T.C.
HACETTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

STUDIES ON SOME ARYLOXYMETHYL THIOSEMICARBAZIDE, 1,3,4-THIADIAZOLE AND 1,2,4-TRIAZOLE-5-THIONE DERIVATIVES

Mohammad Musa SHIRZAD

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ADVISOR
Prof. Dr. Erhan PALASKA

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Öğrenci Adı-Soyadı : Mohammad Musa Shirzad

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Jüri Başkanı: Prof.Dr. Erhan Palaska (Tez Danışmanı)
Hacettepe Üniversitesi

Üye: Prof.Dr. Ayla Balkan
Hacettepe Üniversitesi

Üye: Prof.Dr. Meral Tunçbilek
Ankara Üniversitesi

Üye: Prof.Dr. Rahime Şimşek
Hacettepe Üniversitesi

Üye: Prof.Dr. Birsen Tozkoparan
Hacettepe Üniversitesi

ONAY

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ÖZET

Shirzad, M. M., Bazı Ariloksimetil Tiyosemikarbazit, 1,3,4-Tiyadiazol ve 1,2,4-Triazol-5-tiyon Türevleri Üzerinde Çalışmalar, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Farmasötik Kimya Programı, Yüksek Lisans Tezi, Ankara, 2013. Bu çalışmada, 1-(2-(7-metoksi-2-naftiloksi)asetil)-4-sübstitüe-3-tiyosemikarbazit, 5-((7-metoksi-2-naftiloksi)metil)-2-sübstitüeamino-1,3,4-tiyadiazol ve 3-((7-metoksi-2-naftiloksi)metil)-4-sübstitüe-1,2,4-triazol-5-tiyon yapısında 12 yeni bileşiğin sentezi yapılarak, 2-sübstitüeamino-1,3,4-tiyadiazol ve 4-sübstitüe-1,2,4-triazol-5-tiyon yapısındaki sekiz bileşiğin COX-1 and COX-2 enzimleri üzerindeki inhibitör etkileri incelenmiştir. Bileşiklerin COX-2 enzimi ile etkileşmeleri "Molecular Operating Environment" (MOE) programı kullanılarak yorumlanmıştır.

5-((7-Metoksi-2-naftiloksi)metil)-2-sübstitüeamino-1,3,4-tiyadiazol (**Bileşik 2a-d**) ve 3-((7-metoksi-2-naftiloksi)metil)-4-sübstitüe-1,2,4-triazol-5-tiyon türevleri (**Bileşik 3a-d**) 1-(2-(7-metoksi-2-naftiloksi)asetil)-4-sübstitüe-3-tiyosemikarbazitlerin siklizasyonu ile elde edilmişlerdir. Sentezi yapılan bileşiklerin yapıları IR, ^{1}H-NMR, ^{13}C-NMR, kütle spektrumları ve elemental analiz ile aydınlatılmıştır.

Aktivitesi incelenen bileşikler (**Bileşik 2a-d ve 3a-d**) COX-2 enzimi üzerinde inhibitör etki göstermekle beraber, hiçbiri standart bileşikler NS-398 ve indometazin kadar etkili değildir. Sentez edilen türevler arasında 5-((7-Metoksi-2-naftiloksi)metil)-2-etilamino-1,3,4-tiyadiazolün **(Bileşik 2b)** COX-2 enzimine, 3-((7-metoksi-2-naftiloksi)metil)-4-etil-1,2,4-triazol-5-tiyon ve 3-((metoksi-2-naftiloksi) metil)-4-allil-1,2,4-triazol-5-tiyonun (**Bileşik 3b ve 3c**) COX-1 enzimine karşı seçici olarak en aktif bileşikler olduğu gözlenmiştir. COX-2 enzimi üzerinde yapılan *docking* çalışmaları sonucunda, **Bileşik 2b**'nin her iki enzimde ortak olan hidrofobik kısımlara yerleşerek enzim ile etkileştiği ancak COX-2'ye özgü ve Val349, Tyr355, Leu359 and Leu531 tarafından oluşturulan bölgeye yerleşemedikleri gözlenmiştir.

Anahtar kelimeler: Tiyosemikarbazit, 1,3,4-Tiyadiazol, 1,2,4-Triazol-5-tiyon, Anti-inflamatuar aktivite, Docking, COX-1, COX-2.

Bu çalışma Hacettepe Üniversitesi Bilimsel Araştırmalar Birimi tarafından desteklenmiştir. (Proje no:012D12301)

ABSTRACT

Shirzad, M, M., Studies on Some Aryloxymethyl Thiosemicarbazide, 1,3,4-Thiadiazole and 1,2,4-Triazole-5-thione Derivatives, Hacettepe University Institute of Health Sciences Ms. Sci. Thesis in Pharmaceutical Chemistry, Ankara, 2013. In this study, twelve 1-(7-methoxy-2-naphthyloxyacetyl)-4-subtituted-3-thiosemicarbazide, 5-(7-methoxy-2-naphthyloxymethyl)-2-substituted amino-1,3,4-thiadiazole and 3-(7-methoxy-2-naphthyloxymethyl)-4-substituted-1,2,4-triazole-5-thione derivatives have been synthesized and the compounds having 2-substitutedamino-1,3,4-thiadiazole and 4-substituted-1,2,4-triazole-5-thione structure were evaluated for inhibitory effects on COX-1 and COX-2 enzymes. The interaction between the **Compound 2b** and the COX-2 enzyme was interpreted by using "Molecular Operating Environment" MOE program.

5-(7-Methoxy-2-naphthyloxymethyl)-2-substitutedamino-1,3,4-thiadiazole **(Compounds 2a-d)** and 3-(7-methoxy-2-naphthyloxymethyl)-4-substituted-1,2,4-triazole-5-thione derivatives **(Compounds 3a-d)** were synthesized by cyclization of 1-(7-methoxy-2-naphthyloxyacetyl)-4-subtituted-3-thiosemicarbazides **(Compounds 1a-d)**. Chemical structures of the compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra and elemental analysis.

The synthesized compounds **(Compounds 2a-d and 3a-d)** showed lower inhibitory activities on COX-2 enzyme then standard compounds NS-398 and indomethacin. 2-(7-Methoxy-2-naphthyloxymethyl)-5-ethylamino-1,3,4-thiadiazole (**Compound 2b**) is more selective against COX-2, 3-(7-methoxy-2-naphthyloxy methyl)-4-ethyl-1,2,4-triazole-5-thione and 3-(7-methoxy-2-naphthyloxymethyl)-4-allyl-1,2,4-triazole-5-thione (**Compounds 3b and 3c**) are more selective against COX-1 than rest of the compounds. As a result of the *docking* studies on COX-2 enzyme, it was observed that the **Compound 2b** is fitted and interacted with the hydrophobic parts in the active pocket of COX-2, Val349, Tyr355, Leu359 and Leu531.

Key words: Thiosemicarbazide, 1,3,4-Thiadiazole, 1,2,4-Triazole-5-thione, Anti-inflammatory activity, Docking, COX-1, COX-2.

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ABBREVIATIONS

CAN	Ceric Ammonium Nitrate
CNS	Central Nervous System
COX	Cyclooxygenase
DCC	N,N'-Dicyclohexylcarbodiimide
DCE	Dichloroethane
DMAD	Dimethyl Acetylenedicarboxylate
DMF	Dimethylformamide
DMSO-d_6	Dimethylsulfoxide-d_6
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immuno-Sorbitent Assay
FDA	Food and Drug Administration
GI	Gastrointestinal
GSK-3b	Glycogen Synthase Kinase 3 beta
MAOIs	Monoamine Oxidase Inhibitors
m-CPBA	Meta-Chloroperbenzoic Acid
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NOX-1	Nicotinamide adenine dinucleotide phosphate-oxidase 1
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
PEG-400	Polyethylene Glycol-400
PGG_2	Prostaglandine G_2
PGH_2	Prostaglandine H_2
PGI_2	Prostacyclin
PPA	Polyphosphoric acid
p-TsCl	P-Toluenesulfonyl chloride
SAR	Structure–Activity Relationship
TEA	Triethylamine
THF	Tetrahydrofuran
TMS	Tetramethylsilane
TXA_2	Thromboxane A_2
PTZ	Pentylenetetrazol
MES	Maximal Electroshock Seizure

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1. INTRODUCTION

Inflammation is the immune system's response to infection and injury and has been implicated in the pathogeneses of some diseases. The cyclooxygenase (COX) isozymes that are responsible for inflammation are used as targets of non-steroidal anti-inflammtory drugs (NSAIDs). The NSAIDs are competitive active site inhibitors of both COXs (1).

Generally NSAIDs play singnificant roles in treatment of inflammatory diseases (2). Depending on the chemical structures, NSAIDs have different potencies against COX-1 and COX-2. The variation of activities and side effects of NSAIDs depend on these potencies. NSAIDs with low potency against COX-1 and also a lower potency against COX-2/COX-1 activity ratio and high potency against COX-2, hope to have good anti-inflammatory activity with less side effects on stomach and kidney (3).

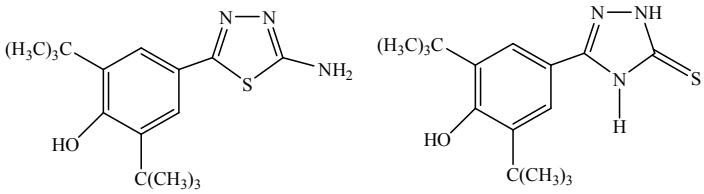
The aspect of inhibitory selectivity of NSAIDs against COXs enzymes becomes an important particularly under the point of view of low risk NSAIDs with reduced side-effects (4). Therefore, the classic NSAIDs are being pushed increasingly into the background, whereas selective COX-2 inhibitors with an attractive pharmacological profile and reduced side-effects are being favored (5). In support of this conclusion, researchers have been started to develope a new generation of compounds with high degree of selectivity for COX-2 with high anti-inflammatory and low ulcerogenic effects (4).

Substituted thiosemicarbazides known as thiourea derivatives and they are valuable building blocks for synthesis of 1,3,4-thiadiazoles, 1,3,4-oxadiazoles and 1,2,4-triazole-3-thiones like five-membered heterocycles. There are many studies with their antimicrobial activities (6, 7). It is well known that, 2-substitutedamino-1,3,4-thiadiazole and 4-substituted-1,2,4-triazole-5-thiones possess analgesic, anti-inflammatory (6-8), antibacterial, antifungal (9-11), anti-tuberculosis (12), antiviral (13), anticonvulsant (14, 15) and anticancer (16, 17) activities.

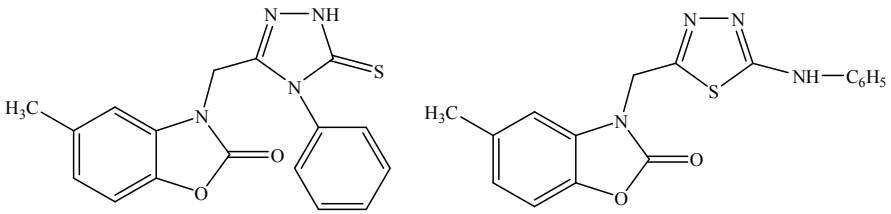
In the past decade to improve the efficacy/safety profile of new NSAIDs, extensive structure–activity relationship (SAR) studies have been carried out using a

wide variety of COX inhibitory profile. Compounds carrying 2-substitutedamino-5-aryl-1,3,4-thiadiazole and 3-aryl-4-substituted-1,2,4-triazole-5-thione rings are addressed higher analgesic and anti-inflammatory activities (6-8, 18-20).

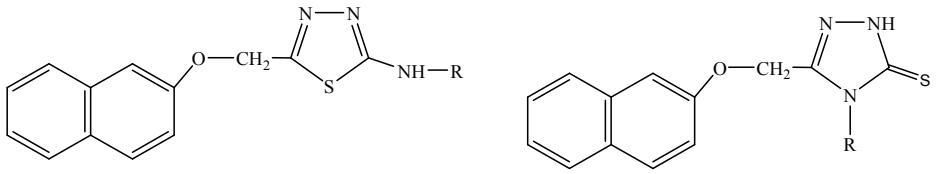
Mullican et al. (21) synthesized and evaluated the anti-inflammatory activity of 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-2-amino-1,3,4-thiadiazole, 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,2,4-triazole-5-thione. They found that the compounds possess high anti-inflammatory activity.



Salgın-Gökşen et al. (19) synthesized 3-[(5-methyl-2-benzoxazolinone-3-yl)methyl]-4-phenyl-1*H*-1,2,4-triazole-5(4*H*)-thiones, 2-phenylamino-5-[(5-methyl-2-benzoxazolinone-3-yl)methyl]-1,3,4-thiadiazoles to evaluate for their anti-inflammatory activities. It was observed that, some of the compounds showed higher analgesic activity when compared with aspirin and morphine, and they were found safe in ulcer incidence.



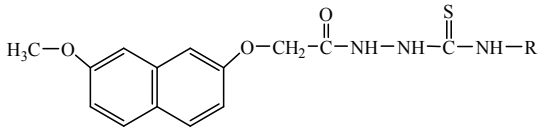
Palaska et al. (8) evaluated the anti-inflammatory activities of 2-(2-naphthyloxymethyl)-5-substitutedamino-1,3,4-thiadiazole and 3-(2-naphthyloxy methyl)-4-substituted-1,2,4-triazole-5-thione derivatives. They found that some of these compounds showed good anti-inflammatory activity.



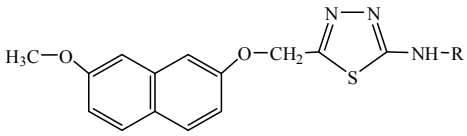
R: alkyl, aryl, allyl

As a part of our continuing efforts in this area, a series of 1-(2-(7-methoxy-2-naphthyloxy)acetyl)-4-substituted-3-thiosemicarbazide (**Compounds 1a-d**) and corresponding 1,3,4-thiadiazole (**Compounds 2a-d**) and 1,2,4-triazole-5-thione (**Compounds 3a-d**) derivatives were synthesized and their structures were confirmed using spectral data and elemental analysis. The anti-inflammatory activities of the compounds were evaluated by inhibition effects on COX-1 and COX-2 enzymes. Furthermore, docking studies for the **compound 2b** were performed in order to gain more insight toward its binding mode.

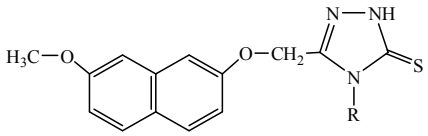
Table 1.1. Structure of synthesized compounds



Compound	R
1a	$-CH_3$
1b	$-C_2H_5$
1c	$-C_3H_5$
1d	$-C_6H_5$



2a	$-CH_3$
2b	$-C_2H_5$
2c	$-C_3H_5$
2d	$-C_6H_5$

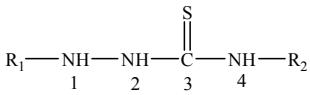


3a	$-CH_3$
3b	$-C_2H_5$
3c	$-C_3H_5$
3d	$-C_6H_5$

2. GENERAL DESCRIPTION

2.1. 1-Acyl-3-thiosemicarbazides

Thiosemicarbazides also known as thiourea derivatives and they are valuable building blocks for the synthesis of five-membered heterocycles. In order to show the position of substituents in thiosemicarbazides according to nomenclature rules numbering starts from nitrogen of hydrazine side.



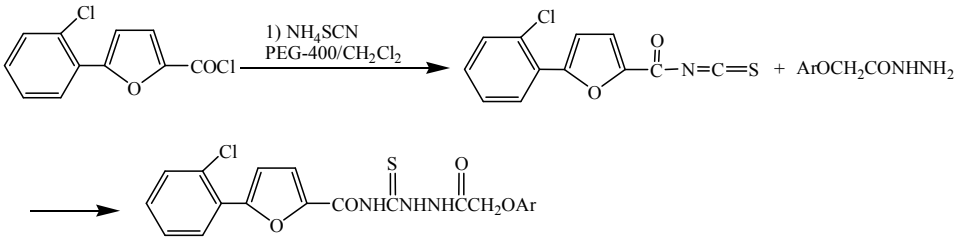
2.1.1. Synthesis

From Alkyl/Aryl Hydrazide and Thiocyanate Derivatives

1-Acyl-3-thiosemicarbazides were synthesized by the reaction of hydrazides and ammonium or potassium thiocyanate in an acidic medium at room temperature (22) or under reflux condition (6, 7, 23).

R: Aryl

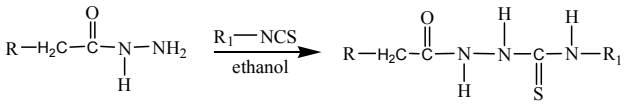
Zheng Li et al. (24) synthesized 5-(2-chlorophenyl)-2-furoylthiocyanate as intermediate product by reaction of 5-(2-chlorophenyl)-2-furoyl chloride with ammonium thiocyanate (catalyzed by polyethylene glycol-400 (PEG-400)) in methylene chloride. Then it was treated with aryloxyacetic acid hydrazide to obtain substituted thiosemicarbazides at room temperature.



Ar: Alkylaryl, halogenaryl, nitoraryl

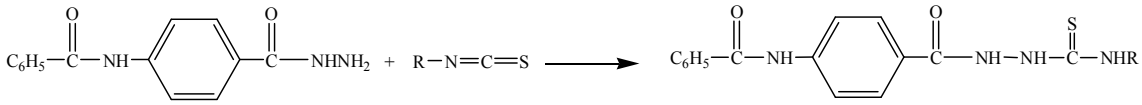
From Alkyl/Aryl Hydrazide and Substituted Isothiocyanate Derivatives

The reaction of alkyl/aryl hydrazides with isothiocyanates gave 1-acyl-3-thiosemicarbazides (25-33).



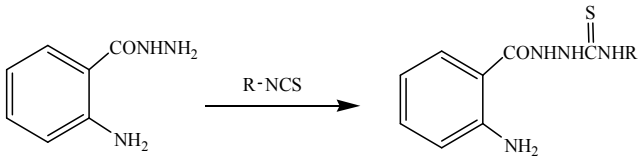
R: Aryl
R_1: Aryl, alkyl, allyl

Karakuş et al. (34) prepared the 1-[4-(benzoylamino)benzoyl]-4-alkyl/aryl-3-thiosemicarbazides by the reaction of 4-(benzoylamino)benzoylhydrazine with substituted isothiocyanates in ethanol.



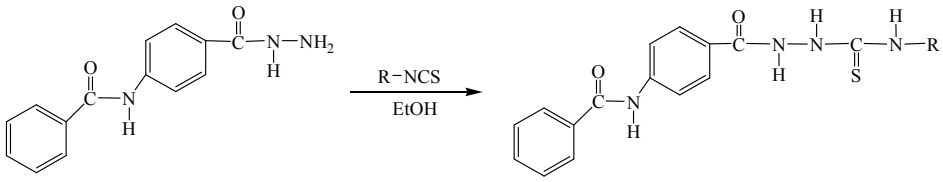
R: Alkyl, cycloalkyl, aryl

Mohsen et al. (35) synthesized 1-acyl-3-thiosemicarbazides from 2-aminobenzoylhydrazines which is treated with alkyl/arylisothiocyanates under reflux condition.



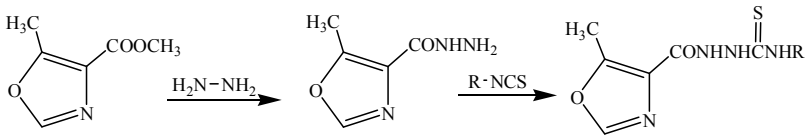
R: alkyl, aryl

Similarly, Küçükgüzel et al. (36) obtained 1-[4-(benzoylamino)benzoyl]-4-alkyl/aryl-3-thiosemicarbazide by the reaction of 4-(benzoylamino)benzoic acid hydrazide with alkyl/arylisothiocyanates.



The 5-methyloxazole-4-carboxylic acid hydrazide was synthesized as intermediate products by reaction of methyl 5-methyloxazole-4-carboxylate and hydrazine hydrate. The synthesized products later treated with substituted

isothiocyanates to give 1-(5-methyloxazol-4-yl)carbonyl-4-substituted-3-thiosemicarbazides (37).

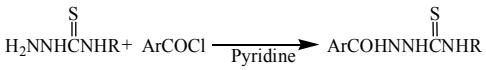


R: Akyl, aryl

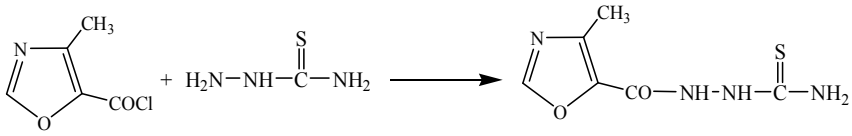
From Carboxylic Acids/Derivatives and Thiosemicarbazides

1-Acyl-3-thiosemicarbazides have been synthesized by the reaction of carboxylic acids and derivatives with thiosemicarbazides (32, 33, 37-40).

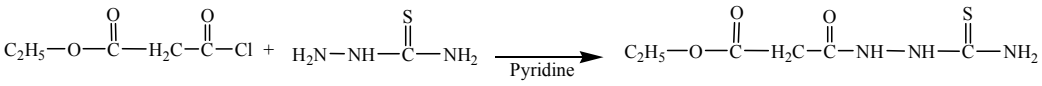
Kane et al. (32) synthesized 1-acyl-4-substituted-thiosemicarbazides from the reaction of 4-subsituted-thiosemicarbazides and aroyl chloride in pyridine.



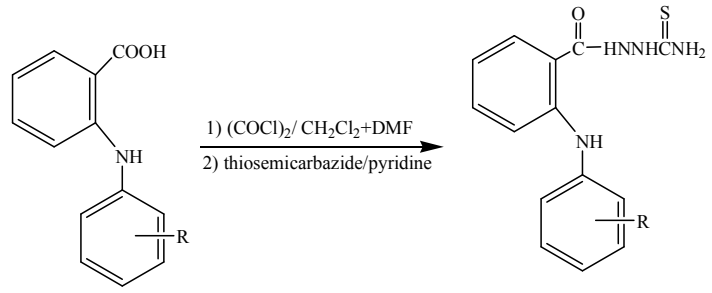
Shafiee et al. (37) prepared 1-(4-methyloxazol-2-yl)-carbonyl-3-thiosemicarbazide derivatives by the reaction of 4-methyloxazole-5-carbonyl chloride and thiosemicarbazide in benzene/pyridine at room temperature.



1-Carbethoxyacetylthiosemicarbazide was synthesized by the reaction of ethyl malonyl chloride and thiosemicarbazide in pyridine at room temperature (38).

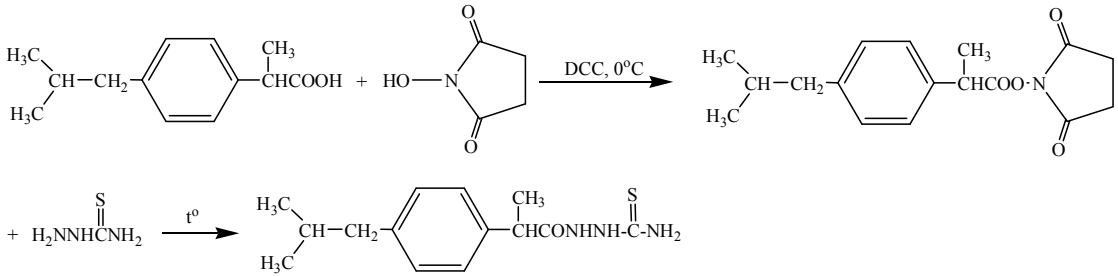


Meclofenamic acid or Flufenamic acid was reacted with oxalyl chloride in dichloromethane to obtain their chloride as intermediates. Then the intermediates sere converted to 1-acyl-3-thiosemicarbazides (39).



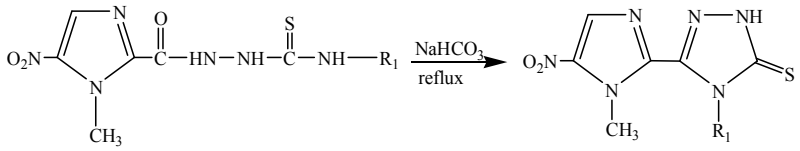
R: 2,6-di-Cl-3-Methyl, 3-CF_3

Tozkoparan et al. (40) reacted ibuprofen with N-hydroxysuccinimide in the presence of DCC. The resulting ester was reacted with thiosemicarbazide to give the desired 4-acyl-3-thiosemicarbazide.



2.1.2. Chemical Properties

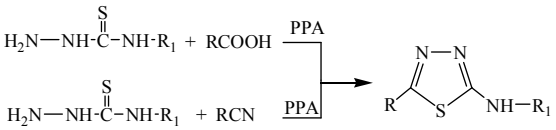
1-Acyl-3-thiosemicarbazides were cyclized with a solution of sodium hydroxide or sodium bicarbonate to corresponding 1,2,4-triazole-3-thione derivatives. For example, 1-(l-methyl-5-nitroimidazole-2-carbonyl)-4-alkylthiosemicarbazides were cyclized with an aqueous solution of sodium bicarbonate to the corresponding 5-(1-methyl-5-nitro-2-imidazolyl)-4-alkyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (41).



R_1: alkyl

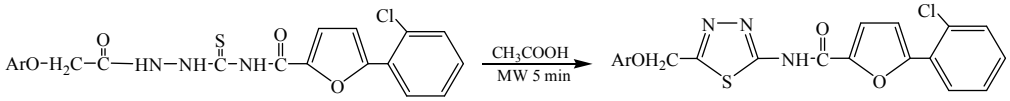
Cyclization of 1-acyl-3-thiosemicarbazides in acidic media gave 2-amino-1,3,4-thiadiazole derivatives.

Golovlyova et al. (42) obtained 2-aminosubstituted-5-substituted-1,3,4-thiadiazole by reaction of thiosemicarbazides with the arylthioacetic acid, arylsulfonylacetic/propionic acids and their nitriles in the presence of polyphosphoric acid (PPA).

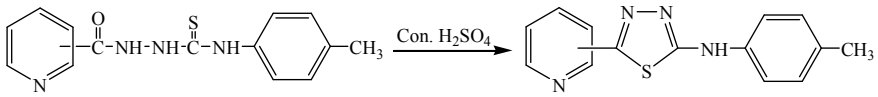


R: 4-$ClC_6H_5SO_2CH_2CH_2$, $BrC_6H_5SO_2CH_2$, 4-$CH_3C_6H_5SCH_2CH_2$, 4-$BrC_6H_5SCH_2$, $C_6H_5CH_2$ **R_1:** H, aryl

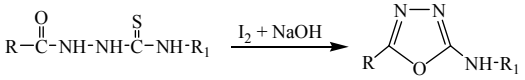
A mixture of 1-aryloxymethyl-4-(5-(2-chlorophenyl)-2-furoyl)thiosemicarbazide and glacial acetic acid was irradiated in microwave oven for to give desired 2-(5-(2-chlorophenyl)-2-furoylamido)-5-aryloxymethyl-1,3,4-thiadiazoles (24).



Zamani et al. (26) synthesized 2-(4-methylphenylamino)-5-pyridyl-1,3,4-thiadiazole, by adding 1-pyridyl-4-(4-methylphenyl)-3-thiosemicarbazide dropwise to concentrated sulfuric acid at 0°C. The resulting solution was stirred for 3h at room temperature and then it allowed to stand overnight.

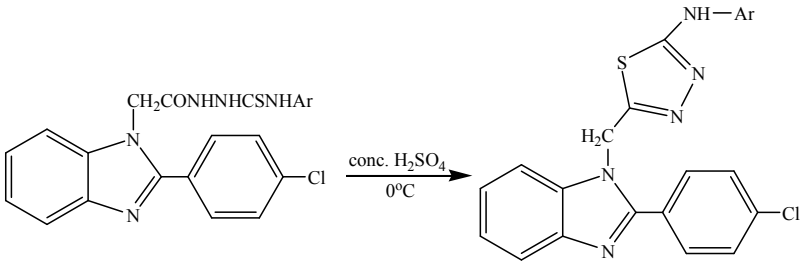


2-Aminosubstituted-5-substituted-1,3,4-oxadiazole derivatives were synthesized by oxidative cyclization of thiosemicarbazide derivatives with iodine/potassium iodide in the presence of sodium hydroxide (8, 42-44).

