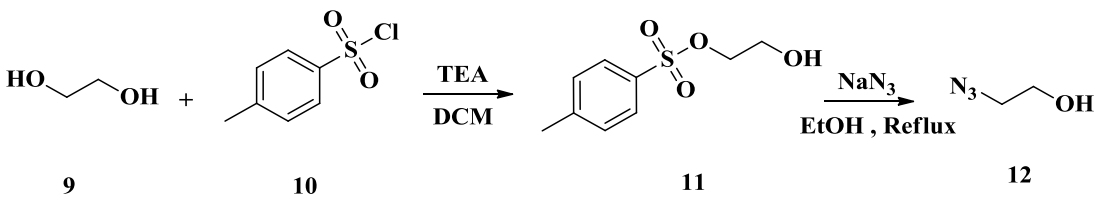



**Scheme (7):** Synthesis of compounds (8a, 8b).

All synthesized compounds were purified by flash chromatography and their structures were confirmed by $^{1}H$ NMR, $^{13}C$ NMR, and HRMS. (See appendix)

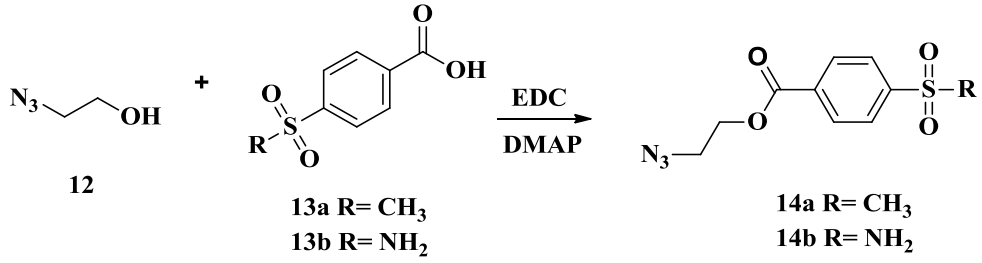
## 3.3 Synthesis and characterization of triazoles with linker

Herein we aim to synthesize triazole with linker between the two-aryl groups **Compounds (15a and 15b)**. For this reason, the linker was firstly synthesized by selective tosylation reaction of ethylene glycol **(9)** using tosyl chloride **(10),** triethylene amine in DCM to obtain **compound (11).** After that, tosyl group was replaced with azide by its reaction with $NaN_3$ in ethanol to produce azidoethanol **compound (12)** as a linker as shown in **Schemes (8).**



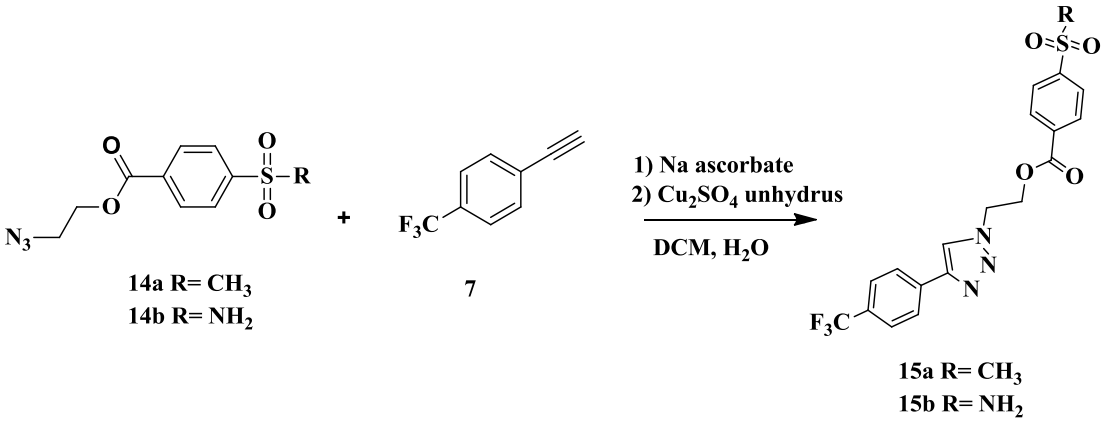

Scheme (8): **Synthesis of compounds (11, 12).**

Once the linker was synthesized, an esterification reaction of **compound (12)** with 4-(methyl sulfonyl)benzoic acid **(13a)** or 4-sulfamoyl benzoic acid **(13b)** using EDC as a coupling agent and DMAP as a catalyst to synthesize **compound (14a)** or **compound (14b),** respectively as shown in **scheme (9)**.