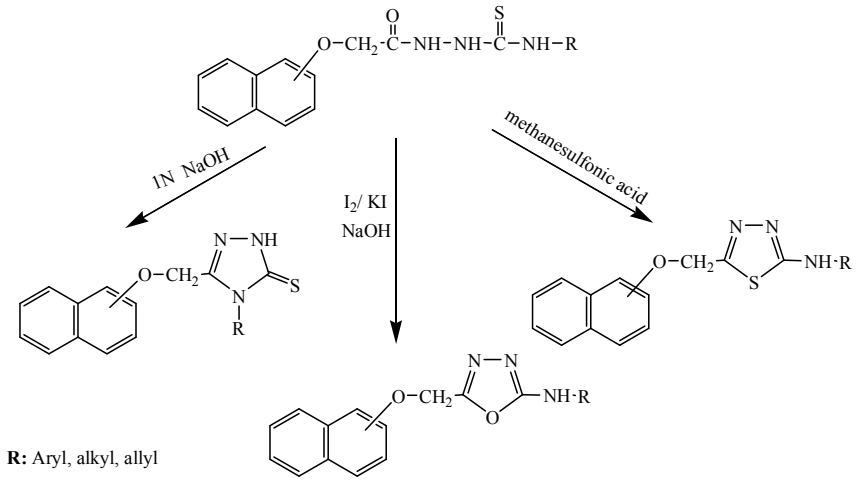


R, R_1: H, alkyl, aryl

Kılcıgil et al. (45) obtained 2-(2-(p-chlorophenyl)benzimidazole-1-yl-methyl)-5-substitutedamino-1,3,4-thiadiazole by cyclization of 1-(2-(*p*-chlorophenyl) benzimidazole-1-yl-methyl)-4-substituted thiosemicarbazides with sulfuric acid at 0°C.



Palaska et al. (8,46) prepared 2-amino-1,3,4-thiadiazole, 2-amino-1,3,4-oxadiazole and 1,2,4-triazole-3-thione derivatives. The reaction started from 1-acyl-3-thiosemicarbazide. The authors reported that, the oxidative cyclization of 1-acyl-3-thiosemicarbazides with iodine/potassium iodide gave 2-amino-1,2,4-oxadiazole derivatives. Additionally, the cyclization of 1-acyl-3-thiosemicarbazides in alkali medium gave 1,2,4-triazole-5-thione derivatives, while in acidic conditions gave 2-amino-1,3,4-thiadiazole derivatives.



2.1.3. Spectral Properties

IR Spectra

In the IR spectra of 1-acyl-4-substituted-3-thiosemicarbazide derivatives, N-H and C=O stretching bands were seen at 3400-3000 and 1740-1640 cm^{-1} respectively (6, 7, 24, 28-30, 47-49). Additionally, thiosemicarbazide derivatives have C=S stretching bands at 1220-1080 cm^{-1} (6, 7, 24, 26, 30, 37, 48-50).

^{1}H-NMR Spectra

In the ^{1}H-NMR spectra of 1-acyl-3-thiosemicarbazides, the N_1-H protons were seen at 10.26-10.50 ppm as a broad singlet, while N_4-H protons at 9.70-9.80 ppm and N_2-H protons at 9.23-9.50 ppm as a singlet (19, 50).

^{13}C-NMR Spectra

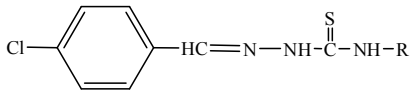
In the ^{13}C-NMR spectrum of 1,4-disubstituted-3-thiosemicarbazide, carbon atoms of thiosemicarbazide give peaks at 165-158 (O=C-N_1) , 168.06 (O=C-N_4) and 182-180 (C=S) ppm respectively (47, 51).

2.1.4. Biological Activity

Antibacterial and Antifungal Activities

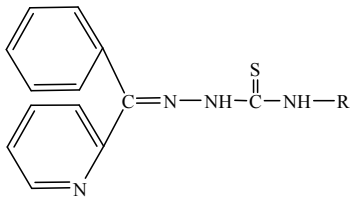
The different thiosemicarbazide derivatives were synthesized and evaluated for biological activities. Some of the synthesized compounds were found to possess antimicrobial activity.

Parul et al. (51) studied some 1,4-disubstituted thiosemicarbazides to evaluate their antimicrobial activity. They found that 1-(4-chlorobenzylidene)-4-substituted-thiosemicarbazides possess potent antibacterial activity against *Bacillus cereus, Staphylococcus epidermidis* and *Moraxella cattarhalis, Staphylococcus saprophyticus* and it was found that the synthesized compounds inhibited the growth of *Candida albicans* and *Aspergillus flavans.* Also they suggested that the activity of these compounds was related to the presence of hydroxyl and chloro groups in aromatic ring.



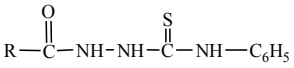
R: ethyl, propyl

The antimalarial activity of the thiosemicarbazones was studied by Pingaew and his co-workers (52). The result of their studies showed that the 2-benzoylpyridine thiosemicarbazones possess valuable activity againts *Plasmodium falciparum*.



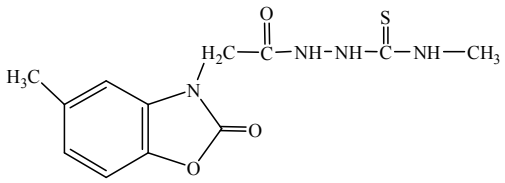
R: H, phenyl

Daoud et al. (53) synthesized and evaluated the antibacterial activity of 4-phenyl-1-acyl-3-thiosemicarbazide derivatives. They found that some of the synthesized compounds show remarkable antibacterial activity against *S. aureus* and *P. aeruginosa*.



R: Aryl

1-[2-(5-Methyl-2-benzoxazolinone-3-yl)acetyl]-4-substitudedthiosemicarbazide derivatives were synthesized and screened for antimicrobial activity. Among them 1-[2-(5-methyl-2-benzoxazolinone-3-yl)acetyl]-4-methylthiosemicarbazide exhibited poor antibacterial activity whereas showed promising antifungal activity against *C. krusei, C. albicans* and *C. parapsilosis* (19).

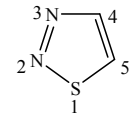


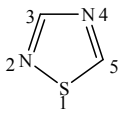
2.2. 2-Amino-1,3,4-thiadiazoles

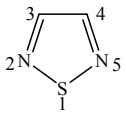
Heterocyclic moieties with N-C-S linkage can be found in a large number of compounds which display a very wide spectrum of biological activity (54, 55). The biological activity of the compounds is mainly depends on their molecular structures. 1,3,4-thiadiazoles are very interesting compounds due to their important applications in many pharmaceutical, biological and analytical field (9). Thiadiazole is a five membered ring system containing hydrogen binding domain, sulfur atom and two-electron donor system. It acts as a constrained pharmacophore. Many drugs containing thiadiazole nucleus are available in the market such as acetazolamide, methazolamide, sulfamethazole and megazol (56, 57).

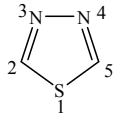
Thiadiazole can act as the bio-isosteric replacement of the thiazole moiety. So it acts like third and fourth generation cephalosporins, hence can be used in antibiotic preparations. During recent years there has been intense investigation of different classes of thiadiazole compounds, many of which known to possess interesting biological properties such as antimicrobial (58-60), antituberculosis (61), anti-inflammatory (58, 62-64), anticonvulsants (14, 65), antihypertensive (66), anticancer (9, 16, 67) and antifungal (10) activities.

Thiadiazole ring system contains two nitrogen and one sulphur atom. They occur in nature in four isomeric forms: 1,2,3-thiadiazole; 1,2,5-thiadiazole; 1,2,4-thiadiazole and 1,3,4-thiadiazole (55).


1,2,3-Thiadiazole


1,2,4-Thiadiazole


1,2,5-Thiadiazole

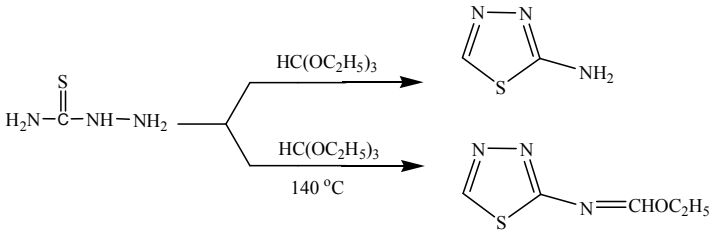

1,3,4-Thiadiazole

2.2.1. Synthesis

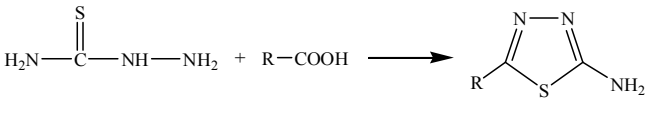
From Thiosemicarbazide and Derivatives

Many synthesis of the 2-amino-1,3,4-thiadiazoles proceed from thiosemicarbazide and derivatives.

2-Amino-1,3,4-thiadiazole was synthesized by the reaction of thiosemicarbazide with excess amount of ethyl orthoformate at high temperature (68).

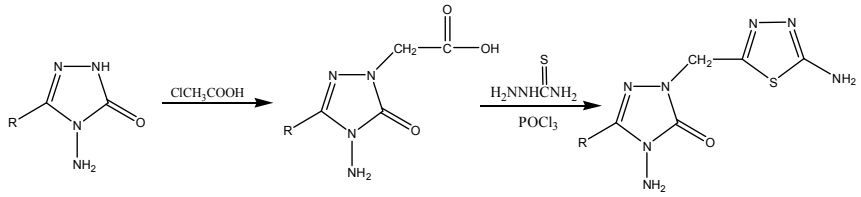


Reaction of carboxylic acid derivatives with thiosemicarbazide gave 2-amino-1,3,4-thiadiazoles (69).



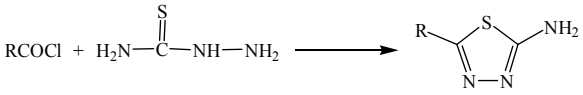
R: Aryl

Demirbaş (70) synthesized acetic acid derivatives as intermediate product by the reaction of 5-alkyl-4-amino-2,4-dihydro-3*H*-1,2,4-triazol-3-one and chloroacetic acid. The synthesized compound was treated with thiosemicarbazides in presence of phosphoryl chloride under reflux condition to prepared corresponding 4-amino-2-[(5-amino-1,3,4-thiadiazol-2-yl)methyl]-5-substituted-2,4-dihydro-3*H*-1,2,4-triazol-3-ones.



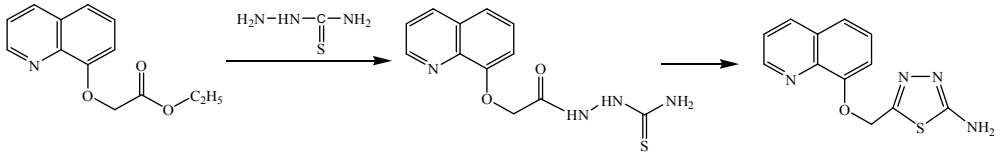
R: aryl, alkyl

Gupta et al. (55) have been reported the synthesis of 2-amino-5-methyl-1,3,4-thiadiazole by reaction of thiosemicarbazide with acyl chloride.

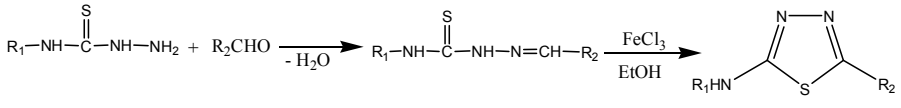


R: Aryl, alkyl

Madhav et al. (59) synthesized 1-(8-quinolinoxyacetyl)-3-thiosemicarbazide by reaction of ethyl-8-quinolinoxy acetate and thiosemicarbazide. This intermediate was added gradually with stirring to anhydrous orthophosphoric acid to obtain desired 2-amino-5-(8-quinolinoxymethyl)-1,3,4-thiadiazole.

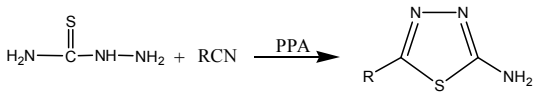


2-Amino-1,3,4-thiadiazoles were synthesized by oxidative cyclization of thiosemicarbazide derivatives by treating them with aldehydes in presence of iron trichloride (14, 57, 71).



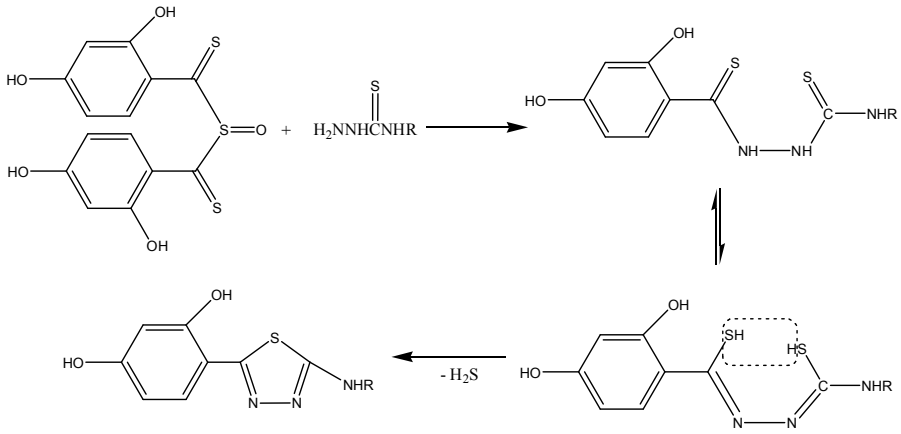
R_1 : Alkyl, Aryl, **R_2 :** Alkylaryl

Alkyl and aryl nitriles were reacted with thiosemicarbazide under acidic conditions to give 1,3,4-thiadiazoles (42).



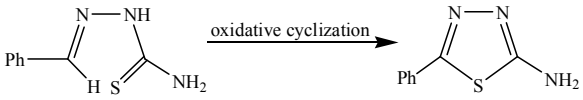
R: Haloaryl

1-(2,4-Dihydroxyphenylcarbonothioyl)-4-substituted-thiosemicarbazides were synthesized as intermediate products by heating of 4-substituted-3-thiosemicarbazides and sulfinyl bis(2,4-dihydroxythiobenzoyl) in methanol. The reaction continued to synthesize 2-substitutedamino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles (72).

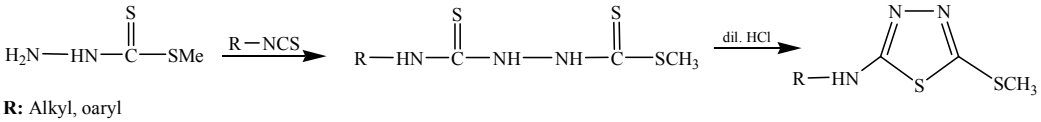


R : Alkyl, Aryl

Oxidative cyclization of thioacylhydrazone by using oxidants such as bromine (73), ferric chloride (14), ammonium ferric sulfate (74) give 2-amino-5-phenyl-1,3,4-thiadiazoles.

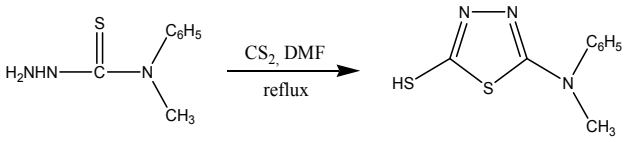


Thiosemicarbazidedithiocarboxylate esters were prepared by the reaction of dithiocarbazide ester and isothiocyanate or thiocyanate.The resulting products in an acidic media or by the heating gave 2-methylmercapto-5-substitutedamino-1,3,4-thiadiazole. Guha and Guha (75) obtained 2-anilino-5-mercapto-1,3,4-thiadiazole by the reaction of methyldithiocarbazate with phenylisothiocyanate in presence of diluated hydrochloric acid.



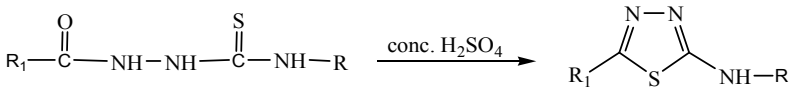
R: Alkyl, oaryl

Spalinska et al. (76) synthesized 5-*N*-methy-*N*-phenyl-1,3,4-thiadiazole-2-thiol by the refluxing 4-phenyl-4-methyl-3-thiosemicarbazide and carbon disulphide in DMF.



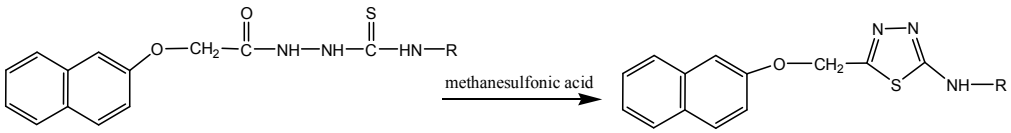
From Monothiodiacylhydrazines:

Monothiodiacylhydrazines (prepared from the acylation of thiosemicarbazides or as intermediates in the reactions of thiohydrazides with carboxylic acids and their derivatives) was cyclized through dehydration with sulfuric, polyphosphoric (PPA) or methanesulfonic acids to give 1,3,4-thiadiazoles (9, 47, 53, 58, 77).



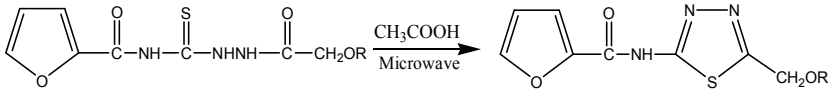
R, R_1: Aryl, alkyl

Palaska et al. (8) produced 2-(2-naphthyloxymethyl)-5-substitutedamino-1,3,4-thiadiazoles by adding methanesulfonic acid dropwise to a solution of 1-acyl-3-thiosemicarbazides in toluen.



R: H, alkyl, allyl, aryl

Wang et al. (78) synthesized 2-(2-furoylamino)-5-aryloxymethyl-1,3,4-thiadiazoles under microwave irradiation condition from 1-aryloxyacetyl-4-furoyl-3-thiosemicarbazides in acetic acid with good yield.

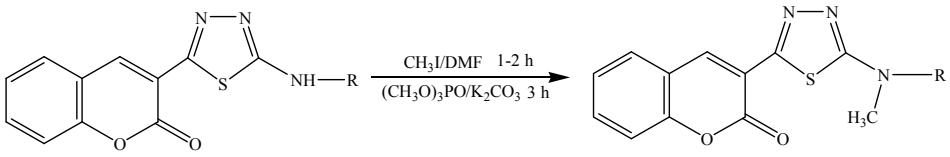


R: Aryl

2.2.2. Chemical Properties

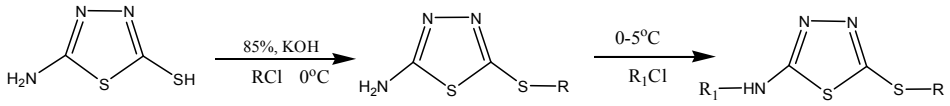
N-alkylation of 2-amino-1,3,4-thiadiazoles give secondary and tertiary amines. Reaction of secondary amines with nitriles give amidines, while acylating agents afforded amides and isocyanates afforded urea derivatives.

The secondary amino group in thiadiazole was alkylated by methyl iodide or trimethyl phosphate in the presence of anhydrous potassium carbonate (79).



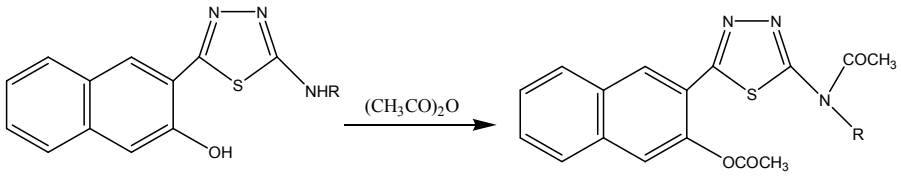
R: Aryl

Sharma et al. (63) used substitution reaction to synthesize 5-(4-substitutedphenylthio)-*N*-(4-substitutedphenyl)-1,3,4-thiadiazol-2-amine from reaction of 2-amino-5-sulfanyl-1,3,4-thiadiazole with 4-chlorobenzene in presence of potassium hydroxide.



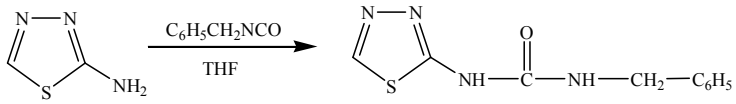
R,R$_1$: haloaryl, alkylaryl

The secondary amine group of the thiadiazole ring was acylated when heated in the presence of acetic anhydride and ethyl orthoformate to afford the amide in good yield (58).

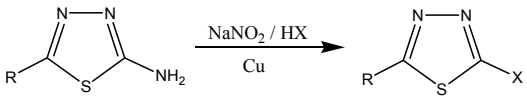


R: aryl, alkyl

2-Amino-1,3,4-thiadiazole reacted with benzyl isocyanate in dry tetrahydrofuran (THF) to afford the 1,3,4-thiadiazole-2-ylurea (80).

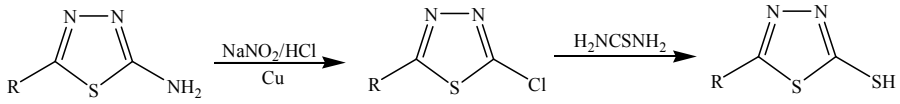


Diazonium salts of 2-amino-1,3,4-thiadiazoles give their 2-halogeno-1,3,4-thiadiazole derivatives according to the Sandmeyer reactions with the presence copper salts as catalyst (81).



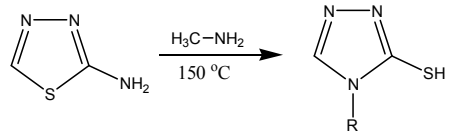
R: H, alkyl; **X:** Cl, Br

Firoozi et al. (82) synthesized 2-mercapto-5-aryl-1,3,4-thiadiazole derivatives from 2-chloro-5-aryl-1,3,4-thiadiazoles and thiourea by heating in ethanol.



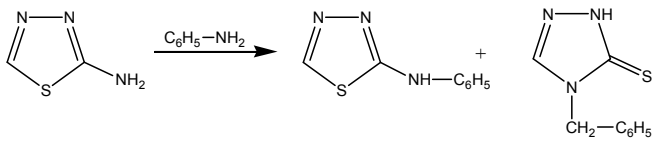
Beside the other chemical properties, although 1,3,4-thiadiazole ring is stable against acids, it is very sensitive against strong nucleophilic attacks. Therefore, in basic medium the 1,3,4-thiadiazole ring is breaks easily.

When a mixture of 2-amino-1,3,4-thiadiazole and methyl amine was heated at 150 ^{o}C, 2-amino-1,3,4-thiadiazole converted into 4-methylamino-1,2,4-triazole-5-thione (83).

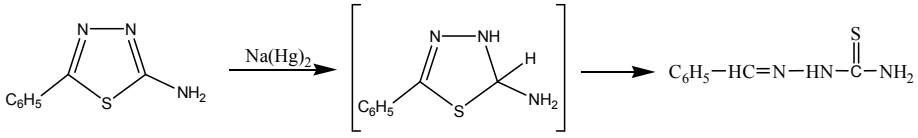


R: H, methyl

The mixture of 2-amino-1,3,4-thiadiazole and benzylamine was heated in xylene and were obtained 2-benzylamino-1,3,4-thiadiazole and 4-benzylamino-1,2,4-triazole-3-thione (84).



Treatment of 2-amino-5-phenyl-1,3,4-thiadiazole with sodium amalgam gave 1-benzyledine-3-thiosemicarbazone (84).



2.2.3. Spectral Properties

IR Spectra

In the IR spectra of 2-amino-5-substituted-1,3,4-thiadiazole derivatives, N-H stretching bands were seen in the regions of 3560-3100 cm^{-1} (6, 10, 15, 16, 18, 24, 31, 60, 63, 65, 72, 78, 85, 86). Additionally, C=N stretching bands appeared at 1680-1600 cm^{-1} (10, 15, 16, 18, 24, 53, 59, 63, 72, 78). While the N-N stretchings bands were observed in 1050-1030 cm^{-1} (31, 15), C-S-C bendings were seen in the region of 750-600 cm^{-1}(9, 15, 16, 31, 72).

^{1}H-NMR Spectra

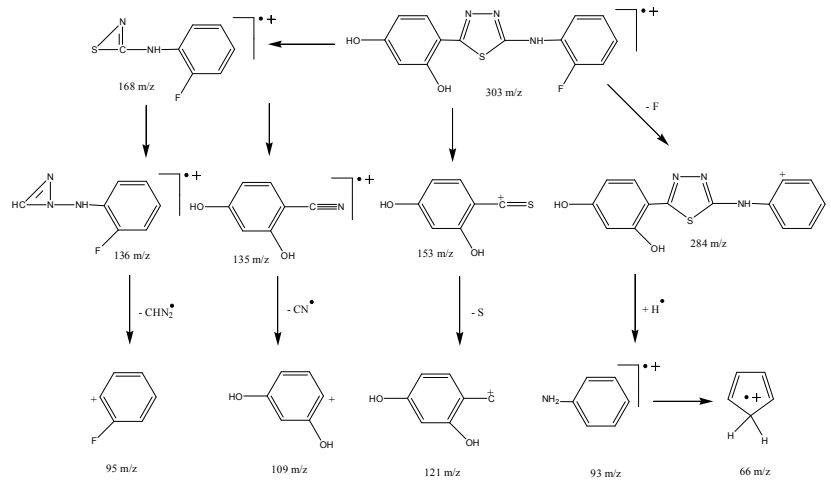
In the ^{1}H-NMR spectra of 2-substitutedamino-5-substituted-1,3,4-thiadiazole derivatives, the signals belongs to amine in the second position of the ring were seen seen as singlet and multiplet between 7.40–13.00 ppm (15, 16, 18, 36, 50, 72, 73, 85, 87).

^{13}C-NMR Spectra

In the ^{13}C-NMR spectrum of in 5-substituted-2-substitutedamino-1,3,4-thiadiazole derivatives C-2 and C-5 bands were observed around 150 and 170 ppm respectively (16, 56, 72, 88).

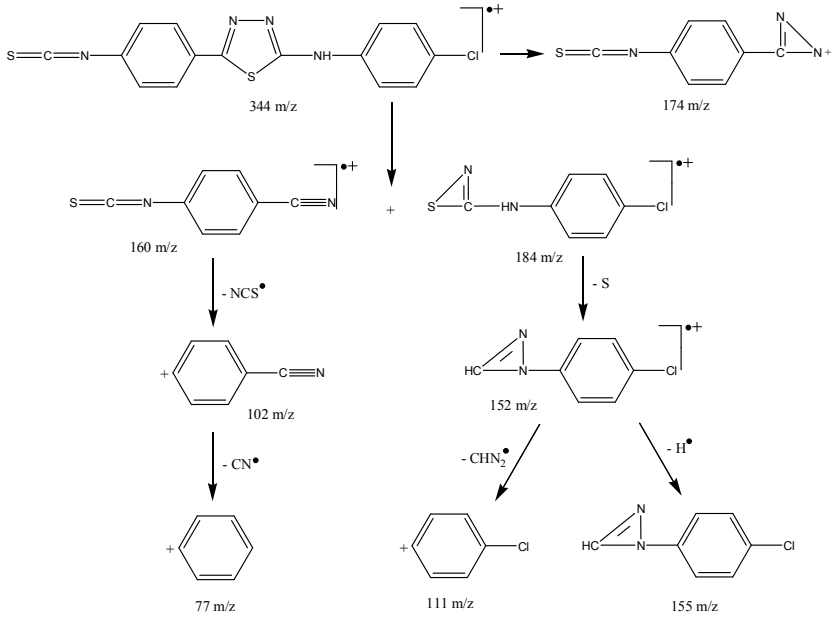
Mass Spectra

The molecular ions of 1,3,4-thiadiazoles are stable, and the M^{+} peaks were seen as expected. One of the principal processes in the fragmentation of the M^{+} ions of 1,2,4-thiadiazoles is the elimination of substituents at 5th position of ring. The resulting 1,2-thiaziren-3-amine and 1*H*-diazirine-3-amine ions is responsible for the entire variety of the pathways of their subsequent fragmentation. Mass fragmentation pathway of 2-(2-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole is given in Scheme 2.1 (16).



Scheme 2.1. Mass fragmentation pathway of 2-(2-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole

The mass fragmentation pathway of *N*-phenyl-*N*'-[4-(5-(4-chlorophenyl)amino-1,3,4-thiadiazole-2-yl)phenyl]thiourea outline in Scheme 2.2. (89).



Scheme 2.2. Mass fragmentation pathway of *N*-phenyl-*N*'-[4-(5-(4-chlorophenyl)amino-1,3,4-thiadiazole-2-yl)phenyl]thiourea

2.2.4. Biological Activities

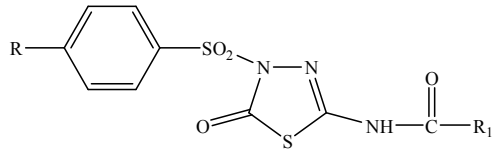
There are several reports in the literature which describe 1,3,4-thiadiazole derivatives have various biological activities. The most relevant and recent studies

have revealed that 1,3,4-thiadiazole derivatives have a broad spectrum of pharmacological activities that can be classified into the following categories.