**Scheme (9):** Synthesis of compounds (14a, 14b).

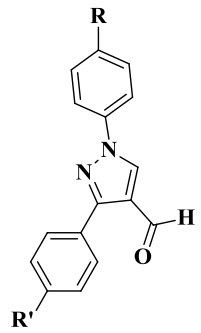
In a final step, triazoles were synthesized using click reaction between **compounds (14a)** or **(14b)** with 4-ethynyl-α,α,α-triflurotoluene **(7)** using sodium ascorbate as a reducing agent and $CuSO_4$ anhydrous in DCM/$H_2O$ (1:1) as shown in **Scheme (10).** All synthesized compounds were purified by flash chromatography and their structures were confirmed by $^{1}H$ NMR, $^{13}C$ NMR, and HRMS. (See appendix)



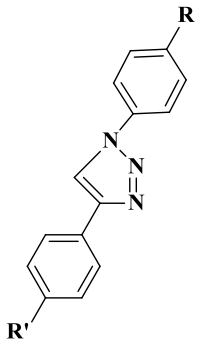

**Scheme (10):** Synthesis of compounds (15a, 15b).

## 3.4 In vitro activity on COX-1 and COX-2 enzymes

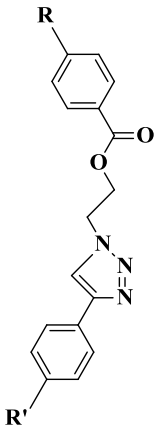
All synthesized compounds were tested for inhibition assay on COX-1 and COX-2 enzymes. Serial concentrations were made in the range (100 µM-0.001 µM) and compared with the activity of celecoxib as a positive control. Then half-maximal inhibitory concentration ($IC_{50}$) and selectivity index (SI) were calculated. Tables **1, 2, 3** below show $IC_{50}$ and SI results for pyrazole core, triazole core without linker and triazole core with linker compounds respectively.




(a)




(b)




(C)

**(a) Pyrazoles:**

**Table 1: half-maximal inhibitory concentration ($IC_{50}$) and selectivity index (SI) for pyrazol core compounds:**

| Compound | R | R' | $IC_{50}$ (COX-1) (µM) | $IC_{50}$ (COX-2) (µM) | SI |
|---|---|---|---|---|---|
| Celecoxib | | | 1.479 | 0.004 | 369.750 |
| (4a) | H | $SO_2CH_3$ | 17.507 | 1.386 | 12.631 |
| (4b) | $SO_2NH_2$ | H | 0.263 | 0.017 | 15.471 |
| (4c) | $SO_2NH_2$ | Br | 18.646 | 1.305 | 14.288 |
| (4d) | $SO_2NH_2$ | $SO_2CH_3$ | 5.375 | 0.098 | 54.847 |
| (4e) | H | $SO_2NH_2$ | 21.306 | 0.551 | 38.668 |
| (4f) | Br | $SO_2CH_3$ | 3.438 | 1.902 | 1.902 |
| (4g) | $NO_2$ | $SO_2CH_3$ | 0.012 | 1.460 | 0.008 |

**(b) Triazoles:**

**Table 2: half-maximal inhibitory concentration ($IC_{50}$) and selectivity index (SI) for triazole core without linker compounds:**

| Compound | R | R' | $IC_{50}$ (COX-1) | $IC_{50}$ (COX-2) | SI |
|---|---|---|---|---|---|
| (8a) | $SO_2NH_2$ | $CF_3$ | 0.145 | 3.324 | 0.044 |
| (8b) | $SO_2CH_3$ | $CF_3$ | 0.075 | 0.226 | 0.332 |

**(C) Triazoles with linker:**

**Table 3: half maximal inhibitory concentration ($IC_{50}$) and selectivity index (SI) for triazole core with linker compounds:**

| Compound | R | R' | $IC_{50}$ (COX-1) | $IC_{50}$ (COX-2) | SI |
|---|---|---|---|---|---|
| (15a) | $SO_2CH_3$ | $CF_3$ | 0.325 | 0.002 | 162.500 |
| (15b) | $SO_2NH_2$ | $CF_3$ | 0.273 | 0.131 | 2.084 |

## 3.5 Discussion

### 3.5.1 Pyrazole core:

From the obtained results, It was noticed that sulfonamide substitution increased COX-2 selectivity when compounds were substituted with sulfonamide at either the N-aromatic ring or the aromatic ring at position 4 (**Compounds 4b-4c**) but when one of the N-aromatic ring was substituted with sulfonamide and the other aromatic ring was unsubstituted (**Compound 4b**) the COX-2 inhibition $IC_{50}$ was better compared to compounds in which the other ring was substituted by sulfone or bromide (**Compound 4c &4d**). **Compound 4b** showed highest inhibition ($IC_{50}$ = 0.017µM). However, When the N-aromatic ring was substituted with sulfonamide and the other aromatic ring was substituted with sulfone **(Compound 4d)** best COX-2 selectivity among the synthesized pyrazole library was obtained. In addition (**Compound 4d**) showed a high COX-2 inhibition activity ($IC_{50}$ = 0.098µM, SI=54.847). It was noticed that Nitro substitution on the N-substitution aromatic ring showed more inhibition towards COX-1 (**Compound 4g**).

### 3.5.2 Trizaoles core:

Triazole compounds have N-aromatic ring either directly attached to the core or through a linker. The results showed that the presence of a linker would improve selectivity of inhibition towards COX-2 (**compounds 15a& 15b**). The presence of a linker in the synthesized compound makes it better fit the extra hydrophobic pocket present in the COX-2 enzyme. This is a possible

explanation for the increased inhibition activity for these compounds compared to compounds without linker. Moreover, results showed that sulfone substitution on the aromatic ring had a better COX-2 inhibition activity than compounds with sulfonamide substituted either in both linker and without linker compounds (**Compounds 8b &15a**). **Compound (15a)** which has a linker and sulfone substitution on one ring and the other ring has CF3 showed the best COX-2 inhibition activity among the whole synthesized library ($IC_{50}$ = 0.002).

Moreover, this compound had a two-fold increase in inhibition activity compared to celecoxib. The results also showed that compounds without linker were more selective towards COX-1 (**Compounds 8a & 8b**). A possible explanation is that these compounds fit better to COX 1 enzyme.

## 4. Conclusion

Variety of selective COX-2 inhibitors were successfully synthesized following simple synthetic approaches based on pyrazole and triazole cores. Vilsmeier-Haack reaction was used in pyrazole derivatives synthesis. Whereas, triazole derivatives with or without linker were synthesized by click reaction. Compounds **4b** and **4d** of pyrazole core were the most potent and selective on COX-2 with $IC_{50}$ values of 0.017 and 0.098 µM, respectively. Regarding the triazole core derivatives, compound **15a** showed the highest COX-2 inhibitory activity compared to Celecoxib with $IC_{50}$ of 0.002 µM as the presence of linker facilitate binding interactions in the side hydrophobic pocket of COX-2 enzyme.

## 5. Limitations and recommendations

Compounds with a variety of substitutions on pyrazole and triazole cores and different linker lengths are recommended to be synthesized in further studies to evaluate their COX-2 inhibitory activity and selectivity. Moreover, an *in vivo* anti-inflammatory and cardiotoxicity studies will be managed to support the obtained *in vitro* results.