Anti-inflammatory and Analgesic Activity

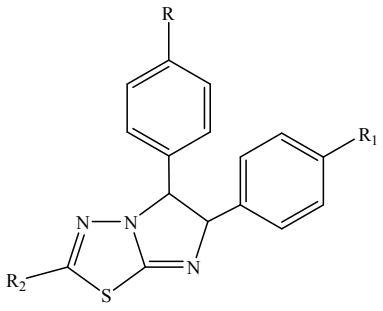
A number of 1,3,4-thiadiazole derivatives were identified as potent anti-inflammatory compounds. Carrageenan-induced foot paw edema (CPE) inhibitory activity of 1,3,4-thiadiazole derivatives were shown equipotent with naproxen, phenylbutazone, hydrocortisone and other NSAIDs (8, 21, 40, 90)

Schenone et al. (86) synthesized *N*-[5-oxo-4-(arylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-amides and evaluated their analgesic and anti-inflammatory activities by using in vivo test. They found that some of the synthesized compounds show good anti-inflammatory activity. Also they reported that to change the substituent (tolyl and p-fluoro-phenyl) caused an increase in activitys.



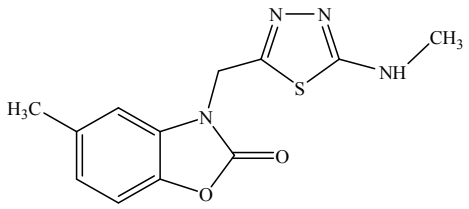
R: H, alkyl
R_1: Aryl, halogen-aryl

Gadad et al. (18) evaluated the biological activity of 2-trifluoromethyl/sulfonamido-5,6-diaryl substitutedimidazo[2,1-b]-1,3,4-thiadiazole derivatives. They found that some of the compounds show selective inhibitory activity toward COX-2 and COX-1. They also exhibit significant anti-inflammatory activity, which is comparable to that of celecoxib in the carrageenan-induced rat paw edema method.

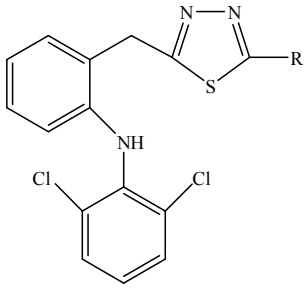


R: H, OCH_3, **R_1:** SCH_3, CH_3, H; **R_2:** CF_3, SO_2NH_2

Gökşen et al. (19) synthesized and evaluated some 1,3,4-thiadiazole derivatives for their analgesic activity. They found that some of the compounds have higher analgesic activity than those of both morphine and aspirin.

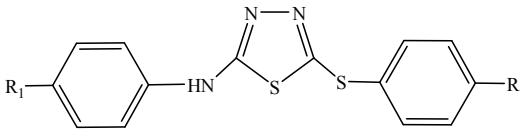


Amir and Shaikha (90) synthesized the 1,3,4-thiadiazole derivatives of diclofenac and screened them for anti-inflammatory activity. The results were shown that the most active comounds possess *p*-fluoro phenyl amino group at the second position. The new synthesized compounds were shown higher anti-inflammatory activity compared to reference drug.



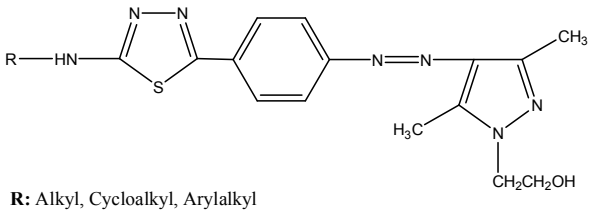
R: Aryl

Sharma et al. (63) synthesized diaryl substituted 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives to evaluated their COX inhibitory activities. Among them some compounds inhibit only COX-2 and others are non-selective. Some of them show higher anti-inflammatory activity than indomethacin and tramadol.



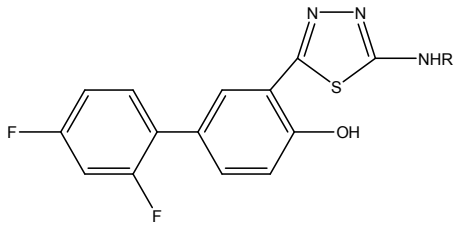
R: sulfonamide
R_1: Halogen, alkyl, sulfonylchloride

Oruç et al. (64) evaluated 2-(substitutedamino)-5-[((1-(2-hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-1,3,4-thiadiazole derivatives for their analgesic activity. They observed that some of them possess good analgesic activity.



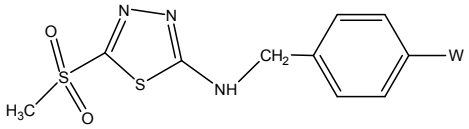
R: Alkyl, Cycloalkyl, Arylalkyl

Küçükgüzel et al. (36) synthesized new 1,3,4-thiadiazoles by replacing the carboxylic acid group of diflunisal with 2-akyl/arylamino-1,3,4-thiadiazole and evaluated their anti-inflammatory activity. They suggested that this replacement caused an increase in anti-inflammatory activity.



R: Alkyl, Aryl

Varandas et al. (62) were synthesized 1,3,4-thiadiazole derivatives and evaluated their anti-inflammatory activity. The in vivo activity study of the new synthesized compounds showed that *p*-fluoro-substituted derivatives are more active than celecoxib in the same molar concentrations.

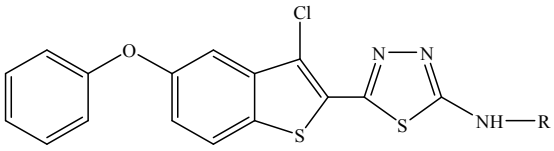


W: H, halogens

Antibacterial and Antifungal Activity

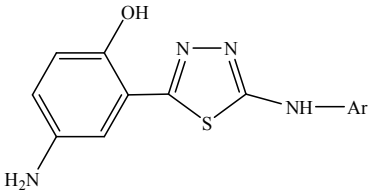
It was shown that 1,3,4-Thiadiazole has a broad spectrum of activity against various pathogens, and extensive research was performed on the synthesis of new potent antibacterial and antifungal agents.

Vasoya et al. (9) evaluated 1,3,4-thiadiazole derivatives for their antimicrobial activity against various microorganisms. It was observed that some of the compounds showed good activity against *E. coli*, *B. megaterium, S. aureus* and *A. niger*.



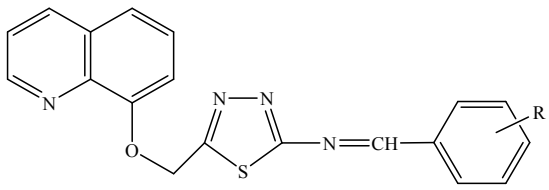
R: Aryl

Hussain et al. (10) studied the 4-amino-2-{5-[(4-substituted phenyl) amino]-1,3,4-thiadiazole-2-yl} phenol derivatives for their antibacterial and antifungal activities. They reported that substitution on amino group in second position of thiadiazole ring caused an increase in antibacterial activity against *S. aureus* and *A. niger*.



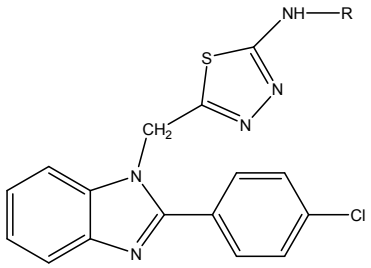
R : Haloaryl

Madhav et al. (59) synthesized new 2-(substituted benzalamino)-5-(8-quinolinoxymethyl)-1,3,4-thiadiazoles and evaluated them for antimicrobial activity. Some of the new synthesized compounds showed favorable inhibitory effect against *E. coli*, *B. substillis, S. aureus* and *Klebsiella pneumoniae*.



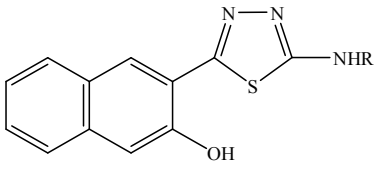
R: Alkoxy, hydroxyl, nitro, halogens, H

Kılcıgil et al. (45) synthesized the 2-(2-(p-chlorophenyl) benzimidazol-1-yl-methyl)-5-substituted-amino-1,3,4-thiadiazoles and evaluated their in vitro antimicrobial activity. All of the tested compounds showed less activity than amipicillin against *E. coli* and *S. aureus*.



R: Aryl, haloaryl

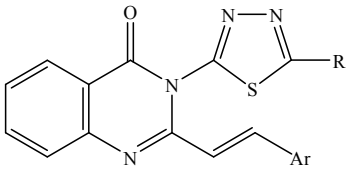
Doğan et al. (58) synthesized and screened 2,5-disubstituted-1,3,4-thiadiazole derivatives for their anticonvulsant and antimicrobial activities. Some of the new synthesized compounds considerd promising anticonvulsants for development of new anticonvulsant agents. Also some of these compounds possess good antimicrobial activity against *E. coli*, *B.substillis* and *S. aureus*.



R : Aryl, Alkyl, arylalkayl

Anticonvulsant Activity

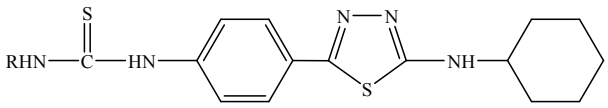
Jatav et al. (14) synthesized some novel 3-[5-substituted phenyl-1,3,4-thiadiazole-2-yl]-2-styrylquinazoline-4(3*H*)-ones and evaluated them for their anticonvulsant, sedative-hypnotic and CNS depressant activities. Some of the synthesized compounds showed anticonvulsant activity while some of them exhibited good sedative-hypnotic and CNS depressant activities.



Ar : Aryl. alkoylaryl
R : Aryl, Haloaryl

Karakuş et al. (65) studied on the *N*-(Alkyl/substituted aryl)-*N*'-[4-*(*5-cyclohexylamino*)*-1,3,4-thiadiazole-2-yl)phenyl]thiourea derivatives to evaluate

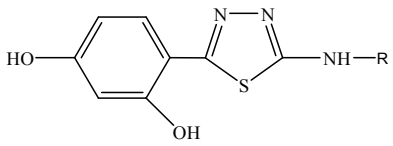
anticonvulsant activity. The result of this study demonstrated that some of the new synthesized compounds have potential effects in pentylenetetrazol (PTZ) induced convulsions and maximal electroshock seizure (MES) tests.



R : Aryl, Alkyl, arylalkayl

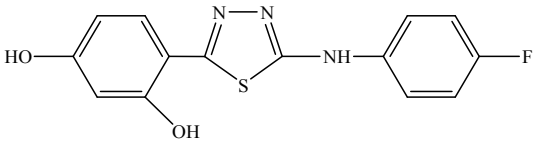
Antitumoral Activity

Matysiak and Opolski (16) synthesized and evaluated N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole derivatives for their antiproliferative activities. The results of screening of synthesized compounds proved that some of them to be more active than reference drug cisplatin.

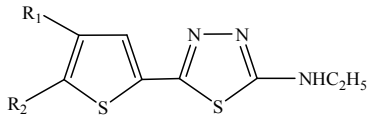


R: Aryl, haloaryl

2,5-disubstituted-1,3,4-thiadiazoles were evaluated for their anticancer activity. The 2-amino-1,3,4-thiadiazole derivative elicites prominent anticancer effects in a range of tumor cell culture with no toxicity for normal cell. *N*-halogenphenyl derivatives of 1,3,4-thiadiazoles possessed the highest antiprofilerative (91).



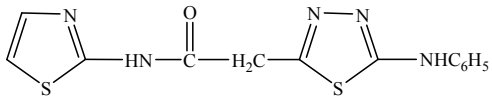
Mavrova et al. (67) synthesized and studied 2,5-disubstituted-1,3,4-thiadiazoles for their anticancer activity. They found that some of the synthesized compounds possessed high cytotoxicity against thymocyte cell and low cytotoxicity against blood lymphocyte.



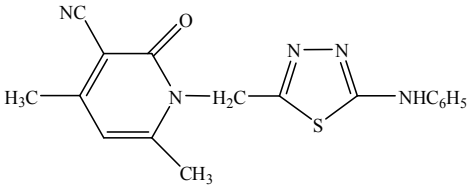
R_1, R_2 : H, alkyl, aryl

Other Activities

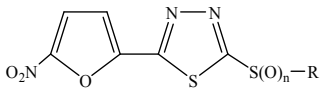
Abdel-Wahab et al. (66) synthesized and evaluated 2-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)-*N*-(thiazol-2-yl)-acetamide for its activity on α-adrenergic receptors. They found that its antihypertensive activity is higher than Minoxidil®.



El-Essawy et al. (13) studied on 4,6-dimethyl-2-oxo-1-((5-(phenylamino)-1,3,4-thiadiazole-2-yl)methyl)-1,2-dihydropyridine-3-carbonitrile. The antiviral screening proved its activity against *Hepatitis B* virus.



Foroumadi et al. (61) synthesized and evaluated 2-(5-nitro-2-furyl)-1,3,4-thiadiazole derivatives for their anti-mycobacterial activity. Some of the synthesized compound showed remarkable activities against *M. tuberculosis*.



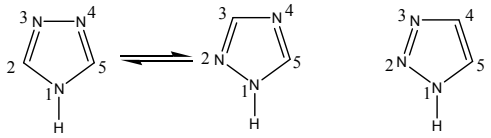
R: Alkyl, aryl

2.3. 1,2,4-Triazole-5-thiones

In the last few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. A large number of 1,2,4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants sedatives, antianxiety, antimicrobial agents and antimycotic activity. Among these heterocycles, the

mercapto- and thione-substituted 1,2,4-triazole ring systems have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives, such as anti-inflammatory and analgesic, antibacterial, antifungal, antimycobacterial and anticancer properties.

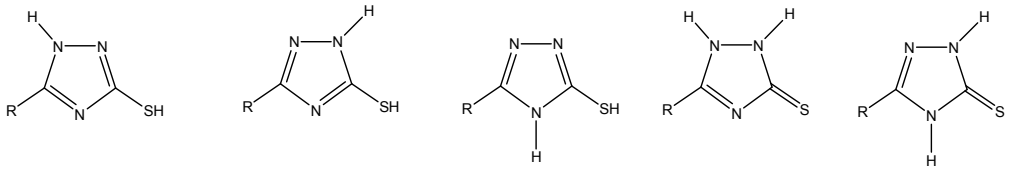
Triazole is a five-membered heterocycle having three nitrogen heteroatoms and two double bonds. There are two possible isomers of triazole depending on the position of nitrogen atom in the ring and are numbered as below.



1,2,4-Triazole 1,2,3-Triazole

Among them, 1,2,4-triazole have drawn great attention to medicinal chemists from two decades due to its wide variety of activity, low toxicity and good pharmacokinetic and pharmacodynamic profiles.

Usually, five possible tautomeric forms of 1,2,4-triazole-5-thione are considered in literature (92).

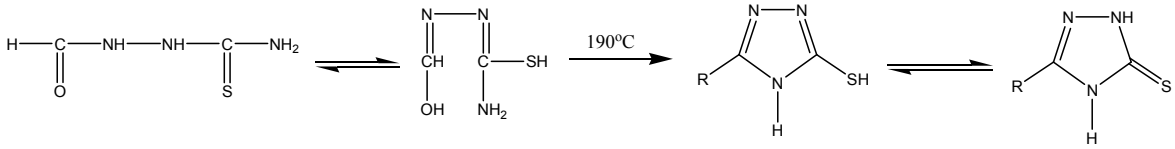


I II III IV V

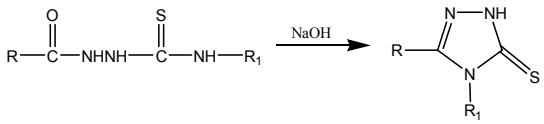
2.3.1. Synthesis

From Thiosemicarbazide and Derivatives

Early methods such as the dry heating of 1-formyl-3-thiosemicarbazide at 190°C gave low yields of 1,2,4-triazole-5-thiones (93).

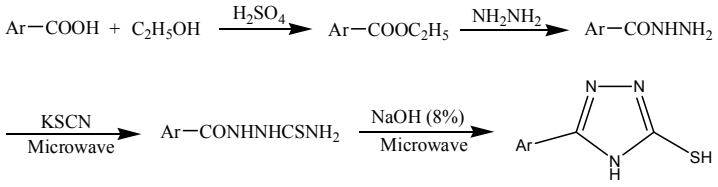


5-Substituted-1,2,4-triazole-3-thione derivatives were synthesized by the cyclisation of 1-acyl-4-substituted-3-thiosemicarbazides in alkali medium (6, 21, 28, 39, 40).



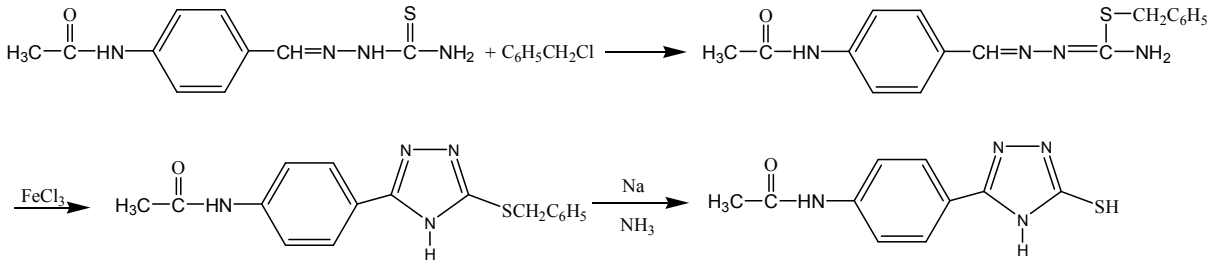
R: Aryl; **R_1:** Alkyl

1-Acyl-3-thiosemicarbazides were prepared as intermediate product from reaction of hydrazide and potassium thiocyanate using microwave irradiation. Cyclization reaction of 1-acyl-3-thiosemicarbazide with the microwave irradiation is carried out in basic condition to give 3-aryl-1,2,4-triazole-5-thiol (94).

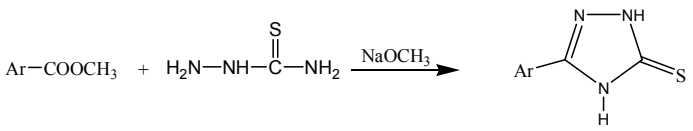


Ar: *o*-$CH_3OC_6H_4$- , *p*-$CH_3OC_6H_4$- , *p*-$O_2NC_6H_4$- , C_6H_5- , 3-pyridinyl

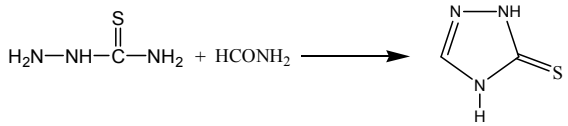
Duschinsky and Gainer (95) reacted the 4-acetamidobenzaldehyde thiosemicarbazone with benzyl chloride and produced 4-acetamidobenzaldehyde-3-benzyl-3-thiosemicarbazone. This compound was cyclized by oxidation with ferric chloride to gave desired 3-(acetamidophenyl)-1,2,4-triazole-5-thione.



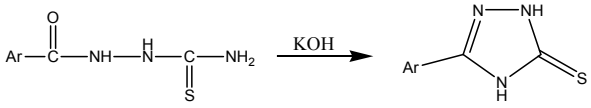
3-Aryl-1,2,4-triazole-5-thione deriavatives were synthesized by reaction of thiosemicarbazides with aromatic carboxylic acid esters in the presence of sodium methoxide (96).



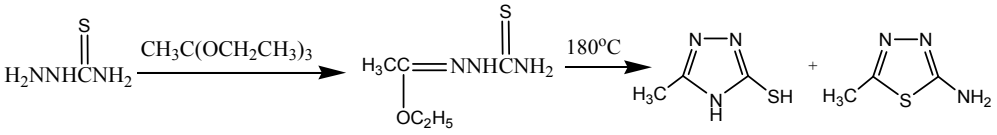
1,2,4-Triazole-5-thione was synthesized by the refluxing thiosemicarbazide derivatives with formamide (97, 98). Similar reactions were repeated with ethyl formate in the presence of sodium methoxide (99, 100).



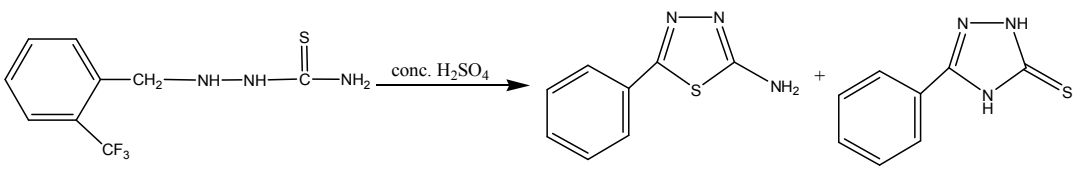
Tozkoparan et al. (101) prepared the 3-aryl-1,2,4-triazole-5-thiones by the reaction from 1-acyl-3-thiosemicarbazides with potassium hydroxide under reflux.



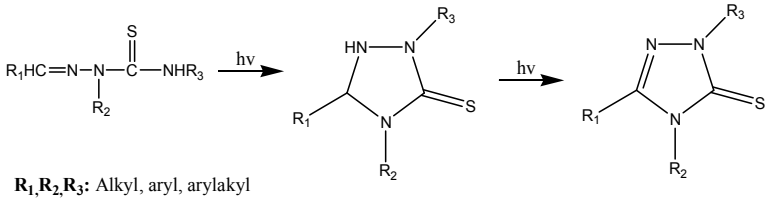
A mixture of thiosemicarbazide and ethyl orthoacetate was heated on the steam-bath, to synthesize thiosemicarbazone. When the resulting compound was heated at 180°C, 2-amino-5-methyl-1,3,4-thiadiazole and 3-methyl-1,2,4-triazole-5-thiol formed in about equal amounts (68).



The cyclization of the *o*-trifluoromethylbenzoyl-3-thiosemicarbazide with concentrated sulfuric acid gave 2-amino-5-(2-trifluoromethyl)phenyl-1,3,4-thiadiazole and a considerable amount of 3-(2-trifluoromethyl)phenyl-1,2,4-triazole-5-thione as a by-product. Formation of 3-(2-trifluoromethyl)phenyl-1,2,4-triazole-5-thione is due to the electronic influence of trifluoromethyl group in ortho position (102).

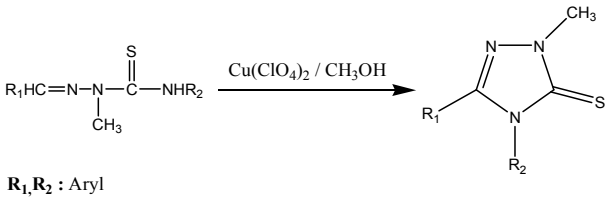


Buscemi and Gruttadauria (103) obtained 1,3,4-trisubstituted-1,2,4-triazole-5-thiones from aldehyde thiosemicarbazones using photoheterocyclization in 366 nm.



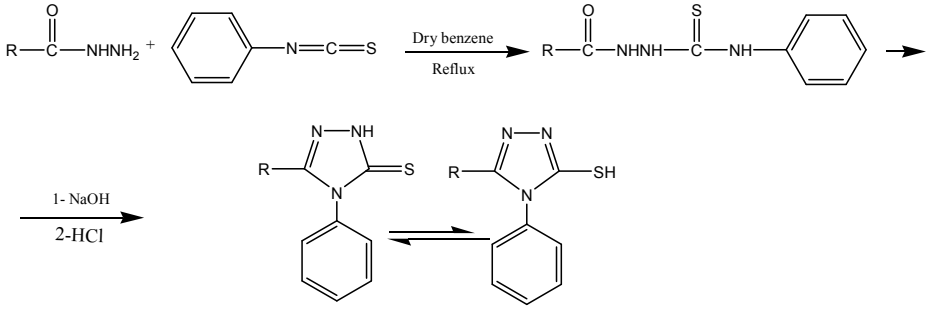
R_1,R_2,R_3: Alkyl, aryl, arylakyl

Same authors synthesized 1-methyl-3,4-diaryl-1,2,4-triazole-5-thiones from 1-benzylidene-2-methyl-4-aryl-3-thiosemicarbazide derivatives in the presence of copper(II)perchlorate (103).



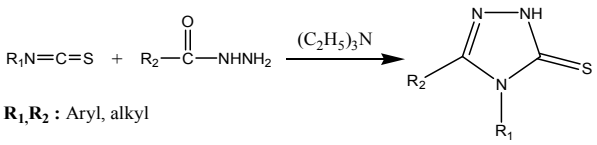
R_1,R_2 : Aryl

Banday and Rauf (104) synthesized a series of 1,2,4-triazole-3-thione derivatives. To obtain the compounds they prepared fatty acid hydrazides from fatty alkenoate as intermediate products. 1-Acyl-3-thiosemicarbazides were obtained by the reaction of hydrazides with phenyl isothiocyanate. The resulting thiosemicarbazides were subjected to intermolecular cyclization in alkaline medium to form 1,2,4-triazole-3-thione derivatives.



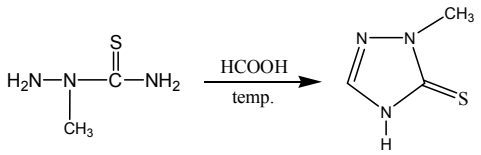
R: Alkenes

Theoclitou et al. (105) produced 3,4-disubstituted-1*H*-1,2,4-triazole-5(4*H*)-thiones from isothiocyanates and acylhydrazine in the presence of triethylamine at high temperature.



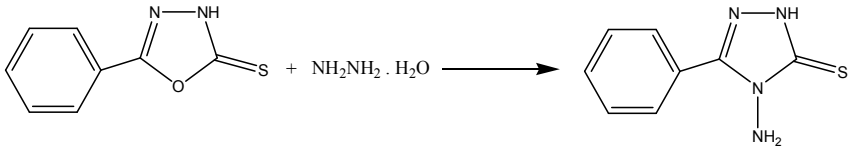
R_1,R_2 : Aryl, alkyl

2-Methyl-3-thiosemicarbazides were heated in formic acid to synthesize 1-methyl-1,2,4-triazole-5-thione derivative (106).

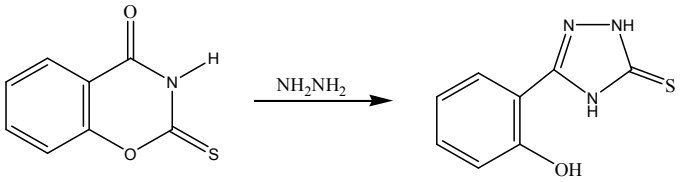


Other Methods of Synthesis

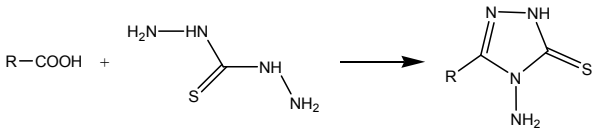
1-Amino-2-aryl-1,2,4-triazole-5-thione derivatives were synthesized by refluxing 2-aryl-1,3,4-oxadiazole-5-thione with hydrazine hydrate in ethanol (107, 108).



3-(2-Hydroxyphenyl)-1,2,4-triazole-5-thione were synthesized by heating the 4-oxo-1,3-benzoxazin-2-thione with hydrazine hydrate (109).

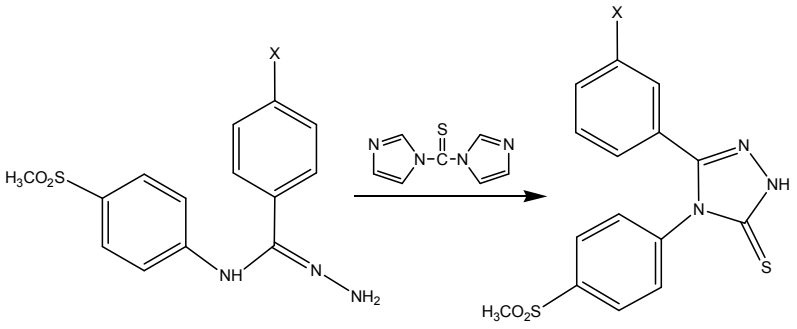


4-amino-3-substituted-1,2,4-triazole-3-thiones were obtained by the reaction of organic acids (propionic acid or acetic acid) with thiocarbohydrazide. (110, 111)



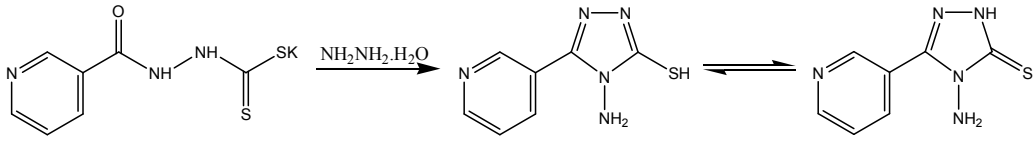
R: Aryl, alkyl

Navidpour et al. (112) synthesized 4-(4-methylsulfonylphenyl)-3-aryl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives by the reaction of *N*-(4-dimethylsulfonylphenyl)-4-substituted benzenecarbohydrazone amide with 1,1'-thiocarbonyldiimidazole under N_2 at room temperature.