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# Appendix

## - Characterization of pyrazoles:

**3- ( 4 -( methylsulfonyl ) phenyl) -1 – phenyl - 1H –pyrazole – 4 - carbaldehyde (compound 4a):**

**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 10.05 (s,1H, CHO), 8.56 (s,1H, CH pyrazole), 8.15 (d, 2H, *J*=9.0 Hz, Ph'H-2, Ph'H-6), 8.04 (d, 2H, *J*=9.1 Hz, Ph'H-3, Ph'H-5), 7.78(d, 2H, *J*=7.5 Hz, Ph H-2, Ph H-6), 7.51(t, 2H, *J*=7.5 Hz, Ph H-3, Ph H-5), 7,41 (t, 1H, *J*=7.5 Hz, Ph H-4), 3.08 (s, 3H, $SO_2CH_3$). **$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 184.9, 151.0, 141.5, 138.9, 136.6, 136.5, 130.2, 129.9, 128.4, 127.7, 122.9, 119.8, 43.9.

**HRMS (ESI, m/z):** calcd. For $C_{17}H_{14}N_2O_3S$ $[M + H]^+$ 327.0803, found 327.0798.

**4- (4 – formyl -3 – phenyl - 1H- pyrazol- 1- yl ) benzene sulfonamide (compound 4b):**

**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 10.02 (s,1H, CHO), 8.59 (s,1H, CH pyrazole), 8.00 (d, 2H, *J*=8.3 Hz, Ph H-2, Ph H-6), 7.89 (d, 2H, *J*=8.4 Hz, Ph H-3, Ph H-5), 7.78(d, 2H, *J*=6.0 Hz, Ph' H-2, Ph' H-6), 7.49-7.43 (m, 5H, *J*=7.5 Hz, Ph' H-3, Ph' H-5, Ph'H-4, $SO_2NH_2$). **$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 185.0, 159.9, 141.6, 141.2, 132.3, 131.8, 131.0, 130.6, 129.8, 129.6, 129.0, 128.9, 128.7, 128.2, 123.0, 119.9, 119.5.

**HRMS (ESI, m/z):** calcd. For $C_{16}H_{13}N_3O_3S$ $[M + H]^+$328.0756, found 328.0750.

**4-(3-(4-bromophenyl)-4-formyl-1H-pyrazol-1-yl) benzene sulfonamide (compound 4c):**

**$^1$H NMR (500 MHz, DMSO):** δ 9.32 (s,1H, CHO), 7.56 (s,1H, CH pyrazole), 7.45-7.42 (m, 2H, Ph H-2, Ph H-6), 7.27-7.16 (d, 6H, Ph H-3, Ph H-5, Ph'H-2, Ph'H-3, Ph'H-5, Ph'H-6 ), 6.97-6.94 (m, 2H, $SO_2NH_2$). **$^{13}$C NMR (125 MHz, DMSO):** δ 184.9, 160.4, 152.1, 142.3, 140.9, 136.8, 132.0, 131.1, 130.7, 128.2, 127.5, 123.4, 123.1, 119.9, 119.0.

**HRMS (ESI, m/z):** calcd. For $C_{16}H_{12}N_3O_3SBr$ $[M + H]^+$ 405.9861, found 405.9855.

**4-( 4- formyl -3 - (4- ( methylsulfonyl ) phenyl )- 1H- pyrazol- 1- yl) benzenesulfonamide (compound 4d):**

**$^1$H NMR (500 MHz, DMSO):** δ 10.05 (s, 1H, CHO), 8.62 (s, 1H, CH pyrazole), 8.12-7.89 (m, 8H, Ph – Ph'), 7.23 (s, 2H, $SO_2NH_2$), 3.08 (s, 3H, SO2CH3). **$^{13}$C NMR (125 MHz, DMSO):** δ 184.9, 160.4, 151.5, 151.0, 148.1, 142.5, 141.7, 140.9, 138.7, 137.1, 136.5, 130.0, 128.2, 127.7, 127.5, 123.3, 123.0, 120.0, 119.1, 43.9.