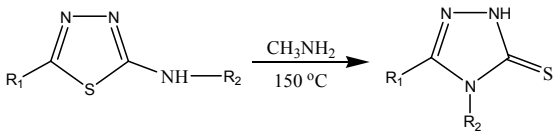


X : H, F, CH_3

To obtain 4-amino-3-(pyridin-3-yl)-2,4-dihydro-1,2,4-triazole-5-thione, a suspension of potassium 3-nicotinoyldithiocarbazate and hydrazide hydrate was refluxed (113).



3,4-Disubstituted-1,2,4-triazole-5-thione derivatives were synthesized by heating 2-amino-5-substituted-1,3,4-thiadiazole derivatives in methylamine (114).

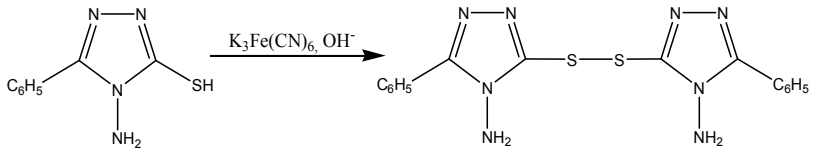


R_1: H; **R_2:** Alkyl

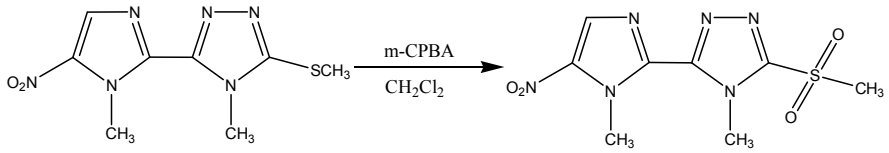
2.3.2. Chemical Properties

Oxidation Reactions

It was reported that in oxidation reaction of 1,2,4-triazole, mercapto groups are easily converted into their methyl ethers with sodium hydroxide and methyl iodide, and these ethers have a slight tendency to lose methanethiol on standing. The mercapto group may be oxidized to disulfide linkage with alkaline apotassium ferricyanide, a reaction that is reversed by dissolution of the disulfide in alkali (115).

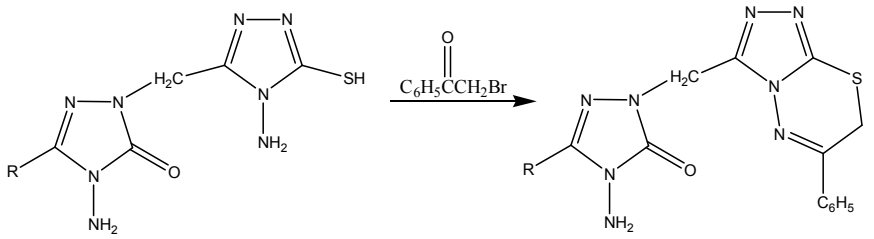


Shafiee et al. (41) prepared 4-methyl-5-(1-methyl-5-nitro-2-imidazolyl)-3-methylsulfonyl-4*H*-1,2,4-triazole by oxidation of 4-methyl-5-(1-methyl-5-nitro-2-imidazolyl)-3-methylthio-4*H*-1,2,4-triazole using (mCPBA) in dichloromethane.



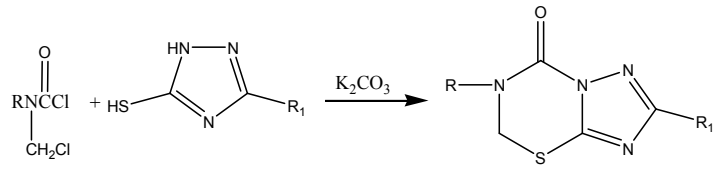
Cyclization Reactions

Demirbaş et al. (116) synthesized 5-substituted-4-amino-2-[(6-phenyl-7*H*-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazin-3-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-ones by the cyclization reaction of 5-substituted-4-amino-2-[4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-ones in the presence of phenacyl bromide.



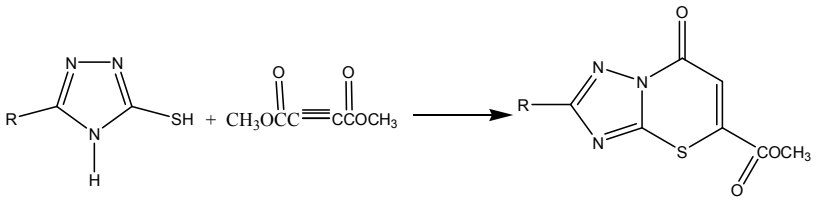
R: Alkyl, aryl

Liu et al. (117) obtained the 2, 6-disubstituted aryl-1,2,4-triazolo[5,1-b]1,3,5-thiadiazin-7-one derivatives by the cyclization reaction of *N*-chloromethyl carbamoyl chloride with 3-aryl-5-mercapto-1,2,4-triazole in the presence of potassium carbonate.



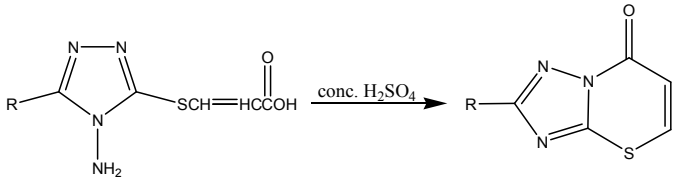
R: Alkyl. **R_1:** aryl

Tozkoparan et al. (118) reported synthesis of 5-carbomethoxy-2-substituted-7H-1,2,4-triazolo[3,2-b]-1,3-thiazine-7-ones by the cyclization of 3-methyl-1,2,4-triazol-5-thiones in the presence of dimethyl acetylenedicarboxylate (DMAD).



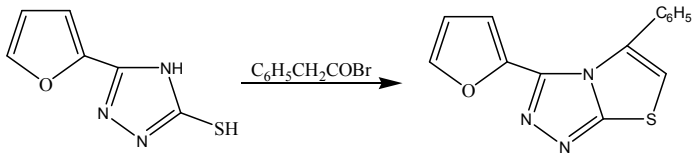
R: Alkyl, aryl, arylalkyl

Heravi et al. (119) reported the synthesis of 2-substituted-[1,2,4]-triazolo[5,1-b][1,3]thiazin-7-ones by cyclization of 5-substituted-3-(4*H*-[1,2,4]-triazol-3-ylsulfanyl)acrylic acids in the presence of concentrated sulfuric acid.



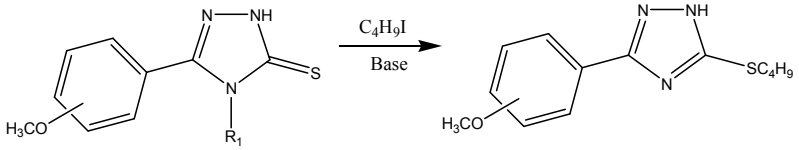
R: Alkyl, aryl, H

Kochhar and Williams (120) synthesized 3-(2-furyl)-5-phenylthiazolo[2,3-c]-1,2,4-triazole by the cyclization reaction of 3-(2-furyl)-1,2,4-triazole-5-thiol/thione with phenyl acetic acid bromide.

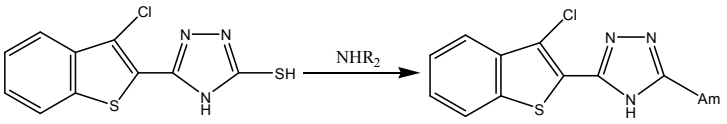


Substitution Reactions

Labanauskas et al. (121) synthesized the 5-butylsulfanyl-3-(of methoxy substituted phenyl)-1*H*-1,2,4-triazoles by the reaction of 3-(methoxy substituted phenyl)-4,5-dihydro-1*H*-1,2,4-triazole-5-thione with 1-iodobutane in alkali medium.

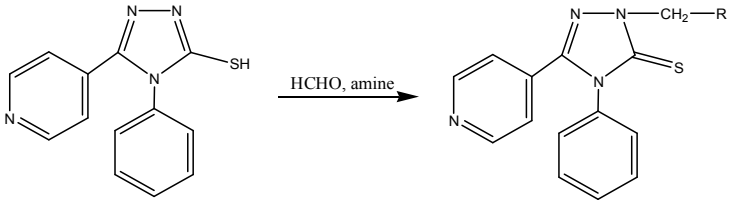


Sharba et al. (48) prepared 3-(3-chloro-1-benzothien-2-yl)-5-(4-morpholinyl/1-piperidinyl/1-piperazinyl)-4*H*-1,2,4-triazole derivatives by refluxing 3-(3-chloro-1-benzothien-2-yl)-4*H*-1,2,4-triazole-5-thiol and secondary amines in dioxane.



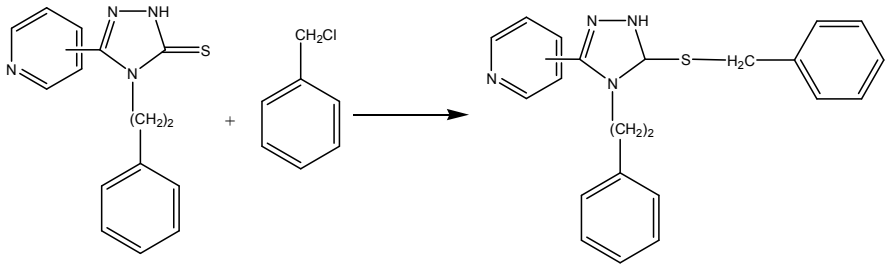
Am: Piperazine, morpholine, piperdine

The reaction of 4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazole-5-thiols with formaldehyde and amine in DMF at room temperature gave corresponding 2-substituted-4-phenyl-5-(pyridin-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-5-thiones (122).

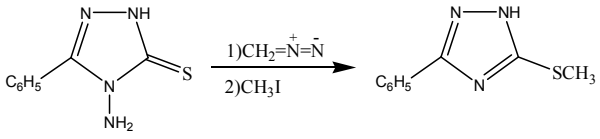


R: Secondary, tertiary amine

Iqbal et al. (123) synthesized the 3-benzylthio-4-(2-phenylethyl)-5-(pyridyl)-1,2,4-triazoles by the reaction of 2,4-dihydro-4-(2-phenylethyl)-5-(pyridyl)-3*H*-1,2,4-triazoles and benzyl chloride in ethanol under reflux condition.



2-Methylthio-1,2,4-triazole was synthesized from the reaction of 3-phenyl-1,2,4-triazole-5-thione with methyl iodide or diazomethane (124).



2.3.3. Spectral Properties

IR Spectra

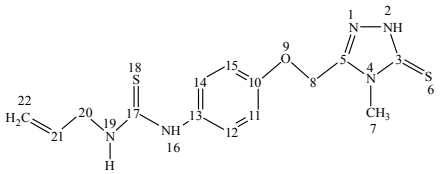
In the IR spectra of 4,5-disubstituted-1,2,4-triazole-5(4*H*)-thione derivatives, stretching bands of N-H of the triazole ring appeared in the region of 3420-3080cm^{-1} (12, 17, 20, 28, 104, 108, 109, 122, 125-131). The characteristic absorption band of C=N and C-N in triazole ring appeared between 1650-1550 (12, 17, 27, 30, 50, 104, 122, 125-133) and 1500-1400 cm^{-1} (15, 131) respectively. The N-H bending bands were seen between 1575-1470, C=S stretching bands appeared between 1380-1150 cm^{-1} (17, 20, 27, 104, 108, 122, 125-127, 131, 132, 134).

^{1}H-NMR Spectra

The ^{1}H-NMR spectrum of 4,5-disubstituted-1,2,4-triazole-5(4*H*)-thione derivatives, the proton attached to nitrogen atom in triazole ring were seen as a singlet between 13–14 ppm (20, 25, 28, 126-128, 131-133, 135).

^{13}C-NMR Spectra

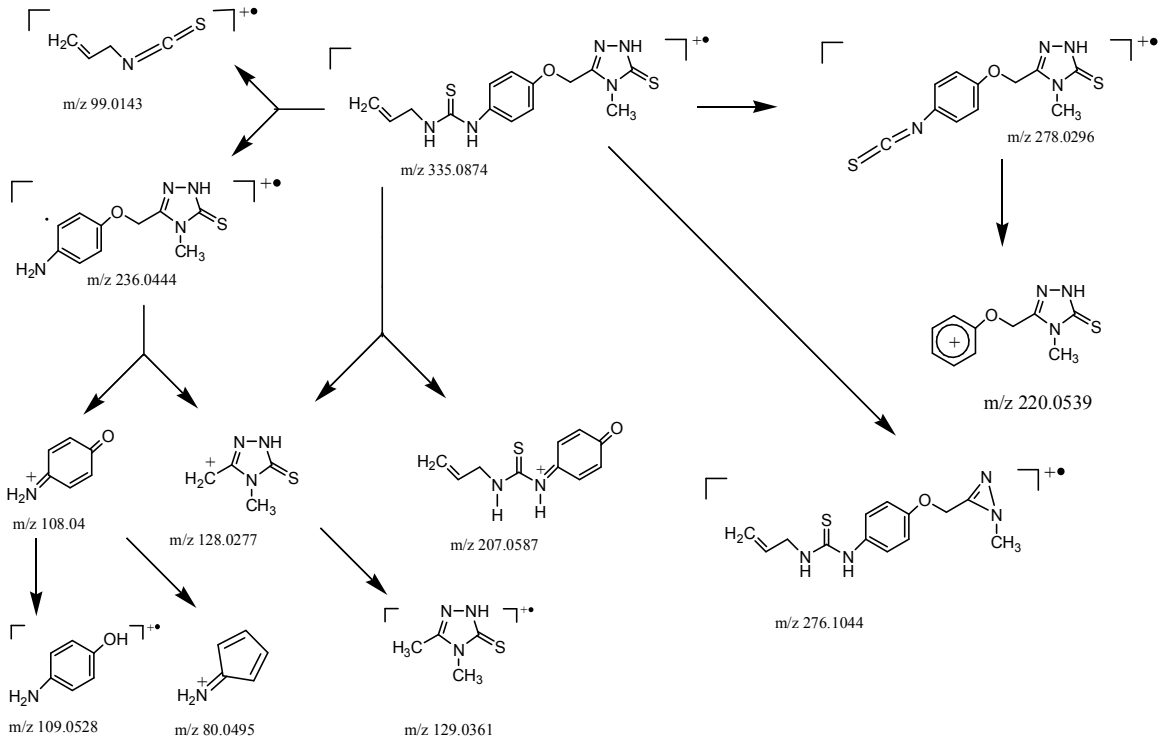
In the ^{13}C-NMR spectrum of 4,5-disubstituted-1,2,4-triazole-5-thiones for C-3 and C-5 were observed in 168.40 and 148.55 ppm respectively (123). Küçükgüzel et al. were elucidated the ^{13}C-NMR spectrum of N-allyl-N'-{4-[4-methyl-5-thioxo-3,4-dihydro-1H-1,2,4-triazol-3-yl)methoxy]phenyl}thiourea and found the data as follow: (C-3) 168.39, (C-5) 149.07, (C-7) 30.89, (C-8) 61.05, (C-10) 155.12, (C-11 and 15) 115.61,)C-12 and 14) 126.59, (C-13) 134.05, (C-17) 180.52, (C-20) 41.01, (C-21) 129.13 and (C-22) 124.39 ppm (25).



Mass Spectra

The mass spectrum of the most of 1,2,4-triazole-3-thione derivatives exhibit the base molecular ion peak at expected m/e, which corresponds to the molecular weight.

Küçükgüzel et al. (25) showed the mass fragmentation of 1-allyl-3-(4-((4-methyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thione-3-yl)methoxy)phenyl) thiourea (Scheme 2.3). The spectrum also exhibits an intense peak at m/z 236.04 which is due to the 5-(4-aminophenoxy)methyl-4-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione fragment resulting from the cleavage of allylisothiocyanate. The peak at m/z 276.10 is consistent with the cleavage of [HCNS].

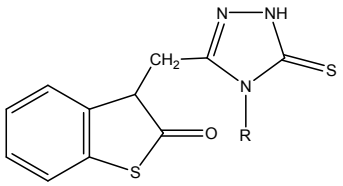


Scheme 2.3. Mass fragmentation pathway of 1-allyl-3-(4-((4-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione-5-yl)methoxy)phenyl)thiourea

2.3.4. Biological Activities

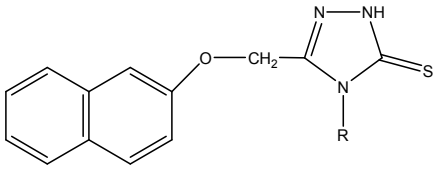
Analgesic and Anti-inflammatory Activities

Gökçe et al. (20) synthesized and evaluated the anti-inflammatory and analgesic activities of some new [(2-oxobenzothiazolin-3-yl)-methyl]-4-alkyl/aryl-1,2,4-triazole-5-thiones. It was found that some of the synthesized compounds possess aspirin-like activity and more potent than novalgine.



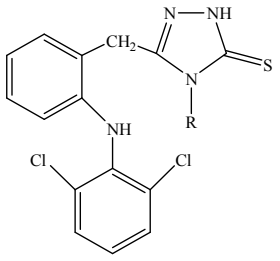
R: Alkyl, aryl

Palaska et al. (8) studied on 3-(2-naphthyloxymethyl)-4-substituted-1,2,4-triazole-5(4*H*)-thione derivatives to evalute their anti-inflammatory activities. Some of the compounds show good anti-inflammatory activities compared with naproxen, indomethacin and phenylbutazone.



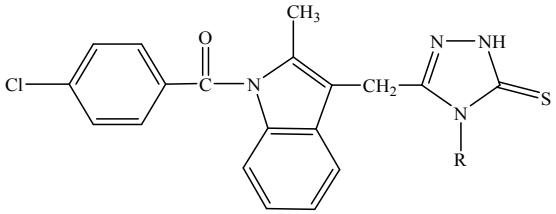
R: Alkyl, aryl

Amir and Kumar (90) replaced the carboxylic acid group of diclofenac with 4-alkyl/aryl-1*H*-1,2,4-triazole-5(4*H*)-thione ring and investigated 3-[(2-(2,6-dichloro anilino)benzyl]4-alkyl/aryl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives for their analgesic and anti-inflammatory activities. They found that this replacement improved their analgesic and anti-inflammatory activities. Additionally, these new synthesized compounds showed low ulcerogenic effects than reference drugs.



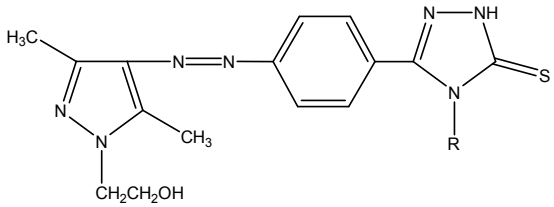
R: Alkylamine, arylamine

Same researchers replaced the carboxylic acid group of indomethacin to 4-n-butyl/cyclohexyl-1*H*-1,2,4-triazole-5(4*H*)-thione and screened for their analgesic, anti-inflammatory and ulcerogenic activities. The results showed that the comounds have higher analgesic and anti-inflammatory activities and lower ulcerogenic activity than reference drug (136).



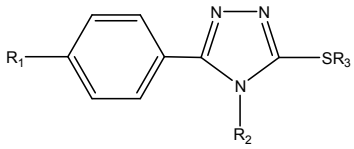
R: Alkyl, cyclohexyl

Oruç et al. (64) examined 3-[4-((1-(2-hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-4-substituted-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives for their analgesic activity and they have been found that their analgesic activity is as same as morphine.



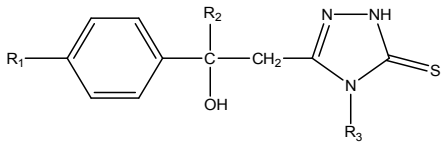
R: Alkyl, Cycloalkyl, Arylalkyl

Navidpour et al. (109) designed a new series of 3-thio and 5-alkylthio-3,4-diaryl-1*H*-1,2,4-triazole-5(4*H*)-thiones to develop their structural-activity relationship. They reported that diarylheterocycles possessed a significant and selectivity inhibition on COX-2 isozyme. According to *in-vivo* test the new synthesized compounds have higher anti-inflammatory activity than celecoxib.



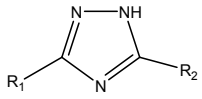
R_1: H, halogen, alkyl, methylsulfonyl
R_2: Aryl
R_3: H, alkyl

Rahman and Hussein (137) investigated a series of 3-[2-(substituted)-2-hydroxyethyl]-4-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones for their analgesic and anti-inflammatory activities, during studies they have been found that compounds structurally similar to indomethacin showed anti-inflammatroy activity and also high analgesic activity than aspirin.



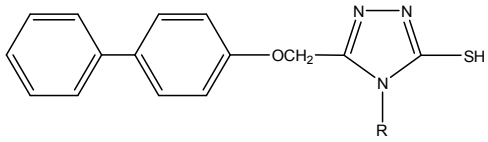
R_1: H, halogen
R_2: H, aryl
R_3: Alkyl, aryl

Tozkoparan et al. (101) studied 3-aryl-5-alkylthio-1,2,4-triazoles and their sulfone derivatives for their anti-inflammatory and analgesic activities. They have been found that sulfone derivatives have higher anti-inflammatory activities. Among the synthesized compounds 2-chloro and 4-chlorophenyl substituted compounds showed higher analgesic and anti-inflammatory activities.



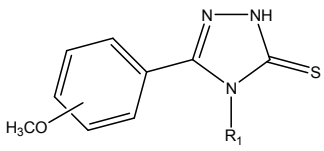
R_1: Aryl
R_2: Thioalkyl, alkylsulfonyl

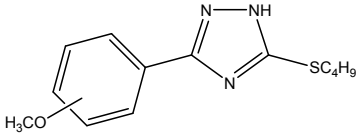
Kumar et al. (50) worked on some 5-[(biphenyl-4-yloxy)methyl]-4-alkyl/aryl-3-mercapto-(4*H*)-1,2,4-triazoles derivatives and studied their analgesic and anti-inflammatory activities. They reported that the synthesized compounds possess higher anti-inflammatory and lower analgesic activities than flurbiprofen.



R: Aryl, alkyl

Labanauskas et al. (121) studied on some 5-substituted-4*H*-1,2,4-triazole-3-thiol derivatives for their anti-inflammatory activity. They proved that some of the synthesized compounds showed same or higher anti-inflammatory activity than aspirin and ibuprofen.

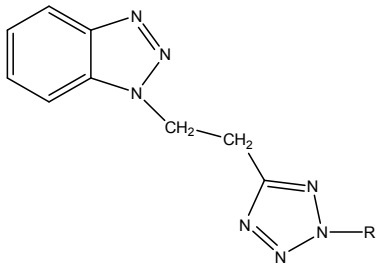




R_1: H, aryl, alkyl

A group of 1-[2-(1*H*-tetrazol-5-yl)ethyl]-1*H*-benzo[d][1,2,4]triazoles were synthesized and evaluated for their anti-inflammatory activity. The result showed

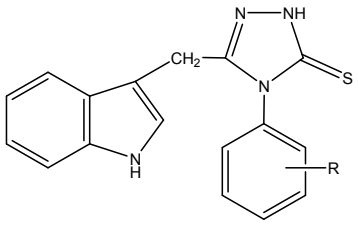
that replacement of active hydrogen in tetrazole moiety with substituted benzoyl and sulfonyl group caused to form the comounds having potent anti-nociceptive and mild anti-inflammatory activity (138).



R: substituted benzoyl and sulfonyl

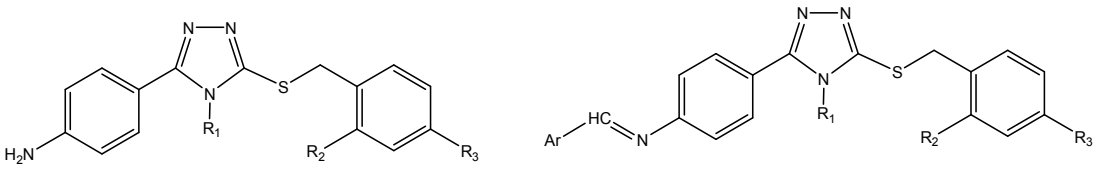
Anticonvulsant Activity

Siddiqui et al. (15) evaluated anticonvulsant activity of a series of 5-(1*H*-indol-3-yl methyl)-4-(substituted aryl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones. They found that some of the synthesized compounds possess higher anticonvulsant activity and show lower neurotoxicity.



R: H, halogen, alkyl, alkoxy

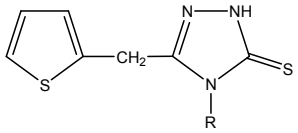
Küçükgüzel et al. (126) synthesized 3-(arylalkylthio)-4-alkyl/aryl-5-(4-aminophenyl)-4*H*-1,2,4-triazole derivatives and evaluated their anticonvulsant activity. Some of the synthesized compounds showed favorable anticonvulsant activity.



R_1: alkyl, aryl; **R_2** and **R_3:** H, halogens

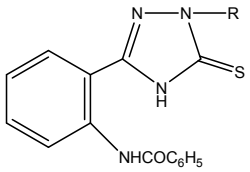
Antifungal and Antibacterial Activities

Wujec et al. (132) evaluated the antifungal activity of 4-substituted-3-(thiophene-2-yl-methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives, they have observed that some of the synthesized compounds have antifungal activity against some species of *Trichophyton spp*.



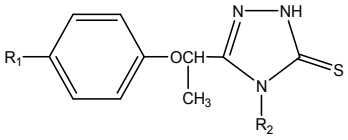
R: Alkyl, arylalkyl, aryl

Kidwai and Mohan (139) showed that 1-substituted-3-[(2-benzoylamino)phenyl]-1,2,4-triazole-5(4*H*)-thione derivative had high antifungal activity against *A. flavus* and *A. niger*.



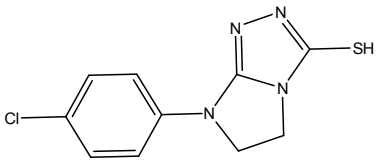
R: H, aryl

Turan-Zitouni et al. (127) elucidated 4-phenyl/cyclohexyl-3-(1-(4-substitutedphenoxy)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives for their antimicrobial activity. They found that some of the compounds have high antifungal activity than ketoconazole.

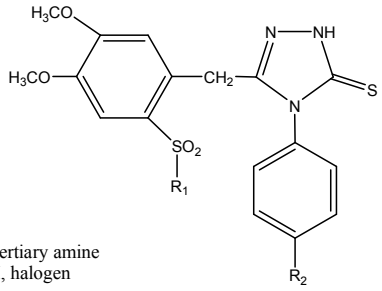


R_1: H, halogen, alkyl
R_2: Alkyl, aryl

Sztanke et al. (133) studied a series of imidazotriazole derivatives for their antimicrobial activity. They reported that some of the imidazo[2,1-c][1,2,4]triazole-3-thiol showed the superior antifungal activity against *C. albicans* and *A. niger* as compared to miconazole.

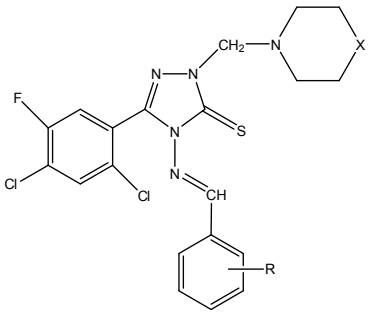


Ezabadi et al. (128) evaluated antifungal activity of 3-[2-(substituted sulfamoyl)-4,5-dimethoxybenzyl]-4-aryl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives against difference *Aspergillus* like *Penicillium funiculosum* and *Trichoderma viride*. They showed that some of the compounds have higher antifungal activity than bifonazol.

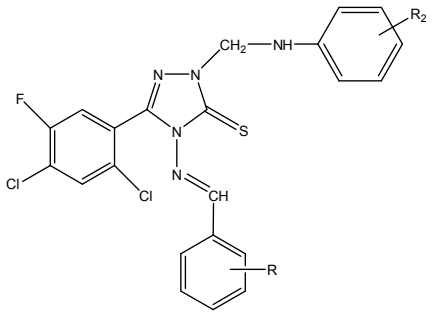


R_1: Tertiary amine
R_2: H, halogen

Karithikeyan et al. (129) synthesized a series of *Schiff* bases of triazole and evaluated for their antibacterial activity. Some of the synthesized compounds exhibited promising antibacterial and antifungal activity.



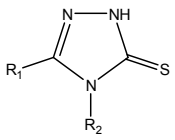
X: O, N-CH_3, CH_2



R_2: 4-F, 3-Cl-4-F

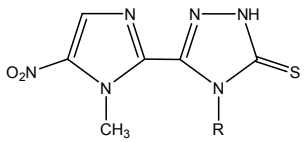
R1: Halogen, amine, alkoxy

Ragenovic et al. (130) studied antibacterial activity of 3-aryl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives and found that those 1,2,4-triazoles bearing an amine group on the 4th position have high antibacterial activities against *E. coli*, *S. aureus* and *B. subtilis*.



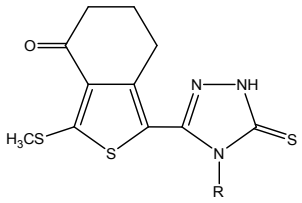
R_1: Ary
R_2: Amine, allyl

Shafiee et al. (41) evaluated antibacterial activity of 3-(1-methyl-5-nitro-2-imidazolyl)-4-substituted-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives and found that some of the compounds are active against *S. aureus* and *B. subtillis*.



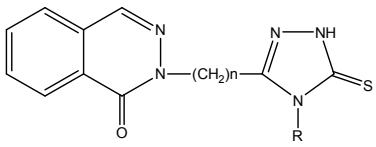
R: Alkyl

Tehranchian et al. (140) studied 1-(4-substituted-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3-yl)-3-methylthio-6,7-dihydrobenzo[c]thiophene-5(4*H*)-one derivatives for their antibacterial activity. They found that some of the compounds have high activity against *S. aureus* and *S. epidermidis*.



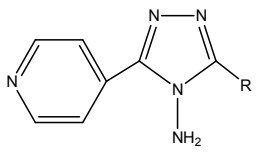
R: Alkyl, cycloalkyl, aryl

Önkol et al. (87) evaluated 3-[(1(2*H*)-phthalazinone-2-yl-ethyl]-4-aryl-1,2,4-triazole-5-thione derivatives for their antibacterial and antifungal activities. They reported that the compounds could be a good starting point for developing better antibacterial and antifungal agents.



R: Aryl

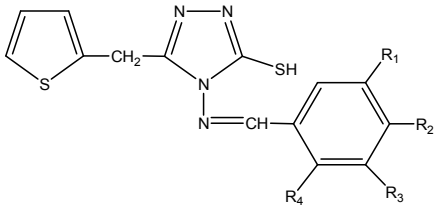
5-Substituted-3-pyridine-1,2,4-triazole derivatives were synthesized and evaluated for their antimicrobial and antifungal activities. Some of the synthesized compounds have remarkable activities against *B. subtilis, S. aureus, P. mirabilis, S. typhi, C. albicans* and *A. niger* (141).



R: aryl

Antituberculosis Activity

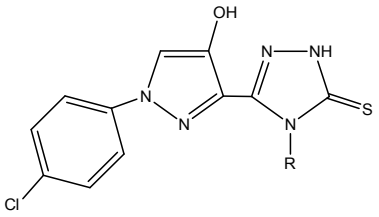
Özdemir et al. (12) evaluated antibacterial activity of 4-arylidenamino-4*H*-1,2,4-triazole-3-thiol derivatives against *M. tuberculosis*. These new synthesized compounds possess higher activity than reference drug (rifampicine).



R_1,R_2,R_3,R_4: H, halogen, alcohol, nitro, alkoxy, alkyl

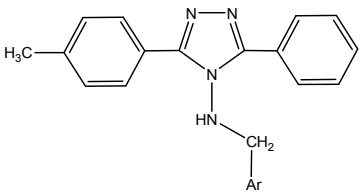
Antitumoral Activity

5-(1-(4-Chlorophenyl)-4-hydroxy-1*H*-pyrazol-3-yl)-4-substituted-1,2,4-triazole-3-thione derivatives were evaluated for antitumoral activity. According to this study some of the compounds showed activity against CNS, leukemia, colon, lung, prostate and breast cancer (17).



R: Cycloalkyl, aryl

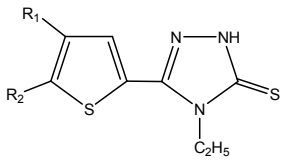
Bekircan et al. (142) evaluated the anticancer activity of 3,5-diaryl-4*H*-1,2,4-triazole derivatives. Some of the compounds were active against leukemia cells and possess antiproliferative activity.



Ar: substituted aryl

Mavrova et al. (67) synthesized and evaluated antitumoral activity of 3,4-disubstituted-1,2,4-triazole-5(4*H*)-thione derivatives. They found that among the

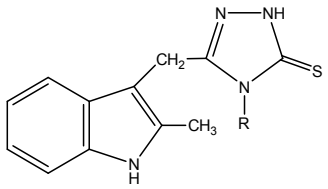
synthesized compounds just 4-ethyl-3-(4,5,6,7-tetrahydro-1-benzothiene-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione was active against tumor cell and has high cytoxicity.



R_1, R_2: Alkyl; **R_1:** H, **R_2**: Aryl

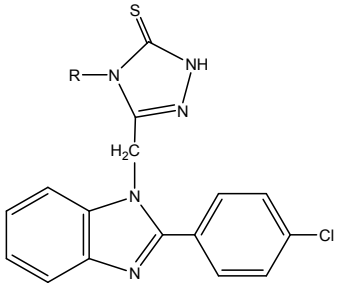
Other Biological Activities

Varvaresou et al. (134) evaluated 3-[(2-methyl-1*H*-3-indolyl)methyl]-4-aryl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives for antidepressant activity. They found that the compounds have higher antidepressant activity than imipramin.



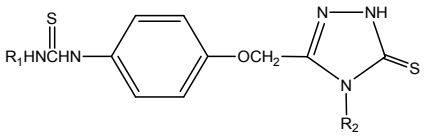
R: Aryl

Kılcıgil et al. (135) synthesized and evaluated 3-(2-(4-chlorophenylbenzimidazole-1-yl)methyl)-4-substitutedphenyl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives for antioxidant activity. They showed that some of the compounds have higher antioxidant activity than reference drugs.



R: Aryl

Küçükgüzel et al. (25) elucidated the antiviral activity of N-alkyl/aryl-N'-{4-[(4-alkyl/aryl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3-yl)methoxy]phenyl} thiourea derivatives. They found that some the compounds possess moderate activities against *Coxsachie B4*, *Herpes simplex* and *Varicella-zoster* viruses.



R_1,R_2: Alkyl, aryl

2.4. Cyclooxygenase Inhibitors

Inflammation may be defined as the series of changes that occur in living tissues following injury or infection, or inflammation is the immune system's response against injury or infection (1). Prostaglandins (PGs) play an important role in the generation of the inflammatory response. The biosynthesis of PGs is dramtically increased in inflammed tissues and contribute in development of cardinal signs of acute inflammation: swelling/redness, pain and fever. The production of PGs depend on activity of enzymes called COXs, these bifunctional enzymes possess both COX and peroxidase activities (143)

Cyclooxygenase (COX) is an enzyme that is responsible for the biosynthesis of important biological mediators called prostanoids (Prostaglandins and thromboxane). Prostaglandin synthesis depends on activity of prostaglandin synthase or COX. During an inflammatory response, both the level and the profile of prostaglandin production change dramatically. In the uninflamed tissues the level of prostaglandin production is very low but in an acute inflammation it increases immediately before the recruitment of leukocytes and the infiltration of immune cells (1).

Cyclooxygenase (COX) enzyme has two catalytic sites. The first, a cyclooxygenase active site, converts arachidonic acid to the endoperoxide PGG_2. The second, a peroxidase active site, then converts the PGG_2 to another endoperoxide, PGH_2. PGH_2 is further processed by specific synthases to form PGs, prostacyclin and thromboxane A_2 (2).

Based on available literatures, in human cell there are at least two types of COX enzymes, called COX-1 and COX-2 (according to the new research third isoform of COX enzyme (COX-3) has recently been identified). COX-1 and COX-2 are the products of two distinct genes, which in humans are localized on chromosomes 9 and

1 (3). The two COXs are genetically found to be different in structure, it related to an amino acid exchange in the active site of enzyme proteins.

The main difference between the two COX active sites is the replacement of Ile523 in COX-1 by Valine, a less bulky amino acid. This replacement creates an adjunct pocket in the COX-2 active site and allows additional interactions with some polar amino acids such as His90 and Arg513. Another difference is that the presence of a Phe503 forces the Leu384 side-chain to point into active site in COX-1 while a smaller amino acid, Leu503 allows Leu384 side-chain to move away from active site and generates an accessible space in the apex of the COX-2 active site (Figure 2.1). These two differences increase the volume of COX-2 active site (144).

Since understanding this structural modification, a new window has been opened to synthesize new drugs with high selectivity inhibtion of COX-1 and COX-2 (4).

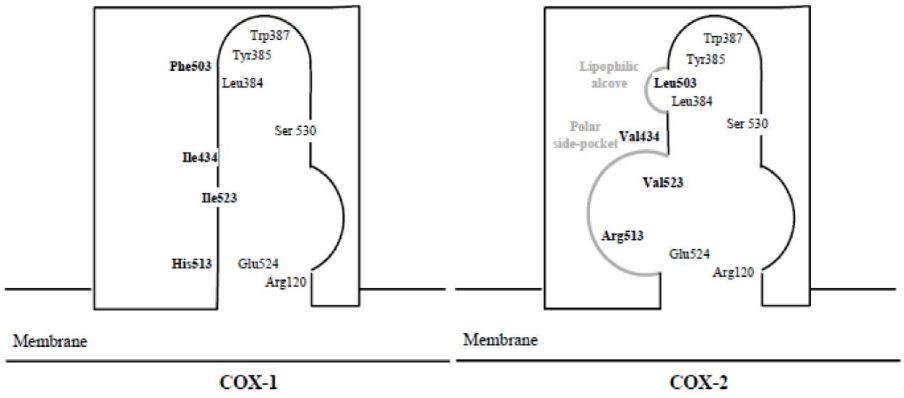


Figure 2.1. Schematic representation of the active site of the two COX-1 and COX-2 isozymes (145).

The activity of COX-1 enzyme includes cytoprotective properties in gasteroentestinal system, control of renal functions in the kidney (4) and in platelets that leads to thromboxane A_2 production, causing aggregation of the platelets to prevent inappropriate bleeding (3). COX-2 plays a significant role in PG formation during pathophysiological states, such as inflammation. One of the important role of COX-2 is in resolution of inflammation, a role that is very important for healing of gastric ulcers (146).

When the highly selective COX-2 inhibitors entered to treatments, their development and clinical usage have been brought a big promise about their safety on gastrointestinal system that have not been fully realized (147). These drugs (coxibs) produce less gastric ulceration than other NSAIDs, but still coxibs cause significant gastric and cardiovascular adverse effects susceptible individuals, especially if they are administered with aspirin. The *Wallace* et al. (148) have been expressed their concern about the usage of NSAIDs. For example the dose-dependent hypertensive effect of some NSAIDs, this is not easily predicted by their inhibition of COX enzyme alone. With regard this reason, they have been suggested to improve safety profile over existing.

In general, there exists virtually very little difference between the therapeutic efficacy of different NSAIDs, as certain patients would respond to one *'drug'* better than another. In reality, it is almost difficult to predict the best suitable drug for a patient; thus, it invariably necessitates arriving at the *best-fit-drug via* trial and error only. Keeping in view the innumerable adverse side effects caused by the NSAIDs their clinical usefulness are restricted drastically. Therefore, patients who are taking such drugs for relatively longer periods should have periodic white-blood cell counts as well as determinations of serum creatinine levels, besides hepatic enzyme activities (149).

2.4.1. Role of Cyclooxygenase (COX) Isozymes in Inflammation

The two COX isozymes, COX-1 and COX-2 are known as targets of nonsteroidal anti-inflammatory drugs (NSAIDs). These drugs inhibit the active site of both COXs. However, both COXs exist as homodimers, only 1 partner is used at a time for substrate (146). NSAIDs block the COXs by inactivating the COX site at one of the monomers of COX dimer and this inactivating cause to stop the prostanoid formation (5). The evaluation of blocking activity of prostanoids by NSAIDs according to their structure has been proved that prostanoids are important mediators involving in promotion of fever, pain and inflammation (150). The most important distinctions between COX-1 and COX-2 are the differential of their tissue distribution. COX-1 in gastrointestinal system, kidney, vascular smooth muscle and platelets (Figure 2.2) (151).The COX-2 is undetectable in most tissues, but it has been seen a dramatically increase in inflamed tissue (152).

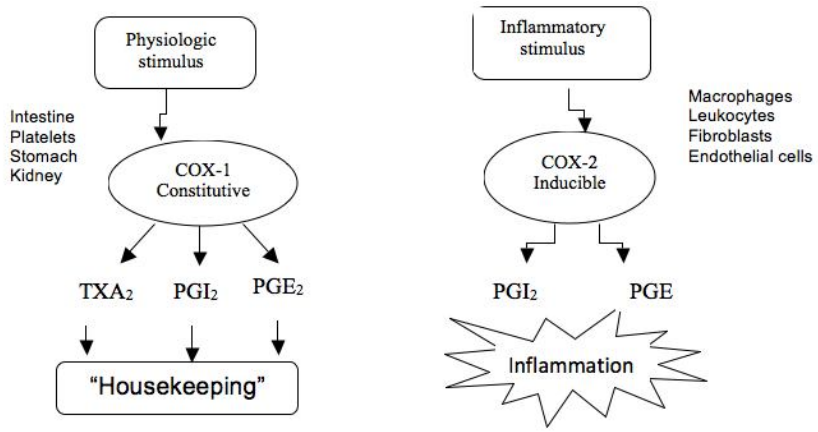


Figure 2.2. The scheme of prostaglandins biosynthesis: Differing roles of COX-1 and COX-2 enzymes.

2.4.2. COX-1 and COX-2 Inhibitors

When the NSAIDs have been started to show anti-inflammatory activity via inhibition of prostaglandin biosynthesis by blocking cyclooxygenase activity, many researchers have been developed different models of in-vivo and in-vitro to study the interaction of NSAIDs with cyclooxgenases (COXs). Whatever the active site of COX isozymes is consisted from a hydrophobic channel which is the site of NSAIDs binding. This channel have areas with high electron density (4).

Most NSAIDs producers, have been focused on COXs as molecular target for these drugs. They are trying to produce NSAIDs with high capacity to bind the active site of COX isozymes and block the their activities (146). COX-1 and COX-2 are different isozymes, distributated in difference tissues and possess their own functions. NSAIDs with non-selectivity against COXs are produce serious side effects, such as gastric ulcer and other side effects (153). Since understanding structural and functional difference between COX-1 and COX-2, producing NSAIDs with selective inhibtion of COX-1 or COX-2 are the priority (143).

There are four major groups of NSAIDs on the basis of their inhibitory activity on COX-1 and COX-2:

Non-selective COX Inhibitors

These drugs inhibit both COX-1 and COX-2. Most NSAIDs are having these characteristics like high dose of aspirin, diclofenac, ibuprofen, naproxen, mefenamic acid, indomethacin, ketoprofen and piroxicam.

Selective COX-1 Inhibitors

Still there aren't any selective COX-1 inhibitors except Aspirin. Aspirin in low doses selectively blocks platelet COX-1 and the resulting synthesis of thromboxane, a proaggregatory prostaglandin, without affecting endothelial cell production of prosacylcine, an antiaggregatory prostaglandin. Aspirin by blocking the biosynthesis of thromboxane in vascular prostaglandin synthesis, hence reducing thrombosis.

Selective COX-2 Inhibitors

Selective COX-2 inhibitors only inhibit the COX-2 enzyme, allowing for the production of the prostaglandins that protect the stomach, while still relieving fever, pain and inflammation. They do not have the anti-platelet effects associated with nonselective NSAIDs and so do not alter clotting. The selective COX-2 inhibitors are: Celecoxib and meloxicam.

Highly selective COX-2 Inhibitors

Nowaday developed highly selective COX-2 inhibitors show only COX-2 inhibition and even in high doses they dont have any COX-1 inhibition effect. However, it is probable that useful drugs will also emerge from this class, as has been shown for the selective inhibitors. These drugs will not inhibit platelet aggregation and should have an excellent gastrointestinal safety profile, as already shown in animal experiments. In light of the concern of the cardiovascular adverse effects of the highly selective COX-2 inhibitors, unfortunately, most of these drugs show high side effect on cardiovascular systems, such as, hypertesion and myocard infraction. Celecoxib, etoricoxib and rofecoxib are the most common highly selective COX-2 inhibitors.

2.4.3. Methods for Determination of COX Activity

Since NSAIDs have been shown the inhibition effect on COX-1 and COX-2 through the inhibition of PG synthesis by interaction with cyclooxygenase, many in vitro assay systems have been developed to investigate the selectivity of non-steroidal anti-inflammatory drugs (4). The most commonly used are classified into 3 groups:

1. Systems using animal enzymes, animal cells, or cell lines, which were the first to be developed.

2. Assays using human recombinant enzymes, human cell lines, or human blood cells (mainly platelets and monocytes), which are the current standards.
3. Newly developed models using human cells that are target cells for the anti-inflammatory and adverse effects of non-steroidal anti-inflammatory drugs. These targets include human gastric mucosa cells, chondrocytes and synoviocytes.

However, when choosing a test system, not only should the characteristics defined above be taken into account, but the more practical aspects, such as the feasibility and the reproducibility of the assay, should also be assessed. This may explain why an ideal test system such as human gastric mucosa tissue to test for COX-1, and human synovial tissue stimulated by interleukin-1 to test for COX-2, has not been validated for routine use.

It is also important to establish the aim of a study when choosing an assay system. If the aim is to investigate the interaction between a drug and the active site of the enzyme at a molecular level, then purified enzymes should be used. For screening or structure-activity relationship studies, human cell lines (which constitutively express either COX-1 or COX-2) or human recombinant enzymes in a microsomal assay (which allow a high throughput) are best suited. If the aim of the study is to investigate the clinical relevance of selective COX-2 inhibition, then the conditions defined above should determine the choice of the assay. In this context, the systems most commonly used are human recombinant enzymes in whole cells and the human whole blood assay. These systems constitute the best compromise to date between the characteristics of an ideal model as defined above and practical feasibility.

2.4.4. Determination of Inhibition of COX Enzyme by Using Enzyme Immunoassay Methods

Enzyme Immunoassay (EIA)

An assay that uses an enzyme-bound antibody to detect antigen. The enzyme catalyzes a color reaction when exposed to substrate. Immunoassays are those assays wherein any anti-body or anti-gen are detected for their presence in a given sample.

ELISA (Enzyme Linked Immuno-Sorbient Assay)

An enzyme-linked immune-sorbent assay (ELISA) involves the reaction of an antibody-enzyme complex with an antigen or antibody held immobile on a solid surface. On incubation of the conjugated enzyme with a suitable substrate, a product is formed. The formation of a product helps to detect presence of the antigen or antibody and its quantity provides a measure of the amount of antigen-antibody reaction that occurred. The ELISA test may be performed in two forms: direct and indirect. ELISA is a type of immunoassay aimed to detect antigen or antibodies present in an inflicted individual for proper diagnosis and further treatment. Among the available immunoassays, ELISA is of the distinguished and widely used types. It is extensively used due to the advantages like rapidity or speed in experimentation, greater sensitivity and specificity for even small amount of test samples. In ELISA test, the reaction is measurable in both qualitative and quantitative terms.

Types of ELISA

Direct ELISA

Direct ELISAs involve attachment of the antigen to the solid phase, followed by an enzyme-labeled antibody. This type of assay generally makes measurement of crude samples difficult, since contaminating proteins compete for plastic binding sites.

Indirect ELISA

Indirect ELISAs also involve attachment of the antigen to a solid phase, but in this case, the primary antibody is not labeled. An enzyme-conjugated secondary antibody, directed at the first antibody, is then added. This format is used most often to detect specific antibodies in sera.

Competitive ELISA

The third type of ELISA is the Competition Assay, which involves the simultaneous addition of 'competing' antibodies or proteins. The decrease in signal of samples where the second antibody or protein is added gives a highly specific result.

Sandwich ELISA

The last type of assay is the sandwich ELISA. Sandwich ELISAs involve attachment of a capture antibody to a solid phase support. Samples containing known or unknown antigen are then added in a matrix or buffer that will minimize attachment to the solid phase. An enzyme-labeled antibody is then added for detection.

2.5. Molecular Docking

Molecular docking has become an increasingly important tool for drug discovery. There are two primary reasons for this evolution: first, there has been an explosive growth of protein structural data not only from X-ray crystallography, but also from NMR and electron microscopy studies. Second, this growth of protein structural data have come significant advances in both computational techniques and hardware. As computers continue to increase in speed and capability, we are able to tackle larger and more complex systems (153, 154)

The aim of molecular docking is to give a prediction of the ligand-receptor complex structure using computation methods. Docking can be achieved through two interrelated steps: first by sampling conformations (search algorithms) of the ligand in the active site of the protein; then ranking these conformations via a scoring function.

2.5.1. Search Algorithms

There are a huge number of possible binding modes between two molecules. Unfortunately, it would be too expensive to computationally generate all the possible conformations. Various search algorithms such as monte carlo, genetic algorithms and molecular dynamics have been developed and widely used in molecular docking software.

2.5.2. Scoring Functions

Scoring function is one of the most important components in molecular docking. The purpose of the scoring function is to predict the strength of the non-covalent interaction between ligand and receptor after they have been docked. Scoring

functions can be classified into three distinct categories: force-field-based, empirical and knowledge-based scoring functions. (155-157).

Force Field Scoring Function

Force field scoring functions are developed based on physical atomic interactions, including Van der Waals (VDW) interactions, electrostatic interactions, and bond stretching/bending/torsional forces.

Empirical Scoring Function

Empirical scoring functions, estimate the binding affinity of a complex on the basis of a set of weighted energy terms. Compared to the force field scoring functions, the empirical scoring functions are much faster in binding score calculations due to their simple energy terms.

Knowledge-based Scoring Function

Knowledge-based scoring functions rely on statistical means to extract rules on preferred, and non preferred atom pair interaction from experimentally determined protein-ligand complexes. The rules are interpreted as pair-potential that are subsequently used to score ligand binding pose.

Nowadays, in docking applications outside these methods, a hybrid method (a combination of quantum mechanic and molecular mechanic methods) is used. In this method, the reaction that occurs in active site of enzyme reaction is calculated with quantum mechanic and the other part is calculated with molecular mechanic.

The following applications were made in the development of the docking technique (155, 158).

Rigid body docking; where both the receptor and small molecule are treated as rigid.

Flexible ligand docking; where the receptor is held rigid, but the ligand is treated as flexible.

Flexible docking; where both receptor and ligand flexibility is considered.

Thus far, the most commonly used docking algorithms use the rigid receptor/flexible ligand model.

3. MATERIALS AND METHODS

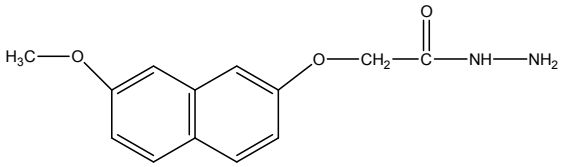
3.1. Chemistry

3.1.1. Materials

7-Methoxy-2-naphthol, ethyl bromoacetate, ethyl isothiocyanate, allyl isothiocyanate, methanesulfonic acid, phosphoryl chloride were purchased from Sigma-Aldrich. Methyl isothiocyanate, phenyl isothiocyanate, hydrazine monohydrate were purchased from Merck. Sodium hydroxide was purchased from Riedel-de Haën. Other reagents and solvents which are used in this study were purchased from Merck and Fluka.

3.1.2. Methods of Synthesis

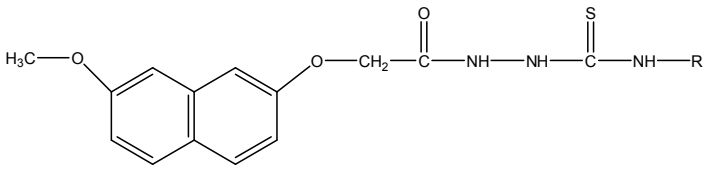
2-(7-Methoxy-2-naphthyloxy)acetylhydrazide



Method A

A mixture of 7-methoxy-2-naphthol 1.74 g (10 mmol), anhydrous potassium carbonate 1.382g (10 mmol) and ethyl bromoacetate 1.67g (10 mmol) were dissolved in anhydrous acetone (50 ml) and refluxed for 6 h. The reaction mixture was filtered and the excess solvent was removed by distillation under reduced pressure. The residue and 0.5 g hydrazine monohydrate (15 mmol) were dissolved in absolute ethanol (50 ml) and refluxed on a water-bath for 2 h. The solid mass was filtered off, dried and recrystallized from ethanol.

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-substituted thiosemicarbazides (Compounds 1a-d)

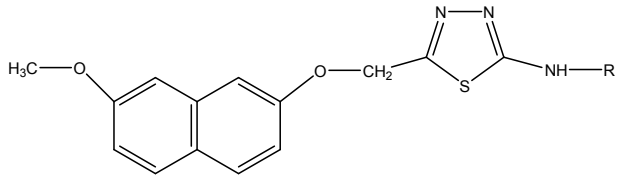


R: CH_3, C_2H_5, C_3H_5 and C_6H_5

Method B

The mixture of 1-(7-methoxy-2-naphthyloxyacetyl)hydrazine (10mmol) and 10 mmol of appropriate substituted isothiocyanate derivatives was dissolved in 30 ml ethanol and refluxed for 4 h. on water-bath. The crude product which precipitated on cooling was filtered, washed with diethyl ether, dried and recrystallized from dioxane–water.

5-((7-Methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4–thiadiazoles (Compounds 2a-d)



R: CH_3, C_2H_5, C_3H_5 and C_6H_5

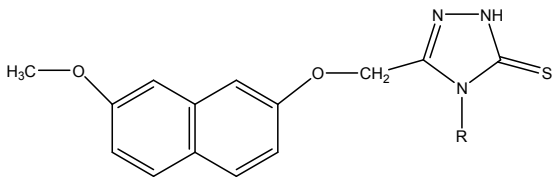
Method C

The appropriate 1-(7-methoxy-2-naphthyloxyacetyl)-4-substituted-3-thiosemicarbazide derivatives (1 mmol) were dissolved in 10 ml toluene and methanesulfonic acid (15 mmol) was added dropwise and refluxed for 45 min. on water-bath. The precipitated product was filtered off and recrystallized from suitable solvents.

Method D

Phosphoryl chloride (5 ml) was added to appropriate 1-(7-methoxy-2-naphthyloxyacetyl)-4-substituted-3-thiosemicarbazide derivatives (1 mmol) and refluxed in oil bath for 3 h. The resulting solution was poured to ice-water mixture. The precipitated product which obtained, filtered off and recrystallized from suitable solvents.

3-((7-Methoxy-2-naphthyloxy)methyl)-4-substituted-1,2,4-triazole-5–thiones (compounds 3a-d)



R: CH_3, C_2H_5, C_3H_5 and C_6H_5

Method E

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-substituted-3-thiosemicarbazide derivatives (4 mmol) were refluxed for 8 h in 40 ml 1N aquoeus sodium hydroxide. The resulting mixture was acidified to pH 2 with 1N HCl. The acidic solution was extracted with 25ml ethyl acetate 3 times, then the organic phase dried with anhydrous sodium sulfate, filtered and distilling off the organic phase and the residue recrystallized from suitable solvents.

3.1.3. Analytical Methods

Melting Points

The melting points of the synthesized compounds were determined by Thomas Hoover Capillary Melting point apparatus.

Thin Layer Chromatography

Materials

TLC aluminum plates: Kieselgel F_{254} Type 60 (Merck) was used in TLC analysis.

Solvent systems: Three different solvent systems were prepared to be used in chromatographic controls of the synthesized compounds.

S – 1: Benzene:Methanol (95:5)

S – 2: Chloroform:Methanol (95:5)

S – 3: n-Hexane:Ethyl acetate:Methanol (60:30:10)

Method

Dragging conditions: The solvents systems were poured to the chambers and kept for 1 hour to adequate saturation.

The reactions were controlled and monitored with TLC by dissolving the synthesized compounds and starting materials with suitable solvents and application of them with microcapillary tubes onto silicagel plates.

Visuallization of TLC results: Visualization of the synthesized compounds and their starting materials were determined by UV light (254/366 nm) and iodine vapour (159).