**HRMS (ESI, m/z):** calcd. For $C_{17}H_{15}N_3O_5S_2$ $[M + H]^+$ 406.0531, found 406.0526.

**4- ( 4- formy l- 1- phenyl -1 H- pyrazol -3 –y l ) benzene sulfonamide (compound 4e):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 9.95 (s,1H, CHO), 9.27 (s,1H, CH pyrazole), 7.95 (d, 2H, *J*=8.6 Hz, Ph' H-2, Ph' H-6), 7.87 (d, 2H, *J*=7.9 Hz, Ph' H-3, Ph' H-5), 7.72(d, 2H, *J*=7.9 Hz, Ph H-2, Ph H-6), 7.52 (t, 3H, *J*=7.6 Hz, Ph H-3, Ph H-5, Ph H-4),7.39 (s, 2H, $SO_2CH_3$). **$^{13}$CNMR (125 MHz, DMSO):** δ 185.1, 160.4, 152.6, 148.9, 139.0, 136.3, 135.4, 131.4, 130.2, 129.6, 128.2, 126.6, 126.2, 122.7, and 119.7.

**HRMS (ESI, m/z):** calcd. For $C_{16}H_{13}N_3O_3S$ $[M + H]^+$ 328.0756, found 328.0750.

**1- ( 4- bromophenyl )- 3 - ( 4 -( methylsulfonyl ) phenyl ) -1H –pyrazole - 4 -carbaldehyde (compound 4f):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 9.96 (s,1H, CHO), 9.38 (s,1H, CH pyrazole), 8.17 (d, 2H, *J*=7.9 Hz, Ph' H-2, Ph' H-6), 8.01 (d, 2H, *J*=7.6 Hz, Ph' H-3, Ph' H-5), 7.93(d, 2H, *J*=10.1 Hz, Ph H-2, Ph H-6), 7.73 (d, 2H, *J*=8.6 Hz, Ph H-3, Ph H-5) , 3.22 (s,3H, $SO_2CH_3$). **$^{13}$C NMR (125 MHz, DMSO):** δ 189.1, 155.8, 146.2, 142.8, 141.4, 137.7, 134.5, 132.3, 127.9, 126.3, 125.7, 48.8.

**HRMS (ESI, m/z):** calcd. For $C_{17}H_{13}N_2O_3SBr$ $[M + H]^+$ 404.9909, found 404.9903.

**3 - ( 4 - ( methylsulfonyl ) phenyl ) -1 - ( 4 –nitrophenyl ) - 1H - pyrazole-4-carbaldehyde (compound 4g):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 10.03 (s,1H, CHO), 9.57 (s,1H, CH pyrazole), 8.37 (d, 2H, *J*=6.7 Hz, Ph H-3, Ph H-5), 8.26 (d, 2H, *J*=7.9 Hz, Ph H-2, Ph H-6), 8.20 (d, 2H, *J*=8.5 Hz, Ph' H-2, Ph' H-6), 8.02 (d, 2H, *J*=7.9 Hz, Ph' H-3, Ph' H5), 3.16 (s,3H, $SO_2NH_2$). **$^{13}$C NMR (125 MHz, DMSO):** δ 184.9, 151.9, 146.5, 143.2, 141.8, 137.6, 136.1, 130.0, 127.7, 125.9, 123.7, 120.3, and 43.9.

**HRMS (ESI, m/z):** calcd. For $C_{17}H_{13}N_3O_5S$ $[M + H]^+$ 372.0654, found 372.0649.

## - Characterization of triazoles:

**Triazoles without linker:**

**4-( 4 - ( 4 -( trifluoromethyl ) phenyl )- 1H -1 , 2 , 3 – triazol -1 –yl ) benzenesulfonamide (compound 8a):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 9.61 (s,1H, CH triazole), 8.18(dd ,4H, *J*=5.8 Hz , *J*=2.8 Hz , Ph H-2, Ph H-6, Ph H-3, Ph H-5 ), 8.09 (d, 2H, *J*=8.5 Hz , Ph' H-3, Ph' H-5), 7.91(d, 2H, *J*=8.2 Hz, Ph' H-2, Ph' H-6), 7.55 (s, 2H, $SO_2NH_2$). **$^{13}$C NMR (125 MHz, DMSO):** δ 146.7, 144.6, 139.0, 134.5, 129.2, 128.9, 128.1, 126.5, 126.4, 125.8, 123.6, 121.6, 120.8.