Spectrometric Analysis

IR Spectra

The IR spectra of synthesized compounds were studied by Perkin Elmer Spectrum BX FT-IR spectrophotometer, with using an Attenuated Total Reflectance (ATR) accessory to obtain spectra of powdered samples in an FT-IR. The frequencies were expressed in wave numbers which have the units of reciprocal centimeter (cm^{-1}).

^{1}H-NMR Spectra

The ^{1}H-NMR spectra of synthesized compounds were studied using tetramethylsilane (TMS) as the internal reference with dimethyl sulfoxide (DMSO-d_6, Merck) as solvent at the Central Laboratory of Faculty of Pharmacy, Ankara University with Varian Mercury 400, 400 MHz. High Performance Digital FT-NMR spectrometer instrument and the chemical shifts were expressed in δ parts per million (ppm).

^{13}C-NMR Spectra

The ^{13}C-NMR spectra of synthesized compounds were studied using tetramethylsilane (TMS) as the internal reference with dimethyl sulfoxide (DMSO-d_6, Merck) as solvents at the Central Laboratory of Faculty of Pharmacy, Ankara University with Varian Mercury 400, 400 MHz. High Performance Digital FT-NMR spectrometer instrument and the chemical shifts were expressed in δ parts per million (ppm).

Mass Spectra

The Mass spectra of synthesized compounds were studied at the laboratory of Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, in Micromass ZQ LC-MS Spectrometer with ESI+ method.

Elemental analysis

The elemental analysis (C, H, N and S) of synthesized comounds were studied at the Central Laboratory of Faculty of Pharmacy, Ankara University with LECO CHN 932. The accuracy was 0.4%.

3.2. Biological Activities

3.2.1. Materials

COX Inhibitor Screening Assay kit (Cayman Chemical Company, 1180 East Ellsworth Road, Ann Arbor, Michigan 48108, USA, Catalog no: 560131) were used for biologic screening assays.

Kit Components

- COX-1 (Ovine)
- COX-2(human recombinant)
- Arachidonic acid (substrate 10mM)
- Heme (hemoglobin, for protein protection)
- Reaction Buffer (100 mM Tris-HCl, pH:8)
- PG Screening EIA Antiserum
- PG Screening AChE Tracer
- PG Screening EIA Standard
- EIA Buffer Concentrate (10X)
- Wash Buffer Concentrate(400X)
- Polysorbate 20
- Mouse Anti-Rabbit IgG Coated Plate
- 96-Well Cover Sheet
- Ellman's Reagent
- Hydrochloric acid
- Potassium hydroxide (0,1M)
- Dimethyl sulfoxide
- Stannous chloride

3.2.2. Method

In this study, COX-1 and COX-2 inhibititory activities of the 5-((7-methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazoles **(compounds 2a-d)** and 3-((7-methoxy-2-naphthyloxy)methyl)-4-subtituted-1,2,4-triazole-5-thiones

(compounds 3a-d) were evaluated by using COX inhibitor screening method in Hacettepe University Faculty of Medicine, Department of Biochemistry.

The COX (ovine) Inhibitor Screening Assay directly measures $PGF_{2\alpha}$ by stannous chloride reduction of COX-derived PGH_2 produced in the COX reaction. In this method as standard we used indomethacin and NS-398.

Preparation of test tubes

1. The test tubes for standard and sample are prepare in different concentrations in DMSO and 20µl solvent add to each test tube.
2. To measure the minimum activity (BC) of both enzymes, the test tubes containing enzymes heat in 100^0C for 3 min to inactivate the enzymes, to measure the maximum activity (IA), the test tubes containing active enzymes is preparing. To avoid the error arising on the enzyme in the possible effect of DMSO's, the DMSO should add at the beginning of the test to the A_{max} tubes.
3. 20µl arachidonic acid add to each test tubes.
4. The prepared test tubes incubate in water-bath for 2 min. The incubation was finishing by adding hydrochloric acid to each test tubes.
5. Remove the test tubes from water-bath and add stanous chloride to each test tubes to avoid the returning of PGH_2 to $PGF_{2\alpha}$.

Measurements

1. To the Enzyme-Linked ImmunoSorbent Assay (ELISA) wells, standards of prostaglandin (S1-S8), the synthesized compounds to be examined for activity (test), a maximum active (IA1 and IA2) and inactive (BC1 and BC2) enzymes samples from their test tubes are applied.
2. To the blank (Blk) wells is adding only Ellman's reagent.
3. The plate was covered by plastic film, to complete the reaction it incubates for 18 hours in room temperature on an orbital shaker.
4. To remove the non-specific binding materials, the plate was washed three times with washing solution.
5. Screening AChE tracer was added to the each well except non-specific binding and blank (Blk) wells.

6. Ellman's reagent was added to the each well and kept at room temperature for 60 min. Then the plate was read at a wavelength between 405 nm (Tecan ELISA Microplate Reader).

The color intensity is opposite to the amount of prostaglandin and therefore directly proportional to the inhibition. To obtain the absorbance of maximum binding (B_{max}), prostaglandin tracer and Ellman's reagent is adding to the B_{max} well (Figure 3.1).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	BC1	BC1	H	H	H	H	H	H	H
B	Blk	S2	S2	BC2	BC2	H	H	H	H	H	H	H
C	NSB	S3	S3	‡	‡	H	H	H	H	H	H	H
D	NSB	S4	S4	‡	‡	H	H	H	H	H	H	H
E	B_0	S5	S5	‡	‡	H	H	H	H	H	H	H
F	B_0	S6	S6	‡	‡	H	H	H	H	H	H	H
G	B_0	S7	S7	H	H	H	H	H	H	H	H	H
H	TA	S8	S8	H	H	H	H	H	H	H	H	H

[Blk (Blank); TA (Total activity); NSB (Non-Specific Binding); B_0 (Maximum Binging); S1-S8 (Standard 1-8); BC1 (Background COX-1); BC2 (Background COX-2); ‡ (100% Initial Activity Samples); H (COX Inhibitor Samples]

Figure 3.1. Sample plate format

To calculate the % inhibition values, all absorbance data should be divided to B_{max}. The data of standard solution (S1-S8) are plotted to the standard curve and calculate the concentration of prostaglandin. By subtracting the concentration of prostaglandin (BC) from concentration of prostaglandins, the value of (X) is putted the following equation.

$$\text{Activity \%} = 100 \times [IA-X] / IA$$

The IC_{50} values (the concentration of the test compound causing 50% inhibition) were calculated from the concentration-inhibition response curves (duplicated determination).

3.3. Molecular Docking

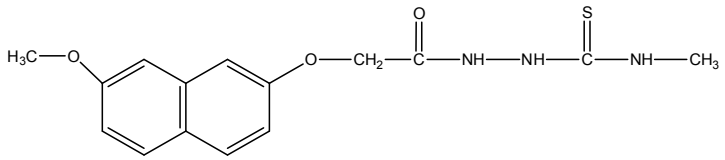
In this study, molecular docking studies were performed with MOE (The Molecular Operating Environment) Version 2011.10, software available from Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, Canada H3A2R7, http://www.chemcomp.com. The ligands were built using the

builder tool of the MOE program and subjected to energy minimization (MMFF94x, gradient: 0.05). The X-ray crystallographic structure of COX-2 complexed with 1-phenylsulfonamide-3-trifluoromethyl-5-(4-bromophenyl)pyrazole SC-558 (PDB: 1CX2) was obtained from the Protein Data Bank. The errors of the protein were corrected by the *Structure Preparation* process in MOE. After correction, hydrogens were added and partial charges (Gasteiger methodology) were calculated. The default Triangle Matcher placement method was used for docking. GBVI/WSA dG scoring function which estimates the free energy of binding of the ligand from a given pose was used to rank the final poses. The ligand–enzyme complex with lowest *S* score was selected.

4. EXPERIMENTAL

4.1. Chemical Data

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-methyl-3-thiosemicarbazide (Compound 1a)



2-(7-Methoxy-2-naphthyloxy)acetyl hydrazide 2.46g (0.01 mol) and methyl isothiocyanate 0.73g (0.01 mol) in ethanol were reacted according to the general synthesis method B and recrystallized from dioxane-water. The yield is 2.68g (84%).

The form of compound is white crystalline powder and the melting point is 193-195 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in acetone, dimethyl sulfoxide and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.26, 0.47, 0.71 respectively.

FT-IR (cm^{-1}); 3338, 3223 (N-H stretching); 3062, 3006 (C-H stretching, aromatic); 2965, 2935 (C-H stretching, aliphatic); 1699 (C=O stretching, amide); 1572 (C=C stretching, aromatic); 1474, 1384 (C-H bending, CH_3, CH_2); 1212 (C=S stretching); 1178, 1162 (C-O stretching, Ar-C-O) and 836, 812 (C-H bending, 1,2,4-trisubstituted benzene).

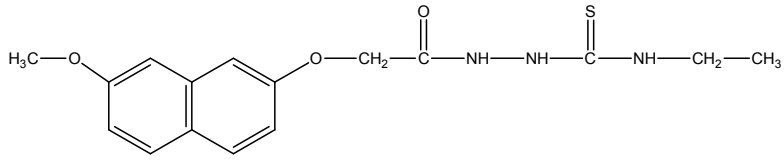
^{1}H-NMR (DMSO-d_6, ppm); δ 2.80 (3H, d, -NHC<u>H</u>$_3$); 3.85 (3H, s, -OC<u>H</u>$_3$); 4.66 (2H, s, -C<u>H</u>$_2$); 7.00-7.80 (6H, m, aromatic protons); 8.00 (1H, d, -N<u>H</u>CH$_3$); 9.32 (1H, s, NHN<u>H</u>CSNH) and 10.10 (1H, s, -N<u>H</u>NHCSNH).

MS (*m/z*); 320 $[M+H]^+$; 342 $[M+Na]^+$, 102 (100%), 175, 118, 87, 74, 57.

Elemental analysis; for $C_{15}H_{17}N_3O_3S$, (MW: 319.1 g/mol);

	C%	H%	N%	S%
Calculated:	56.41	5.37	13.16	10.04
Found:	56.01	5.36	13.24	9.97

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-ethyl-3-thiosemicarbazide (Compound 1b)



2-(7-Methoxy-2-naphthyloxy)acetyl hydrazide 1.23g (0.005 mol) and ethyl isothiocyanate 0.435g (0.005 mol) in ethanol were reacted according to the general synthesis method B and crystallized from n-hexane-ethyl acetate. The yield is 1.2g (72%).

The form of compound is white crystalline powder and the melting point is 185-187 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in dimethyl sulfoxide, acetone and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.25, 0.44, 0.74 respectively.

FT-IR (cm^{-1}); 3263, 3200 (N-H stretching), 3066, 3026 (C-H stretching, aromatic); 2968 (C-H stretching, aliphatic); 1694 (C=O stretching, amide); 1546 (C=C stretching, aromatic); 1464, 1387 (C-H bending, CH_3, CH_2); 1209 (C=S stretching); 1177,1026 (C-O stretching, Ar-C-O); 859, 832 (C-H bending, 1,2,4-trisubstituted benzene).

^{1}H-NMR (DMSO-d_6,ppm); 1.07 (3H, t,-NHCH$_2$C$\underline{H}_3$); 3.60 (2H, m, -NHC$\underline{H}_2$CH$_3$); 3.85 (3H, s, -OC$\underline{H}_3$); 4.67 (2H, s, -C$\underline{H}_2$); 7.00-7.80 (6H, m, aromatic protons); 8.02 (1H, t, -N$\underline{H}$CH$_2$CH$_3$); 9.25 (1H, s,-NHN$\underline{H}$CSNH) and 10.10 (1H, s, -N$\underline{H}$NHCSNH).

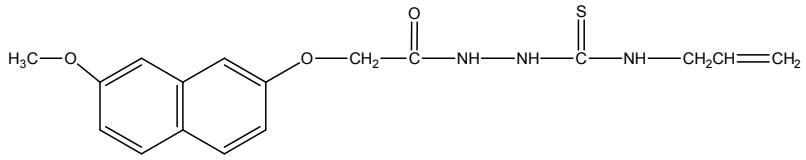
MS (*m/z*); 334 $[M+H]^+$; 355 $[M+Na]^+$, 269, 175, 118, 87 and 57 (100%).

Elemental analysis; for $C_{16}H_{19}N_3O_3S$, (MW: 333.41 g/mol);

	C%	H%	N%	S%
Calculated:	57.64	5.74	12.60	9.62
Found:	57.65	5.54	11.70	8.83

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-allyl-3-thiosemicarbazide

(Compound 1c)



2-(7-Methoxy-2-naphthyloxy)acetyl hydrazide 1.23g (0.005 mol) and allyl isothiocyanate 0.5g (0.005 mol) in ethanol were reacted according to the general synthesis method B and crystallized from cyclohexane-ethylacetate. The yield is 1.4g (81%).

The form of compound is white crystalline powder and the melting point is 188-190 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in acetone, dimethyl sulfoxide and chloroform at room temperature. Insoluble in diethyl ether, benzene, cyclohexane and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.23, 0.55, 0.77 respectively.

FT-IR (cm^{-1}); 3305, 3148 (N-H stretching); 3002 (C-H stretching, aromatic); 2965, 2939 (C-H stretching, aliphatic); 1695 (C=O stretching, amide); 1549, 1513 (C=C stretching, allyl and aromatic); 1480, 1383 (C-H bending, CH_3, CH_2); 1208 (C=S stretching); 1182, 1166 (C-O stretching, Ar-C-O); 860, 831 (C-H bending, 1,2,4-trisubstituted benzene).

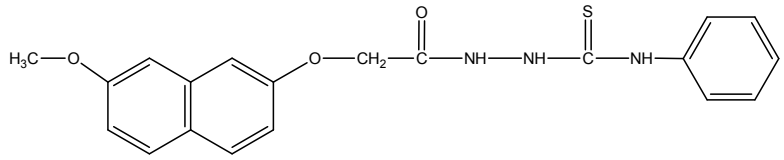
^{1}H-NMR (DMSO-d_6, ppm); 3.85 (3H, s, -OC$\underline{H}_3$); 4.10 (2H, t,-NHC$\underline{H}_2$CH=CH_2); 4.67 (2H, s, -C$\underline{H}_2$); 5.05 (1H, dd, -NHCH$_2$CH=C$\underline{H}_2$; H_A; J_{AB}: 1.6 Hz, J_{AX}: 10 Hz); 5.15 (1H, dd, -NHCH$_2$CH=C$\underline{H}_2$; H_b; J_{AB}: 1.6 Hz, J_{BX}: 17.2 Hz); 5.80 (1H, m, -NHCH$_2$C$\underline{H}$=CH_2); 7.00-7.80 (6H, m, aromatic protons); 8.20 (1H, bs,-N$\underline{H}$CH$_2$); 9.37 (1H, s, -NHN$\underline{H}$CSNH) and 10.16 (1H, bs, -N$\underline{H}$NHCSNH).

MS (*m/z*); 346 $[M+H]^+$; 368 $[M+Na]^+$, 311, 175, 118, 87 and 57 (100%).

Elemental analysis; for $C_{17}H_{19}N_3O_3S$, (MW: 345.42 g/mol);

	C%	H%	N%	S%
Calculated:	59.11	5.54	12.17	9.28
Found:	59.42	5.31	11.78	8.94

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-phenyl-3-thiosemicarbazide (Compound 1d)



2-(7-Methoxy-2-naphthyloxy)acetyl hydrazide 1.23g (0.005 mol) and phenyl isothiocyanate 0.675g (0.005 mol) in ethanol were reacted according to the general synthesis method B and crystallized from dioxane-water. The yield is 1.54g (81%).

The form of compound is white crystalline powder and the melting point is 170-172 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in acetone, dimethyl sulfoxide and chloroform at room temperature. Insoluble in carbon tetrachloride, diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.21, 0.62, 0.72 respectively.

FT-IR (cm^{-1}); 3560, 3440, 3184 (N-H stretching); 3041 (C-H stretching, aromatic); 3041 (C-H stretching, aliphatic); 1681 (C=O stretching, amide); 1633 (N-H bending); 1547 (C=C stretching, aromatic); 1469, 1395 (C-H bending, CH_3, CH_2), 1216 (C=S stretching); 1178, 1156 (C-O stretching, Ar-C-O); 867, 820, 746, 698 (C-H bending, 1,2,4-trisubstituted and monosubstituted benzene).

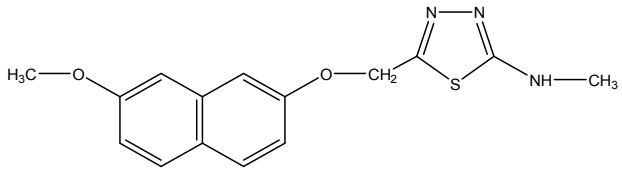
^{1}H-NMR (DMSO-d_6, ppm); 3.83 (3H, s, -OC<u>H</u>$_3$); 4.73 (2H, s, -C<u>H</u>$_2$); 7.00-7.80 (11H, m, aromatic protons); 9.71 (2H, s, -NHN<u>H</u>CSN<u>H</u>) and 10.36 (1H, s, -N<u>H</u>NHCSNH).

MS (*m/z*); 382 $[M+H]^+$; 404 $[M+Na]^+$, 311, 269, 229, 175, 105, 73 and 57 (100%).

Elemental analysis; for $C_{20}H_{19}N_3O_3S$, (MW: 381.45 g/mol);

	C%	H%	N%	S%
Calculated:	62.97	5.02	11.02	8.41
Found:	62.69	5.08	11.14	8.39

5-((7-Methoxy-2-naphthyloxy)methyl)-2-methylamino-1,3,4-thiadiazole (Compound 2a)



1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-methyl-3-thiosemicarbazide 0.319g (1 mmol) and phosphoryl chloride (5ml) were reacted according to the general synthesis method D and crystallized from acetone-water (3:1). The yield is 0.217g (68%).

The form of compound is white crystalline powder and the melting point is 147-149 ^{0}C. It is soluble in hot ethanol, methnol, ethyl acetate; freely soluble in dimethyl sulfoxide and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.37, 0.55, 0.65 respectively.

FT-IR (cm^{-1}); 3202 (N-H stretching); 3040 (C-H stretching aromatic); 2938 (C-H stretching aliphatic); 1634 (C=N stretching, thiadiazole); 1585 (C=C stretching, aromatic); 1454, 1409, 1347 (C-H bending, CH_3, CH_2); 1263, 1158 (C-O stretching, Ar-O-C); 871, 825 (C-H bending, 1,2,4-trisubstituted benzene).

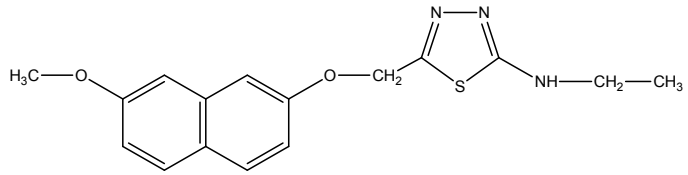
^{1}H-NMR (DMSO-d_6, ppm); 2.90 (3H, d, -C<u>H</u>$_3$); 3.90 (3H, s, -OC<u>H</u>$_3$); 5.40 (2H, s, -C<u>H</u>$_2$); 7.00-7.80 (6H, m, aromatic protons) and 7.90 (1H, q, N<u>H</u>).

MS (*m/z*); 302 $[M+H]^+$(100%); 324 $[M+Na]^+$, 175, 128, 101, 87 and 60.

Elemental analysis; for $C_{15}H_{15}N_3O_2S$, (MW: 301.36 g/mol);

	C%	H%	N%	S%
Calculated:	59.78	5.02	13.94	10.64
Found:	60.02	5.31	13.84	10.36

5-((7-Methoxy-2-naphthyloxy)methyl)-2-ethylamino-1,3,4-thiadiazole (Compound 2b)



1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-ethyl-3-thiosemicarbazide 0.333g (1 mmol) and phosphoryl chloride (5ml) were reacted according to the general synthesis method D and crystallized from acetone-water (3:1). The yield is 0.184g (55%).

The form of compound is white crystalline powder and the melting point is 160-162 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in dimethyl sulfoxide, acetone and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.38, 0.58, 0.72 respectively.

FT-IR (cm^{-1}); 3178 (N-H stretching); 3050 (C-H stretching, aromatic); 2967 (C-H stretching, aliphatic); 1633 (C=N stretching, thiadiazole); 1538 (C=C stretching, aromatic); 1469, 1453, 1344 (C-H bending, CH_3, CH_2); 1261, 1026 (C-O stretching, Ar-O-C); 871, 825 (C-H bending, 1,2,4-trisubstituted benzene).

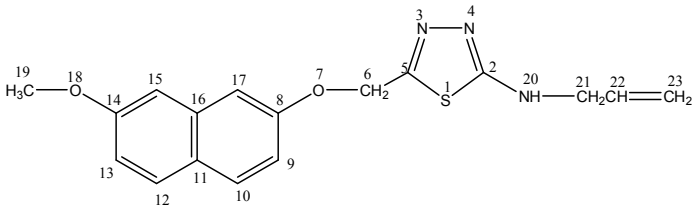
^{1}H-NMR (DMSO-d_6, ppm); 1.20 (3H, t, -$CH_2C\underline{H}_3$); 3.20 (2H, m, -$C\underline{H}_2CH_3$); 3.80 (3H, s, - $OC\underline{H}_3$); 5.40 (2H, s, -$C\underline{H}_2$); 7.00-7.80 (6H, m, aromatic protons) and 7.9 (1H, t, N$\underline{H}$).

MS (*m/z*); 316 $[M+H]^+$ (100%); 338 $[M+Na]^+$, 174, 142, 119, 87and 60.

Elemental analysis; for $C_{16}H_{17}N_3O_2S$, (MW: 315.39 g/mol);

	C%	H%	N%	S%
Calculated:	60.93	5.43	13.32	10.17
Found:	60.59	5.40	13.01	9.87

5-((7-Methoxy-2-naphthyloxy)methyl)-2-allylamino-1,3,4-thiadiazole (Compound 2c)



1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-allyl-3-thiosemicarbazide 0.345g (1 mmol) and phosphoryl chloride (5ml) were reacted according to the general synthesis method D and crystallized from acetone-water (3:1). The yield is 0.241g (70%).

The form of compound is white crystalline powder and the melting point is 158-160 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in dimethyl sulfoxide, acetone and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.35, 0.62, 0.76 respectively.

FT-IR (cm^{-1}); 3306 (N-H stretching); 3150, 3006 (C-H stretching, aromatic); 2960, 2940 (C-H stretching, aliphatic); 1696 (C=N stretching, thiadiazole); 1551, 1514 (C=C stretching, allyl, aromatic); 1384, 1300 (C-H bending, CH_3, CH_2, CH); 1260, 1056 (C-O stretching, Ar-O-C); 860, 832, 724 (C-H bending, 1,2,4-trisubstituted benzene).

^{1}H-NMR (DMSO-d_6, ppm); 3.70 (3H, s, -OC$\underline{H}_3$); 5.40 (2H, s, -C$\underline{H}_2$); 5.05 (1H, dd, -NHCH$_2$CH=C$\underline{H}_2$; H_A; J_{AB}:1.6 Hz, J_{AX}: 9.0 Hz); 5.15 (1H, dd, -NHCH$_2$CH=C$\underline{H}_2$; H_B; J_{AB}:1.6 Hz, J_{BX}:31.0 Hz); 5.90 (1H, m, -CH$_2$C$\underline{H}$=CH$_2$); 3.80 (2H,t, -C$\underline{H}_2$-CH=CH$_2$); 7.0-7.8 (6H, m, aromatic protons) and 8.0 (1H, t, N$\underline{H}$).

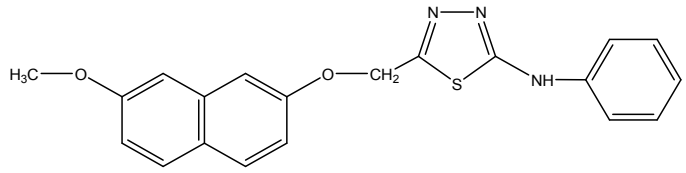
^{13}C-NMR (DMSO-d_6, ppm); 47.55 (C_{21}), 55.79 (C_{19}), 64.93 (C_6), 106.07 (C_{15}), 107.96 (C_{17}), 116.42 (C_{23}), 116.87 (C_9), 116.98 (C_{13}), 124.82 (C_{11}), 129.75 (C_{10}), 129.95 (C_{12}), 135.02 (C_{22}), 136.18 (C_{16}), 154.47 (C_{14}), 156.57 (C_8), 158.55 (C_5, thiadiazole), 170.38 (C_2, thiadiazole).

MS (*m/z*); 328 [M+H]$^+$ (100%); 350 [M+Na]$^+$, 174, 154, 102, 88 and 60.

Elemental analysis; for $C_{17}H_{17}N_3O_2S$, (MW: 327.31 g/mol);

	C%	H%	N%	S%
Calculated:	62.36	5.23	12.83	9.79
Found:	61.85	5.21	12.38	9.40

5-((7-Methoxy-2-naphthyloxy)methyl)-2-phenylamino-1,3,4-thiadiazole (Compound 2d)



1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-phenyl-3-thiosemicarbazide 0.381g (1 mmol) and phosphoryl chloride (5ml) were reacted according to the general synthesis method D and crystallized from acetone-water (3:1). The yield is 0.217g (68%).

The form of compound is white crystalline powder and the melting point is 210-212 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in acetone, dimethyl sulfoxide and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.43, 0.68, 0.87 respectively.

FT-IR (cm^{-1}); 3258 (N-H stretching); 3194, 3135 (C-H stretching, aromatic); 3053 (C-H stretching, aliphatic); 1633 (C=N stretching, thiadiazole); 1556 (C=C stretching, aromatic); 1495, 1446 (C-H bending, CH_3, CH_2); 1216, 1027 (C-O stretching, Ar-O-C); 874, 830, 751 (C-H bending, 1,2,4-trisubstituted benzene, monosubstituted benzene).

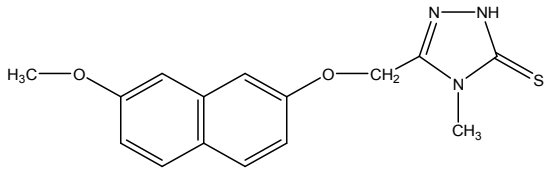
^{1}H-NMR (DMSO-d_6, ppm); 3.90 (3H, s, -OC<u>H</u>$_3$); 5.40 (2H, s, -C<u>H</u>$_2$); 7.00-7.90 (11H, m, aromatic protons) and 10.40 (1H, s, N<u>H</u>).

MS (*m/z*); 364 $[M+H]^+$; 386 $[M+Na]^+$ (100%), 218, 190, 136, 73 and 56.

Elemental analysis; for $C_{20}H_{17}N_3O_2S$, (MW: 363.43 g/mol);

	C%	H%	N%	S%
Calculated:	66.10	4.71	11.56	8.82
Found:	66.15	4.60	11.61	8.71

3-((7-Methoxy-2-naphthyloxy)methyl)-4-methyl-1,2,4-triazole-5-thione (Compound 3a)



1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-methyl-3-thiosemicarbazide 0.638g (0.002 mol) and 1N sodium hydroxide (40ml) were reacted according to the general synthesis method E and crystallized from ethanol. The yield is 0.350g (55%).

The form of compounds is white crystalline powder and the melting point is 221-223 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in acetone, dimethyl sulfoxide and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.62, 0.75, 0.81 respectively.

FT-IR (cm^{-1}); 3153 (N-H stretching, triazole); 3052 (C-H stretching, aromatic); 2921, 2765 (C-H stretching, aliphatic); 1633 (C=N stretching, triazole); 1585 (C=C stretching); 1395 (C-N stretching, triazole); 1396, 1341 (C-H bending, CH_3, CH_2); 1258 (C=S stretching); 1215, 1158 (C-O stretching, Ar-O-C); 825, 792 (C-H bending, 1,2,4-trisubstituted benzene).

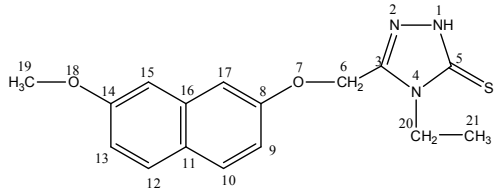
^{1}H-NMR (DMSO-d_6, ppm); 3.50 (3H, s, -C<u>H</u>$_3$), 3.90 (3H, s, -OC<u>H</u>$_3$), 5.30 (2H, s, -C<u>H</u>$_2$), 7.00-7.80 (6H, m, aromatic protons) and 13.85 (1H, s, N<u>H</u>, triazole).

MS (*m/z*); 302 $[M+H]^+$; 324 $[M+Na]^+$ (100%), 302, 175, 102, 87, 71 and 57.

Elemental analysis; for $C_{15}H_{15}N_3O_2S$, (MW: 301.36 g/mol);

	C%	H%	N%	S%
Calculated:	59.78	5.02	13.94	10.64
Found:	59.67	5.22	13.85	10.47

3-((7-Methoxy-2-naphthyloxy)methyl)-4-ethyl-1,2,4-triazole-5-thione (Compound 3b)



1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-ethyl-3-thiosemicarbazide 0.666g (0.002 mol) and 1N sodium hydroxide (40ml) were reacted according to the general synthesis method E and crystallized from ethanol. The yield is 0.395g (59.3%).

The form of compound is white crystalline powder and the melting point is 204-206 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in acetone, dimethyl sulfoxide and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.53, 0.7, 0.8 respectively.

FT-IR (cm^{-1}); 3091 (N-H stretching, triazole); 3050 (C-H stretching, aromatic); 2911, 2762 (C-H stretching, aliphatic); 1633 (C=N stretching, triazole); 1606, 1582 (N-H bending, C=C stretching, aromatic); 1497, 1462, 1350 (C-H bending, CH_3, CH_2); 1367 (C-N stretching, triazole); 1257 (C=S stretching); 1213, 1029 (C-O stretching, Ar-O-C); 826, 787 (C-H bending, 1,2,4-trisubstituted benzene).

^{1}H-NMR (DMSO-d$_6$, ppm); 1.30 (3H, t, -$CH_2C\underline{H}_3$); 3.90 (3H, s, -$OC\underline{H}_3$); 4.00 (2H, q, -$C\underline{H}_2CH_3$); 5.40 (2H, s, -$C\underline{H}_2$); 7.00-7.80 (6H, m, aromatic protons) and 13.90 (1H, s, N$\underline{H}$, triazole).

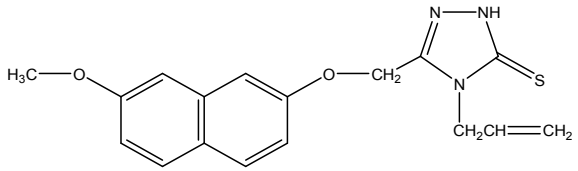
^{13}C-NMR (DMSO-d$_6$, ppm); 14.09 (C_{21}); 41.00 (C_{20}); 55.82 (C_{19}); 60.66 (C_6); 106.08 (C_{15}); 107.95 (C_{17}); 116.15 (C_9); 117.14 (C_{13}); 124.93 (C_{11}); 129.82, 130.06 (C_{10}, C_{12}); 136.15 (C_{16}); 148.59 (C_3 triazole); 156.41 (C_{14}); 158.61 (C_8); 167.87 (C_5 triazole).

MS (*m/z*); 316 $[M+H]^+$; 338 $[M+Na]^+$, 301, 175, 142, 102, 87, 74 and 56 (100%).

Elemental analysis; for $C_{16}H_{17}N_3O_2S$, (MW: 315.39 g/mol);

	C%	H%	N%	S%
Calculated:	60.93	5.43	13.32	10.17
Found:	60.83	5.38	13.09	9.90

3-((7-Methoxy-2-naphthyloxy)methyl)-4-allyl-1,2,4-triazole-5-thione (Compound 3c)



1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-allyl-3-thiosemicarbazide 0.690g (0.002 mol) and 1N sodium hydroxide (40ml) were reacted according to the general synthesis method E and crystallized from ethanol. The yield is 0.328g (47.5%).

The form of compound is white crystalline powder and the melting point is 148-150 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in acetone, dimethyl sulfoxide and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.46, 0.71, 0.84 respectively.

FT-IR (cm^{-1}); 3092 (N-H stretching, triazole); 3049 (C-H stretching, aromatic); 2924, 2763 (C-H stretching, aliphatic); 1633 (C=N stretching, triazole); 1583 (C=C stretching); 1394 (C-H bending); 1349 (C-N stretching, triazole); 1229 (C=S stretching); 1212, 1158 (C-O stretching, Ar-O-C); 827, 760 (C-H bending, 1,2,4-trisubstituted benzene).

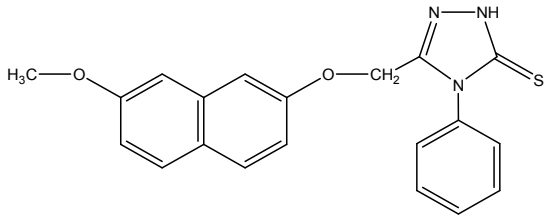
^{1}H-NMR (DMSO-d$_6$, ppm); 3.85 (3H, s, -OC$\underline{H}_3$); 4.70 (2H, d, -C$\underline{H}_2$CH=CH$_2$); 5.10 (1H, dd, -NHCH$_2$CH=C$\underline{H}_2$; H_A; J_{AB}: 1.6 Hz, J_{AX}: 17 Hz); 5.20 (1H, dd, -NHCH$_2$CH=C$\underline{H}_2$; H_B; J_{AB}: 1.2 Hz, J_{BX}: 10.2 Hz); 5.30 (2H, s, -C$\underline{H}_2$); 5.90 (1H, m, -CH$_2$C$\underline{H}$=CH$_2$); 7.00-7.80 (6H, m, aromatic protons) and 13.95 (1H, s,N$\underline{H}$ triazole).

MS (*m/z*); 328 [M+H]$^+$; 349 [M+Na]$^+$, 301, , 175, 154, 102 (100%), 87, 74 and 58.

Elemental analysis; for $C_{17}H_{17}N_3O_2S$, (MW: 327.4 g/mol).

	C%	H%	N%	S%
Calculated:	62.36	5.23	12.83	9.79
Found:	62.08	5.25	12.72	9.63

3-((7-Methoxy-2-naphthyloxy)methyl)-4-phenyl-1,2,4-triazole-5-thione (Compound 3d)



1-(2-(7-Methoxy-2-naphthoxy)acetyl)-4-methylthiosemicarbazide 0.381g (0.001 mol) and 1N sodium hydroxide (40ml) were reacted according to the general synthesis method E and crystallized from ethanol. The yield is 0.245g (55%).

The form of compound is white crystalline powder and the melting point is 191-193 ^{0}C. It is soluble in hot ethanol, methanol, ethyl acetate; freely soluble in acetone, dimethyl sulfoxide and chloroform at room temperature. Insoluble in diethyl ether, benzene and water.

R_f values in TLC at S-1, S-2 and S-3 solvent systems are 0.38, 0.67, 0.86 respectively.

FT-IR (cm^{-1}); 3080 (N-H stretching, triazole); 3034 (C-H stretching, aromatic); 2909, 2757 (C-H stretching, aliphatic); 1627 (C=N stretching, triazole); 1494 (C=C stretching, aromatic); 1386 (C-N stretching, triazole); 1330 (C-H bending); 1254 (C=S stretching); 1210, 1026 (C-O stretching, Ar-O-C); 840, 760 (C-H bending, 1,2,4-trisubstituted benzene, monosubstituted benzene).

^{1}H-NMR (DMSO-d_6, ppm); 3.85 (3H, s, -OC<u>H</u>$_3$); 5.05 (2H, s, -C<u>H</u>$_2$); 6.80-7.70 (11H, m, aromatic protons) and 14.10 (1H, s, N<u>H</u>, triazole).

MS (*m/z*); 363 $[M+H]^+$ (100%); 386 $[M+Na]^+$, 335, 301, 190 , 175, 104, 87 and 73.

Elemental analysis; for $C_{20}H_{17}N_3O_2S$, (MW: 363.43 g/mol);

	C%	H%	N%	S%
Calculated:	66.10	4.71	11.56	8.82
Found:	65.73	5.22	11.64	8.83

4.2. Biological Activities

The inhibitory effects of the eight synthesized compounds on COX-1 and COX-2 enzymes were evaluated. NS-398 and indomethacin were used as reference compounds. The results are given in Table 4.1.

Table 4.1. In vitro COX-1 and COX-2 enzyme inhibition data for the synthesized compounds

Compound	R	COX-1 $IC_{50}(\mu M)^a$	COX-2 $IC_{50}(\mu M)^a$	Selectivity Index (SI^b)
2a	CH_3	>250	>250	n.d.[c]
2b	C_2H_5	>250	150.2	>1.6
2c	C_3H_5	>250	230.2	>1.1
2d	C_6H_5	136.3	>250	<0.5
3a	CH_3	154.5	>250	<0.6
3b	C_2H_5	45.6	176.5	0.3
3c	C_3H_5	31.8	>250	<0.1
3d	C_6H_5	166.4	>250	<0.7
NS-398		213.2	2.1	101.5
Indomethacin		0.67	18.5	0.036

[a] The in vitro test compound concentration required to produce 50% inhibition of enzymatic activity. The result (IC_{50}, μM) is the mean of two determinations acquired using the COX Inhibitor Screening Assay Kit (Catalog No. 560131, Cayman Chemicals Inc., Ann Arbor, MI, USA)
[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).
[C] Not determined

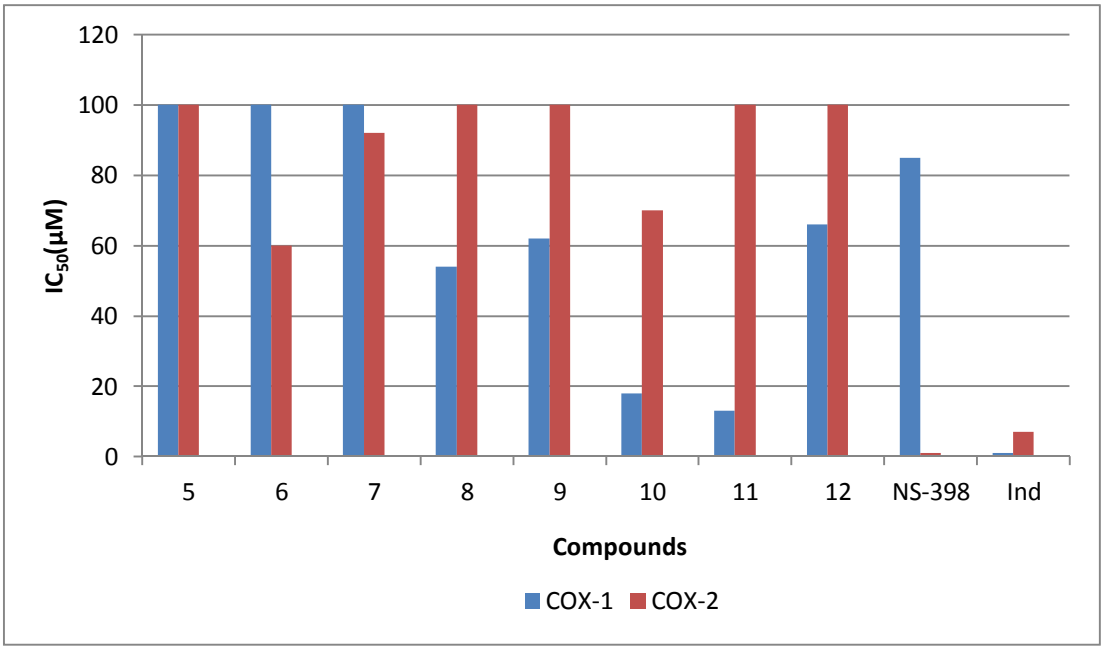


Figure 4.1. The IC_{50}(µM) values of COX-1 and COX-2 enzymes

4.3. Molecular Docking

Docking studies were performed with **compound 2b** and COX-2 enzyme. The orientation and interactions of the most energetically favored conformation of **compound 2b** in COX-2 active side are showed in **Figure 4.2**. Docking studies showed that the naphthyl ring of the **compound 2b** fitted into the hydrophobic cavity formed Val349, Tyr355, Leu359 and Leu531. A hydrogen bond occurred between C=O moiety of Leu352 and CH_2. Although thiadiazole moiety did not fill the adjunct pocket, but the thiadiazole ring formed an arene-cation interaction with His90.

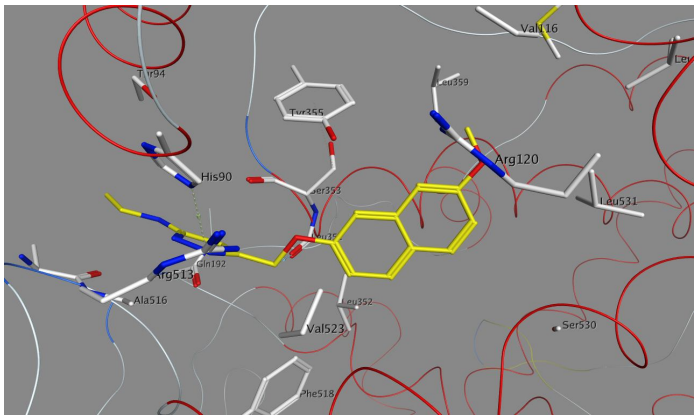


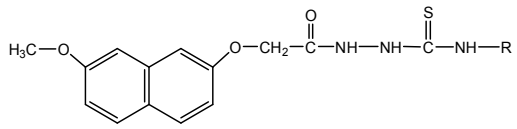
Figure 4.2. The orientation of **compound 2b** in COX-2 active site (**compound 2b** is shown as yellow, residues are shown as white)

5. DISCUSSION

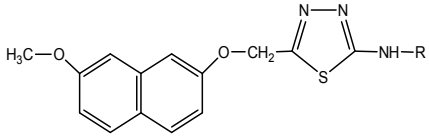
In this study, twelve new 1-(2-(7-methoxy-2-naphthyloxy)acetyl)-4-substituted-3-thiosemicarbazide (**Compounds 1a-d**), and corresponding 5-((7-methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazole (**Compounds 2a-d**) and 3-((7-methoxy-2-naphthyloxy)methyl)-4-subtituted-1,2,4-triazole-5-thione derivatives (**Compounds 3a-d**) were synthesized (**Table 5.1**).

All the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra and elemental analysis. Also, the IC_{50} (μM) values of COX-1 and COX-2 enzymes and indices of selectivity of 2-substitutedamino-1,3,4-thiadiazoles and 4-subtituted-1,2,4-triazole-5-thiones were counted.

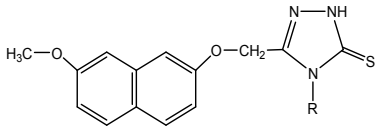
Table 5.1. Melting points, yields % and cyrstallization solvents of synthesized compounds



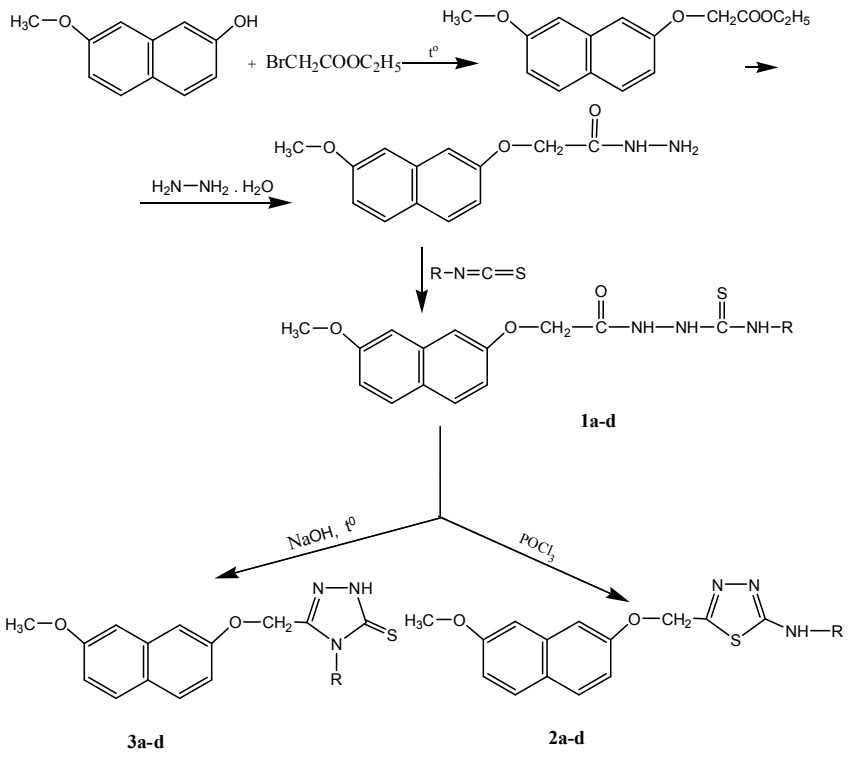
Compound	R	Melting Point (°C)	Yield %	Cryst. Solvents
1a	$-CH_3$	193-195	84	Dioxane-Water
1b	$-C_2H_5$	185-187	72	n-Hexane-Ethylacetate
1c	$-C_3H_5$	188-190	81	Cyclohexane-Ethylacetate
1d	$-C_6H_5$	170-172	81	Dioxane-Water



2a	$-CH_3$	147-149	68	Acetone-Water
2b	$-C_2H_5$	160-162	55	Acetone-Water
2c	$-C_3H_5$	158-160	70	Acetone-Water
2d	$-C_6H_5$	210-212	68	Acetone-Water



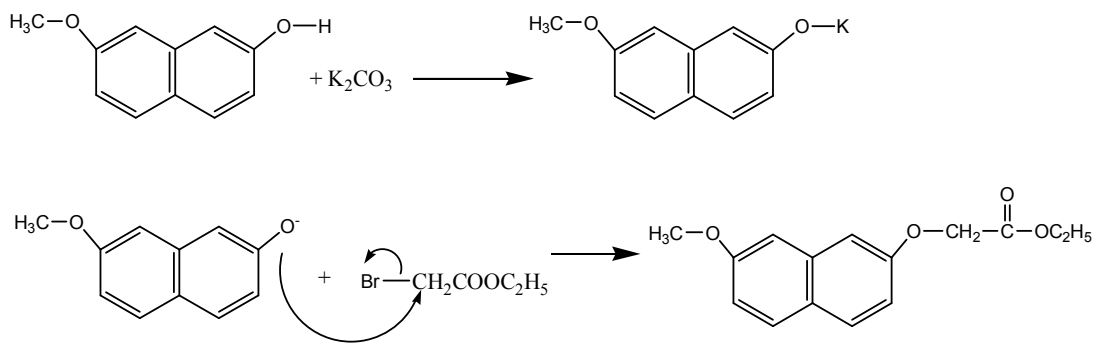
3a	$-CH_3$	221-223	55	Ethanol
3b	$-C_2H_5$	204-206	59.3	Ethanol
3c	$-C_3H_5$	148-150	47.5	Ethanol
3d	$-C_6H_5$	191-193	55	Ethanol

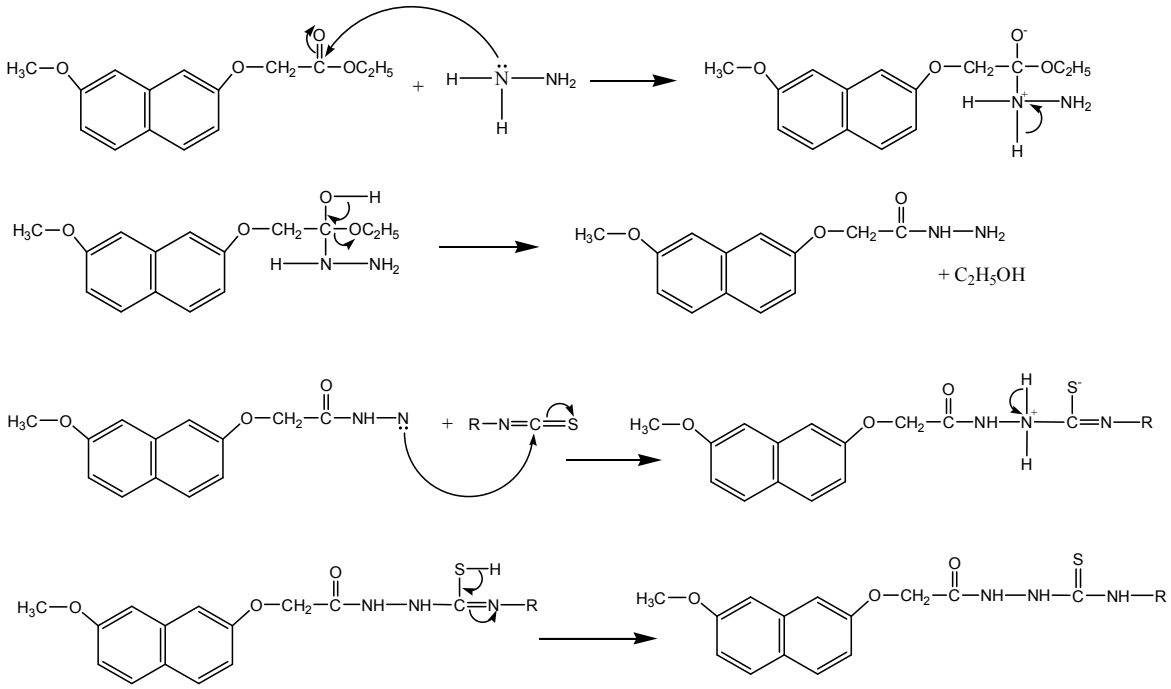


R: Methyl, ethyl, allyl and phenyl

Scheme 5.1. General synthesis pathway of the compounds

According to the synthesis method (**Scheme 5.1**), the starting material 7-methoxy-2-naphthol was reacted with ethylbromoacetate in presence of anhydrous potassium carbonate and heated about 6 h. in the oil-bath. The resulting product ethyl-2-(7-methoxy-2-naphthyloxy)acetate was reacted with hydrazine monohydrate to yield 2-(7-methoxy-2-naphthyloxy)acetylhydrazine. The mechanism of this reaction proposed as follows:



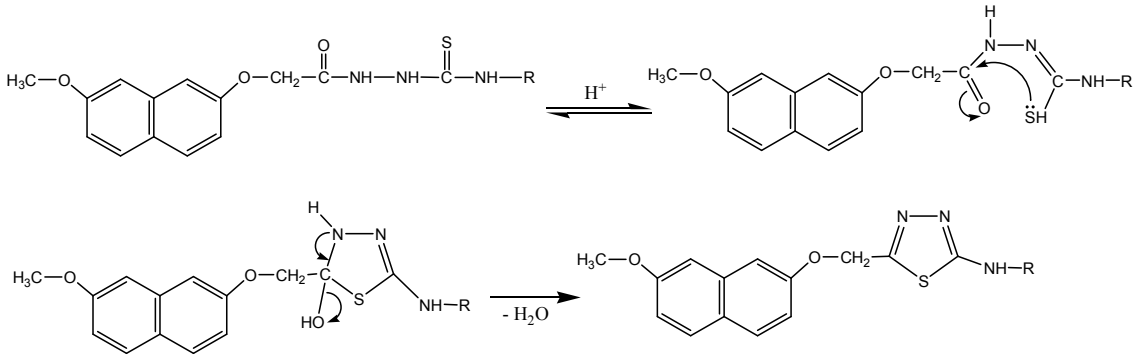


R: Aryl, alkyl, allyl

There are various methods of synthesis to obtain of 2-substitutedamino-5-substituted-1,3,4-thiadiazoles in literature. Among them two methods were used preferably. According to the first method, thiosemicarbazide derivatives were dissolved in toluene and methanesulfonic acid was added dropwise and refluxed for 50 min. The precipiated product which obtained was filtered, dried and recrystallized from suitable solvents.

Based on the second method, phosphoryl chloride ($POCl_3$) was added to the thiosemicarbazide derivatives and refluxed for 2-3 h. in oil-bath at 70^0C. The resulting solution was slowly and carefully poured into ice-water. The precipiated product which obtained was filtered, dried and recrystallized from suitable solvents.

We tried two reported methods to obtain target compounds. At the end we preferred the second method to synthesize 5-(7-methoxy-2-naphthyloxy)methyl-2-substituted amino-1,3,4-thiadiazole derivatives, because the synthetic processes are easier and work up was easy. Additionally, the products were pure and obtain in good yields. Mechanism of this reaction proposed to be as follows:



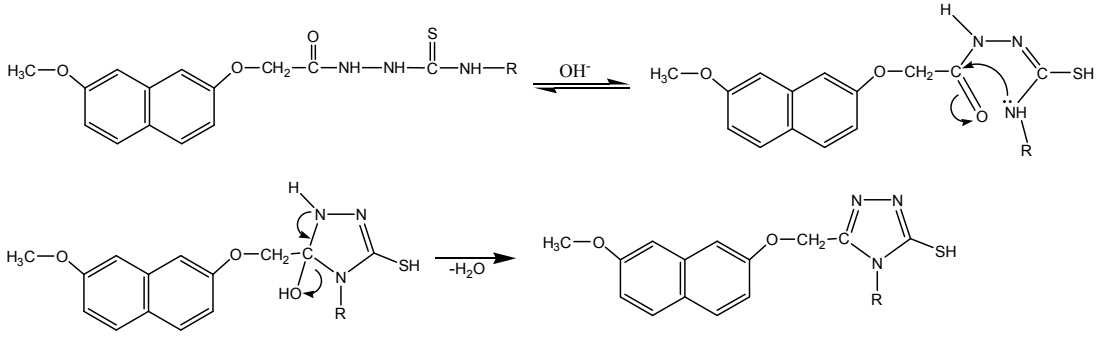
R: Aryl, alkyl, allyl

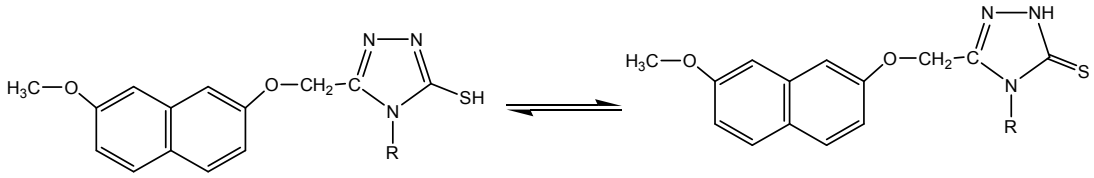
In literature, 3,4-disubstituted-1,2,4-triazole-5-thione derivatives were synthesized using different methods, but two methods were used frequently than the others.

According to the first method, thiosemicarbazide derivatives were dissolved in 1N sodium hydroxide and refluxed for 8 h on water-bath. The mixture was acidified to pH 2 with 1N hydrochloric acid. Then the acidic solution was extracted with ethylacetate 3 times, the organic phase was dried with anhydrous sodium sulfate. The excess solvent was filtered and removed under reduced pressure and the residue was recrystallized from suitable solvents.

Based on the second method, hydrazine derivatives were dissolved in methanol and isothiocyanate derivatves were added and refluxed for 10 h on water-bath. The precipitated products were filtered, dried and recrystallized from suitable solvents.

We synthesized 3-(7-methoxy-2-naphthyloxy)methyl-4-substituted-1,2,4-triazole-5-thione derivatives with the first method. In this method, the purification and crystallization processes were easy. Additionally, the products were pure and obtain in good yields. Mechanism of this reaction is suggested as:





R: Aryl, alkyl, allyl

The chemical structures of 1-(2-(7-methoxy-2-naphthyloxy)acetyl)-4-substituted-3-thiosemicarbazides (**Compounds 1a-d**), 5-((7-methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazoles (**Compounds 2a-d**) and 3-((7-methoxy-2-naphthyloxy)methyl)-4-subtituted-1,2,4-triazole-5-thiones (**Compounds 3a-b**) were characterized and identified by IR, ^{1}H-NMR, ^{13}C-NMR, Mass (ESI+) spectra and elemental analysis.

All spectral data were in accordance with the assumed structures.

In the IR spectra of 1-(7-methoxy-2-naphthyloxy)acetylhydrazide N-H stretching bands were seen between 3320–3200 cm^{-1}. Other stretching bands were observed at 3024 cm^{-1} (C-H stretching aromatic), 2917 cm^{-1} (C-H stretching aliphatic), 1667 and 1630 cm^{-1} (C=O and C=C stretching) respectively.

1-(2-(7-Methoxy-2-naphthyloxy)acetyl-4-substituted-3-thiosemicarbazide derivatives (**Compounds 1a-d**) have N-H stretching bands at 3560-3300 cm^{-1}, C=O and C=S stretching bands at 1700-1680 and 1260-1210 cm^{-1} respectively.

In the IR spectra of 5-((7-methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazole derivatives **(Compounds 2a-d)** absorption bands were seen as expected. N-H stretching bands were observed at 3300-3200 cm^{-1}. Aromatic and aliphatic C-H stretching bands were seen at 3100-3000 and 2960-2900 cm^{-1} respectively. 1,3,4-Thiadiazole derivatives have C=N stretching bands at 1700-1630 and aromatic C=C stretching absorption bands at 1580-1500 cm^{-1}. C-S stretching and C-H bending bands of 1,2,4-trisubstituted benzene were seen at 1100-1000 and 840-820 cm^{-1} respectively.