**HRMS (ESI, m/z):** calcd. For $C_{15}H_{11}N_4O_2F_3S$ $[M + H]^+$ 369.0633, found 369.0628.

**1-(4-(methylsulfonyl) phenyl)-4-(4-(trifluoromethyl) phenyl)-1H-1, 2, 3-triazole (compound 8b):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 9.62 (s,1H, CH triazole), 8.23(d ,2H, *J*=8.9 Hz , Ph' H-3, Ph'H-5 ), 8.18 (dd, 4H, *J*=8.5 Hz , *J*=8.2 Hz, Ph H-2, Ph H-6, Ph H-3, Ph H-5 ), 8.87(d, 2H, *J*=8.2 Hz, Ph' H-2, Ph' H-6), 3.28 (s, 3H, $SO_2CH_3$). **$^{13}$C NMR (125 MHz, DMSO):** δ 146.8, 141.1, 140.3, 134.4, 129.6, 129.2, 128.9, 126.5, 126.4, 125.7, 121.7, 121.0, 44.0.

**HRMS (ESI, m/z):** calcd. For $C_{16}H_{12}N_3O_2F_3S$ $[M + H]^+$ 368.0681, found 368.0675.

**Triazoles with linker:**

**2-hydroxyethyl 4-methylbenzenesulfonate (compound11):**

**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 7.79 (d, 2H, *J*=8.4 Hz, $H_2OT_s$), 7.34 (d, 2H, *J*=7.9 Hz, $H_2OT_s$), 4.11 (t, 2H, *J*=4.3 Hz, $CH_2O$), 3.79 (t, 2H, *J*=4.6 Hz, $CH_2O$), 2.42 (s, 3H, $CH_3$), 2.00 (BS, 1H, OH). **$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 145.1, 132.6, 130.0, 128.0, 71.7, 60.7, 21.7.

**2-azidoethanol (compound 12):**

**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 3.73 (t, 2H, *J*=4.1 Hz, $CH_2OH$), 3.40 (t, 2H, *J*=4.7 Hz, $CH_2N_3$), 2.25 (BS, 1H, OH). **$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 61.8, 53.5.

**2-azidoethyl 4-(methylsulfonyl) benzoate (compound 14a):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 8.17 (d,2H, *J*=7.9 Hz, Ph H-2, Ph H-6), 8.07 (d,2H, , *J*=8.2 Hz, Ph H-3, Ph H-5), 4.47 (t, 2H, *J*=4.9 Hz ,$CH_2O$), 3.67 (t, 2H, *J*=5.2 Hz, $CH_2N_3$), 3.24 (s, 3H, $SO_2CH_3$). **$^{13}$C NMR (125 MHz, DMSO):** δ 164.9, 145.4, 134.1, 130.6, 128.0, 64.8, 49.8, 43.7.

**2-azidoethyl 4-sulfamoylbenzoate (compound 14b):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 8.12 (d,2H, *J*=8.5 Hz, Ph H-2, Ph H-6), 7.94 (d,2H, , *J*=8.5 Hz, Ph H-3, Ph H-5), 7.53 (s, 2H, $SO_2NH_2$), 4.45 (t, 2H, *J*=4.9 Hz, $CH_2O$), 3.66 (t, 2H, *J*=4.9 Hz, $CHN_3$).**$^{13}$C NMR (125 MHz, DMSO):** δ 165.0, 148.7, 132.6,130.4, 127.7, 126.6, 125.5, 64.6, 49.8.

**2-(4-(4-(trifluoromethyl) phenyl)-1H-1, 2, 3-triazol-1-yl) ethyl 4-(methylsulfonyl) benzoate (compound 15a):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 8.84 (s,1H, CH triazole), 8.10 (d, 2H, *J*=8.6 Hz, Ph' H-3, Ph' H-5 ), 8.02 (dd, 4H, *J*=6.4 Hz , *J*=1.8 Hz, Ph H-2, Ph H-6, Ph H-3, Ph H-5), 7.78(d, 2H, *J*=8.2 Hz, Ph' H-2, Ph' H-6), 8.86 (t, 2H, *J*=4.6 Hz, $CH_2O$), 4.74 (t, 2H, *J*=4.9 Hz, $CH_2N$), 3.28 (s, 3H, $SO_2CH_3$). **$^{13}$CNMR (125 MHz, DMSO):** δ 164.7, 145.6, 135.1, 134.0, 130.6, 127.9, 126.1, 123.6, 64.2, 49.3, and 43.6. **HRMS (ESI, m/z):** calcd. For $C_{19}H_{16}F_3N_3O_4S$ $[M + H]^+$440.0892, found 440.0885.

**2-(4-(4-(trifluoromethyl) phenyl)-1H-1, 2, 3-triazol-1-yl) ethyl 4-sulfamoylbenzoate (compound 15b):**

**$^{1}$H NMR (500 MHz, DMSO):** δ 8.86 (s,1H, CH triazole), 8.07-8.03 (m,4H, Ph H-2, Ph H-6, Ph H-3, Ph H-5 ), 7.92-7.90 (m, 2H, Ph' H-3, Ph' H-5), 7.80-7.77(m, 2H, Ph' H-2, Ph' H-6), 7.52 (bs, 2H, $SO_2NH_2$), 4.86 (t, 2H, *J*=4.0 Hz, $CH_2O$), 4.73(t, 2H, *J*=3.9 Hz, $CH_2N$). **$^{13}$C NMR (125 MHz, DMSO):** δ 164.9, 148.7, 145.5, 135.1, 132.4, 130.4, 128.6, 126.5, 123.7, 64.0, 49.4. **HRMS (ESI, m/z):** calcd. For $C_{18}$ $H_{15}N_4O_4F_3S$ $[M + H]^+$ 441.0844, found 441.0839.

**جامعة النجاح الوطنية**

**كلية الدراسات العليا**

# " مثبطات سيكلو اكسيجيناز 2 الانتقائية: التصنيع والتقييم البيولوجي "

**إعداد**

**هديل يوسف فندي صوافطة**

**إشراف**

**د. محي الدين عسالي**

**د. مراد أبو الحسن**

**قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الصيدلانية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.**

**2019**

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## الملخص

تم تصنيع مشتقات البايرزولات ثنائية الحلقة والتريازولات لإنتاج سلسلة من مثبطات انزيم السايكلو اكسيجيناز الانتقائية. ولتصنيع سلسلة هذه المشتقات، تم اتباع تفاعل فيلمسمير – هاك مع تفاعل النقر في تصنيع مشتقات البايرزول والتريازول على التوالي. تم تقييم جميع المركبات المنتجة في المختبر بواسطة دراسات التثبيط على انزيمي سايكلو اكسيجيناز 1 و2. خمسة مركبات كانت الأكثر فعالية وانتقائية في تثبيط انزيم سايكلو اكسيجيناز2 مع قيم تركيز المادة الموافق للتثبيط النصفي في نطاق 0.551 –0.002 مايكرومولار. بالنسبة لمشتقات البايرزولات ثنائية الحقلة أظهر المركب 4ب أفضل تثبيط بتركيز مادة موافق للتثبيط النصفي = 0,017 مايكرومولار حيث ارتبطت الحلقة العطرية N بمجموعة السلفون أميد والحلقة العطرية الأخرى كانت غير متفرعة. مع ذلك، أظهر المركب 4د أفضل انتقائية على انزيم السايكلواكسيجيناز بتركيز مادة موافق للتثبيط النصفي = 0,098 مايكرومولار ومعامل انتقاء= 54,847 حيث ارتبطت الحلقة العطرية N بـ بمجموعة السلفون أميد والحلقة العطرية الأخرى بمجموعة السلفون. بالنسبة لمشتقات التريازولات ثنائية الحلقة، كان المركب 15أ هو الأكثر فعالية بين كل مجموعة المركبات بما في ذلك المركب المرجعي (سيليكوكسيب) بتركيز مادة موافق للتثبيط النصفي = 0,002 مايكرومولار ومعامل انتقاء = 162,5حيث أن وجود رابط اضافي يمكن أن يسهل الربط مع المنطقة الكارهة للماء الإضافية لإنزيم سايكلو اكسيجيناز2.