3-((7-Methoxy-2-naphthyloxy)methyl)-4-substituted-1,2,4-triazole-5-thione derivatives **(Compounds 3a-d)** have N-H stretching bands at 3200-3150 cm^{-1}. Aromatic C-H stretching bands were observed at 3050-3000 cm^{-1}, aliphatic C-H, C=C and C=N stretching bands were seen at 2950-2900, 1640-1630 and 1520-1500

cm^{-1} respectively. All 1,2,4-triazole-5-thione derivatives have C-S stretching bands at 1030-1025 cm^{-1} and 1,2,4-trisubstituted benzene C-H bending bands at 850-820 cm^{-1}.

IR spectrum of **Compound 2c** represents the IR absorption bands of 5-((7-methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazole derivatives (**Figure 5.1**). N-H stretching band was observed at 3306 cm^{-1}. Other bands were observed at 3150, 3006 (C-H stretching aromatic); 2960, 2940 (C-H stretching aliphatic); 1696 (C=N stretching); 1551, 1484 (C=C stretching, allylic and aromatic); 1208, 1021 (C-O stretching, Ar-O-C); 1056 (C-S stretching); 860, 832 cm^{-1} (C-H bending, 1,2,4-trisubstituted benzene).

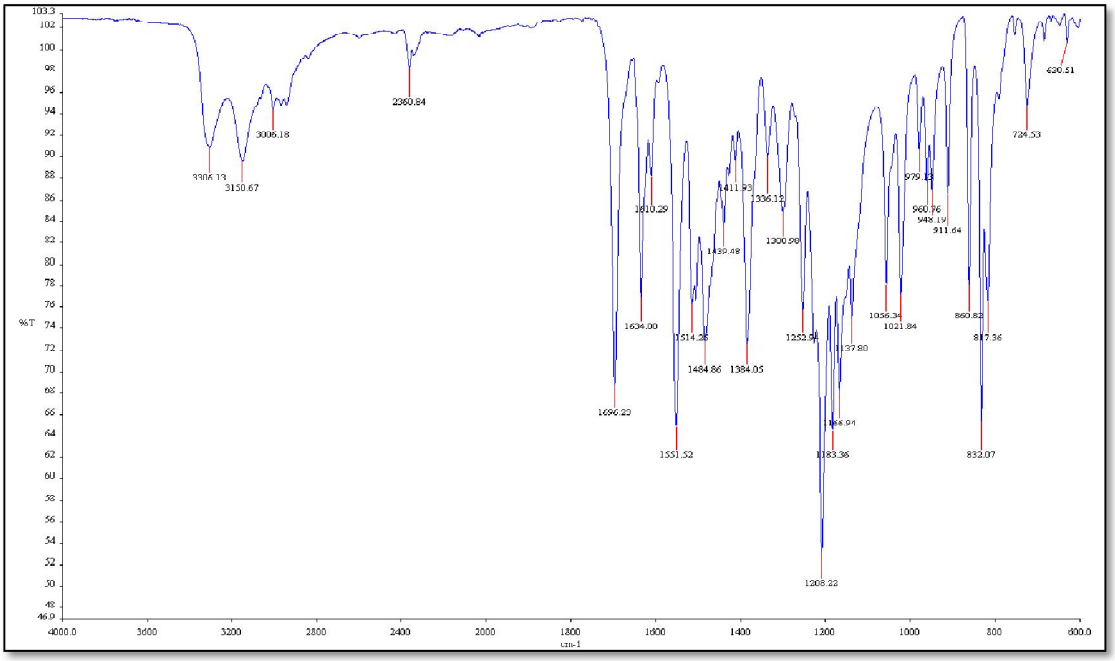


Figure 5.1. The IR spectrum of **Compound 2c**

IR spectrum (cm^{-1}) of **Compound 3b** represents the IR absorption bands of 3-((7-methoxy-2-naphthyloxy)methyl)-4-substituted-1,2,4-triazole-5-thione derivatives (**Figure 5.2**). In the spectra, N-H stretching band was observed at 3091 cm^{-1}. Other absorption bands were observed at 3050 (C-H stretching, aromatic); 2911 (C-H stretching, aliphatic); 1633 (C=N stretching); 1606, 1582 (N-H bending, C=C stretching, aromatic); 1497, 1462, 1350 (C-H bending, CH_3, CH_2); 1367 (C-N stretching, triazole); 1257 (C=S stretching); 1213, 1029 (C-O stretching, Ar-O-C); 826, 787 cm^{-1} (C-H bending, 1,2,4-trisubstituted benzene).

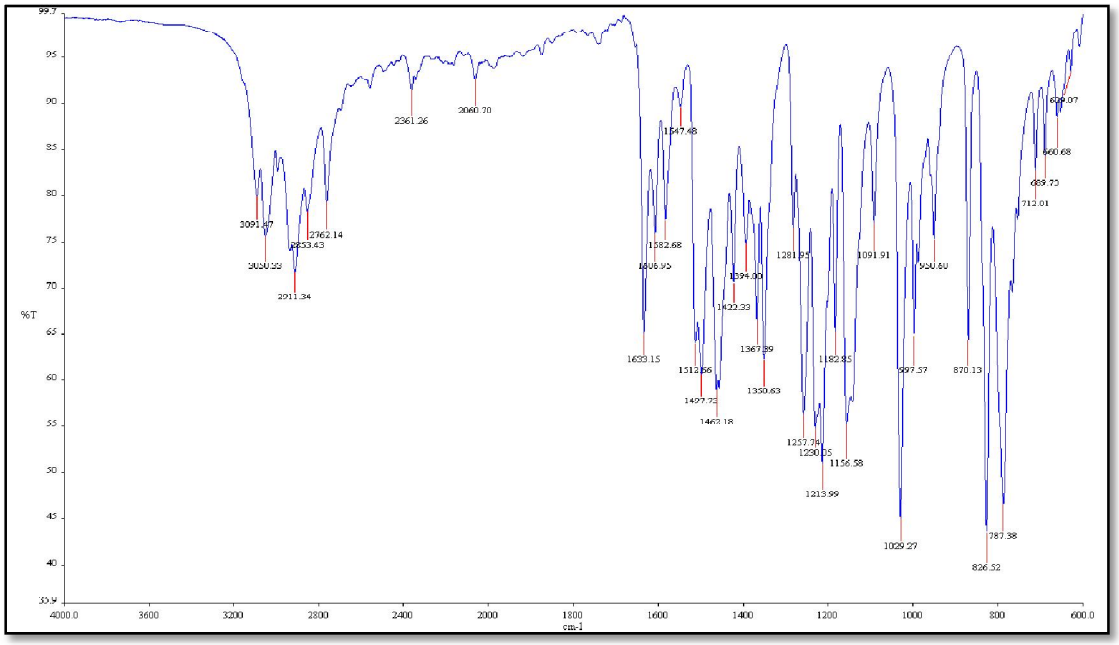


Figure 5.2. The IR spectrum of **Compound 3b**

In the ^{1}H-NMR spectra of the synthesized compounds, all protons were seen according to the expected chemical shift and integral values.

In the ^{1}H-NMR spectra of the 1-(7-methoxy-2-naphthyloxy)methyl)-4-substituted-3-thiosemicarbazide derivatives (**Compounds 1a-d)**, methoxy protons were observed as singlet at 3.85-3.90 ppm. The methylenic protons of aryloxymethyl groups were seen as a singlet at 4.66-4.73 ppm. The aromatic protons of naphthyl and phenyl groups were observed as multiplet at 7.00-7.80 ppm. While the N_4-H protons of thiosemicarbazide were seen at 8.00-8.20 ppm, N_2-H and N_1-H protons were observed as singlet at 9.25-9.71 and 10.10-10.36 ppm respectively. Methyl protons were seen as a doublet at 2.80 (NH-C<u>H</u>$_3$), the methylene and methyl protons of ethyl substituent (NH-C<u>H</u>$_2$-C<u>H</u>$_3$) were observed as a multiplet at 3.60 and as a triplet at 1.07 ppm respectively. The methylenic protons (NH-C<u>H</u>$_2$-CH=CH$_2$) of allyl group adjacent to the N_4 of thiosemicarbazide were seen as a triplet at 4.10 ppm. Vinylic methylene (H_A ve H_B) and methine protons (H_X) (NH-CH$_2$-C<u>H</u>=C<u>H</u>$_2$) of allyl substituent were observed as doublet of doublet at 5.04 (H_A) and 5.15 (H_B) and as a triplet at 5.80 (H_X) ppm respectively (H_A; J_{AB}: 1.6 Hz, J_{AX}: 10 Hz; H_B; J_{AB}: 1.6 Hz, J_{BX}: 17.2 Hz).

In the ^{1}H-NMR spectra of the 5-((7-methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazoles (**Compounds 2a-d),** methoxy protons were

observed as singlet at 3.70-3.90 ppm. The methylenic protons of aryloxymethyl groups were seen as a singlet at 5.40-5.50 ppm. The aromatic protons of naphthyl and phenyl groups were observed as multiplet at 7.00-7.80 ppm. The proton of N-H adjacent to the thiadiazole ring were seen at 7.80-10.40 ppm. Methyl protons were seen as a doublet at 2.90 (NH-C$\underline{H}_3$), the methyl and methylene protons of ethyl substituent (NH-C$\underline{H}_2$-C$\underline{H}_3$) were observed as a triplet at 1.20 and as a multiplet at 3.20 ppm respectively. The methylenic bridge protons (NH-C$\underline{H}_2$-CH=CH$_2$) of allyl group were seen as a triplet at 3.80 ppm. Vinylic methylene (H_A ve H_B) and methine protons (H_X) (NH-CH$_2$-C$\underline{H}$=C$\underline{H}_2$) of allyl substituent were observed as doublet of doublet at 5.10 (H_A) and 5.20 (H_B) and as a triplet at 5.90 (H_X) ppm respectively (H_A; J_{AB}: 1.6 Hz, J_{AX}: 9.0 Hz; H_B; J_{AB}: 1.6 Hz, J_{BX}: 31.0 Hz).

In the ^{1}H NMR spectra of 3-((7-methoxy-2-naphthyloxy)methyl)-4-subtituted-1,2,4-triazole-5-thiones **(Compounds 3a-d**) all protons were seen according to the expected chemical shift and integral values. The protons of methoxy substituent were observed as singlet at 3.80-3.90 ppm. Methylenic protons of aryloxymethyl groups were seen as a singlet at 5.10-5.30 ppm. The aromatic protons of naphthyl and phenyl groups were observed as multiplet at 7.00-7.80 ppm. The N-H protons of 1,2,4-triazole ring were seen as a singlet at 13.90-14.10 ppm. While the protons for methyl substituent were seen as a singlet at 3.50 ppm, methyl end methylene protons of ethyl substituent (NH-C$\underline{H}_2$-C$\underline{H}_3$) were observed as a triplet at 1.30 and as a quartet at 4.10 ppm respectively. The methylenic protons (NH-C$\underline{H}_2$-CH=CH$_2$) of allyl group adjacent to the triazole ring were seen as a triplet at 4.70 ppm. Vinylic methylene (H_A ve H_B) and methine protons (H_X) (NH-CH$_2$-C$\underline{H}$=C$\underline{H}_2$) of allyl substituent were observed as doublet of doublet at 5.10 (H_A) and 5.20 (H_B) and as a triplet at 5.90 (H_X) ppm respectively (H_A; J_{AB}: 1.2 Hz, J_{AX}: 17.0 Hz; H_B; J_{AB}: 1.2 Hz, J_{BX}: 10.2 Hz).

^{1}H-NMR spectra of 1,3,4-thiadiazole derivatives were represented with **Compound 2c** (Figure 5.3). The protons were observed at δ 3.70 (3H, s, -OC$\underline{H}_3$); 3.80 (2H, t, -C$\underline{H}_2$CH=CH$_2$); 5.10 (1H, dd, -CH$_2$CH=C$\underline{H}_2$); 5.20 (1H, dd, -CH$_2$CH=C$\underline{H}_2$); 5.40 (2H, s, -C$\underline{H}_2$); 5.90 (1H, m, -CH$_2$C$\underline{H}$=CH$_2$); 7.00-7.80 (6H, m, aromatic protons) and at 8.00 (1H, t, N$\underline{H}$).

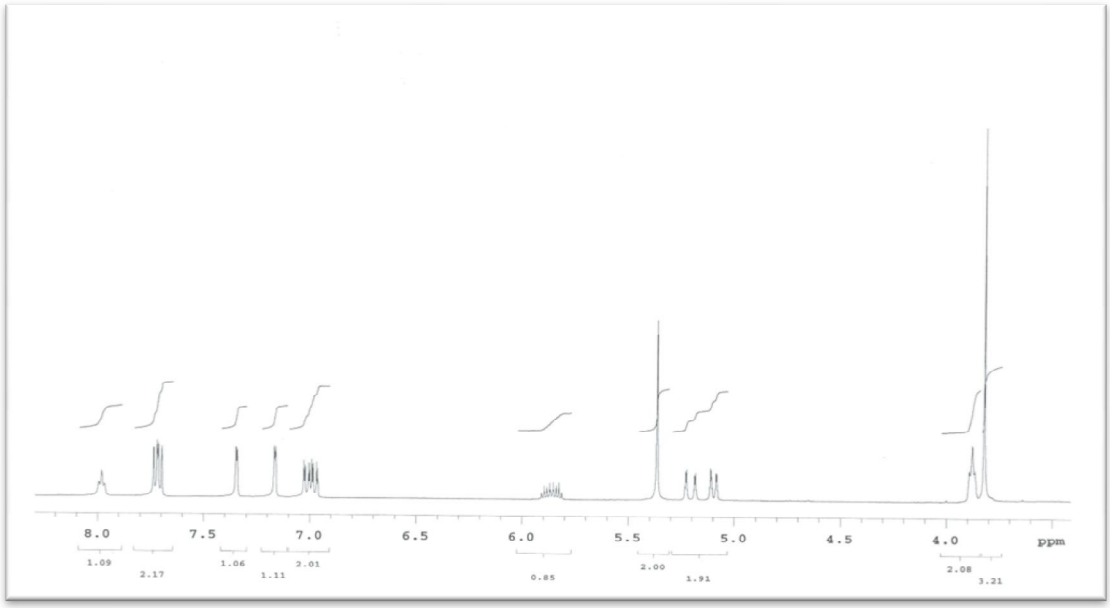


Figure 5.3. The ^{1}H-NMR spectrum of **Compound 2c**

^{1}H-NMR spectra of 1,2,4-triazole-3-thione derivatives were represented with **Compound 3b** (Figure 5.4). The protons were seen at δ 1.30 ppm (3H, t, -$CH_2C\underline{H}_3$); 3.90 (3H, s, -$OC\underline{H}_3$); 4.00 (2H, t, -$C\underline{H}_2CH_3$); 5.40 (2H, s, -$C\underline{H}_2$); 7.00-7.80 (6H, m, aromatic protons) and 13.90 (1H, broad s, N<u>H</u> triazole).

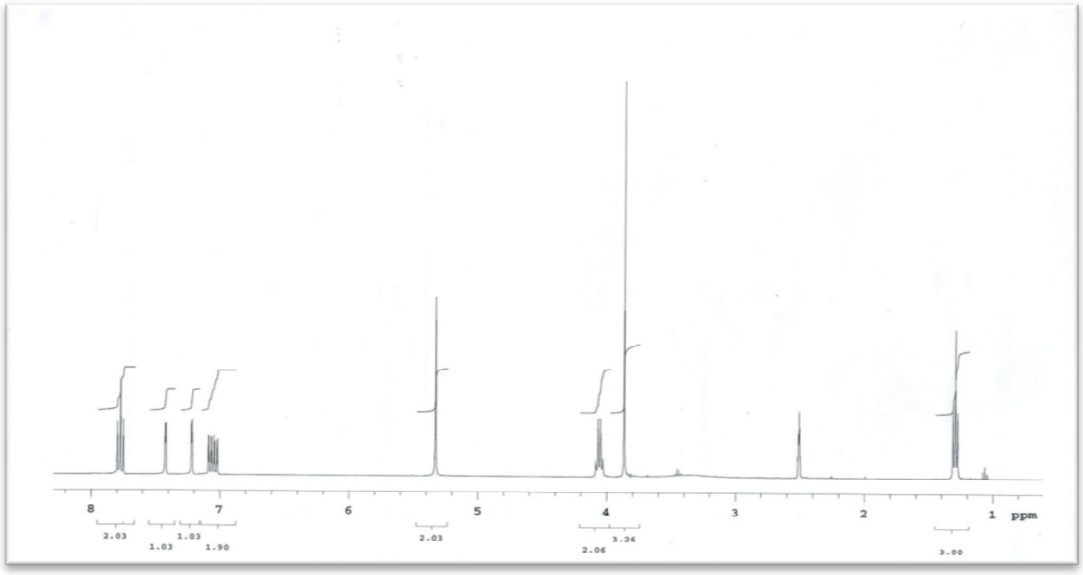
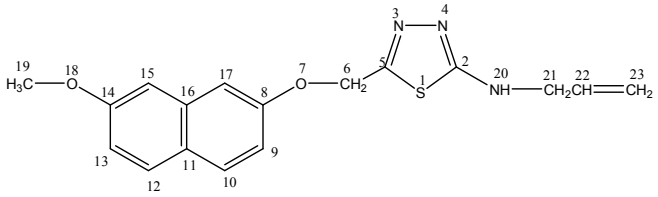


Figure 5.4. The ^{1}H-NMR spectrum of **Compound 3b**

The ^{13}C-NMR **Compound 2c** was chosen to evaluated the structures of 5-((7-methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazole (Figure 5.5). In the ^{13}C-NMR spectra, all carbons were observed according to the expected

chemical shifts. Methylenic carbons were seen at 64.93 ppm, the methoxy carbon at 55.79 and the aromatic carbons were seen at 106.07-156.57 ppm respectively. The carbons belong to thiadiazole ring were observed at 158.55 (C_2) and 170.38 (C_5) ppm, the allylic carbons (-$\underline{C}H_2$-$\underline{C}H$=$\underline{C}H_2$) were appeared at 47.55, 135.02 and 116.42 ppm.



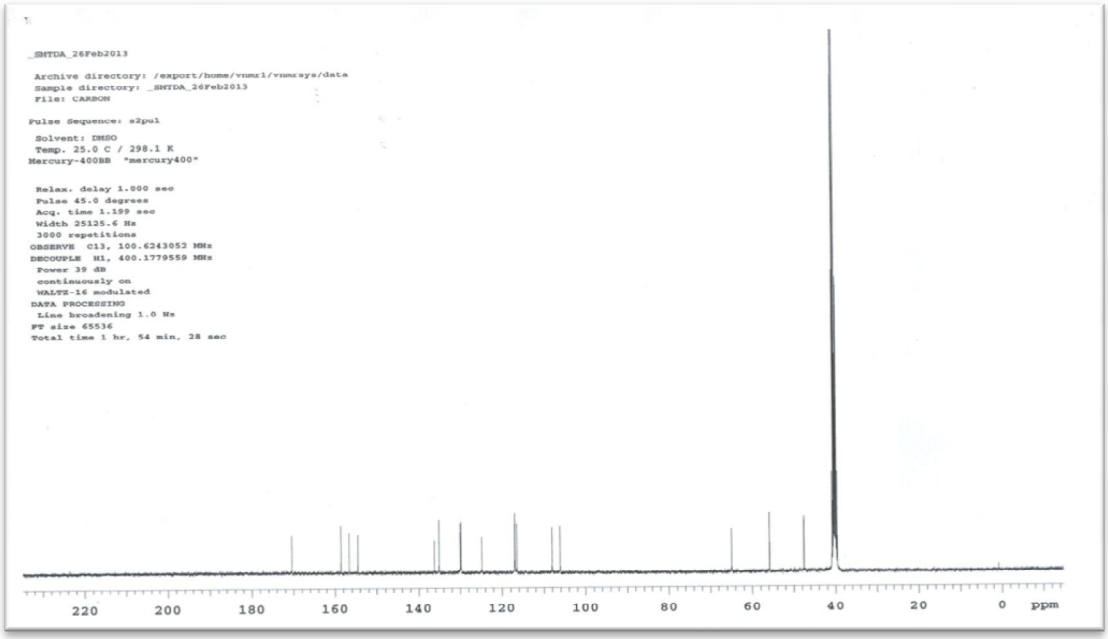
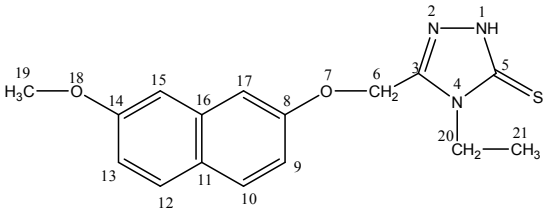


Figure 5.5. The ^{13}C-NMR spectrum of **Compound 2c**

The ^{13}C-NMR **Compound 3b** was selected to evaluated the structures of 3-((7-methoxy-2-naphthyloxy)methyl)-4-subtituted-1,2,4-triazole-5-thiones (Figure 5.6). The ^{13}C-NMR spectra, all carbons were appeared according to the expected chemical shifts. Methylenic carbon was seen as at 60.66 and the methoxy carbon was observed at 55.82 ppm. The aromatic carbons were observed at 106.08-156.41 ppm, the carbons belong to triazole ring at 148.59 (C_2), 167.87 ppm (C_5) and the aliphatic carbons (C_{21} and C_{20}) were seen at 14.09 and 41.00 ppm respectively.



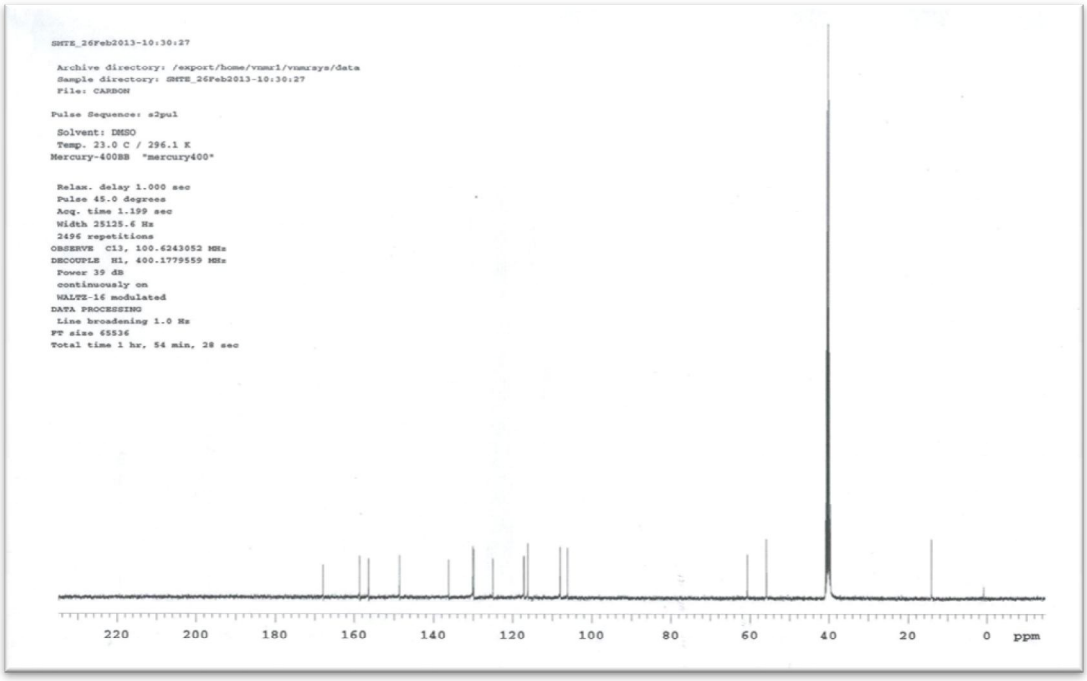


Figure 5.6. The ^{13}C-NMR spectrum of **Compound 3b**

The Mass spectra of synthesized compounds were studied in Micromass ZQ LC-MS Spectrometer with ESI+ method.

The mass spectrum of 1-(7-methoxy-2-naphthyloxy)acetylhydrazide have two intensive peaks which were observed at *m/z:* 247 for protonated molecular ion $[M+H]^+$ and m/z 269 for molecular ion with sodium $[M+Na]^+$.

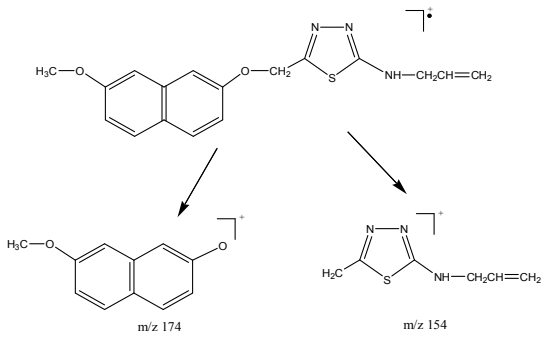
In the Mass spectra of 1-(7-methoxy-2-naphthyloxy)acetyl-4-substituted-3-thiosemicarbazide derivatives (**Compounds 1a-d**) protonated molecular ions $[M+H]^+$ and molecular ions with sodium $[M+Na]^+$ were observed at *m/z:* 320 $[M+H]^+$ and 342 $[M+Na]^+$; 334 $[M+H]^+$ and 356 $[M+Na]^+$; 346 $[M+H]^+$ and 368 $[M+Na]^+$; 382$[M+H]^+$ and 404 $[M+Na]^+$ respectively.

In the Mass spectra of 5-((7-methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazoles **(Compounds 2a-d),** all ions have been found as expected. Protonated molecular ions $[M+H]^+$ and molecular ions with sodium $[M+Na]^+$ were

observed at *m/z;* 302 $[M+H]^+$ and 324 $[M+Na]^+$; 316 $[M+H]^+$ and 338 $[M+Na]^+$; 328 $[M+H]^+$ and 350 $[M+Na]^+$; 364 $[M+H]^+$ and 386 $[M+Na]^+$ respectively.

In the Mass spectra of 3-((7-methoxy-2-naphthyloxy)methyl)-4-subtituted-1,2,4-triazole-5-thiones **(Compounds 3a-d**) all ions have been found as expected. Protonated molecular ions $[M+H]^+$ and molecular ions with sodium $[M+Na]^+$ were observed at *m/z;* 302$[M+H]^+$ and 324$[M+Na]^+$; 316 $[M+H]^+$ and 338 $[M+Na]^+$; 328 $[M+H]^+$ and 350 $[M+Na]^+$; 364 $[M+H]^+$ and 386 $[M+Na]^+$ respectively.

The mass spectra of 2-substitutedamino-1,3,4-thiadiazole derivatives were illustrated with **Compound 2c** (Figure 5.7). The protonated molecular ion $[M+H]^+$ was seen at 328 and molecular ion with sodium $[M+Na]^+$ was observed at 350 (*m/z*). The fragmentation pathways for **Compound 2c** is proposed to be as follows: the fragments of this compound are 3-methyl-2-allylamino-1,3,4-thiadiazole and 7-methoxy-2-naphthyloxy.



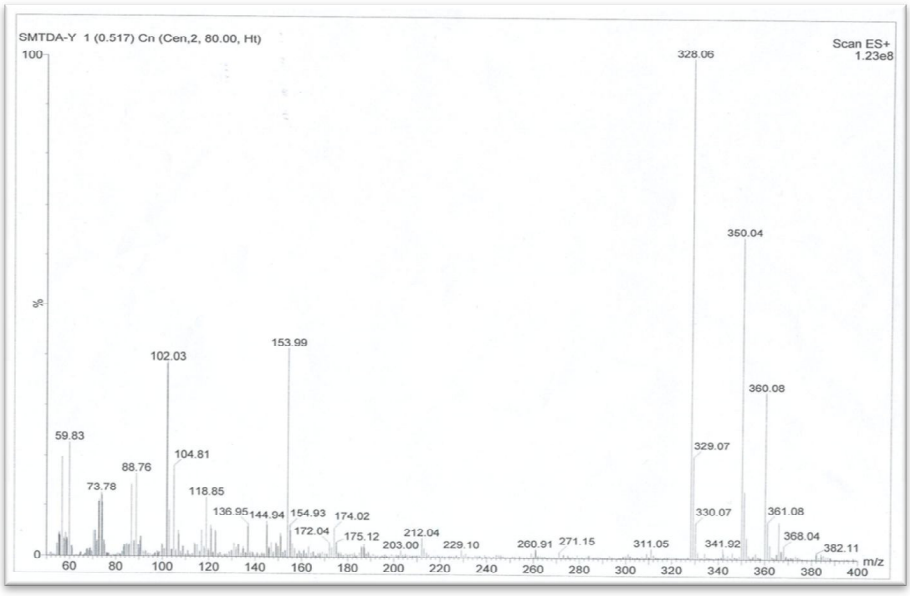
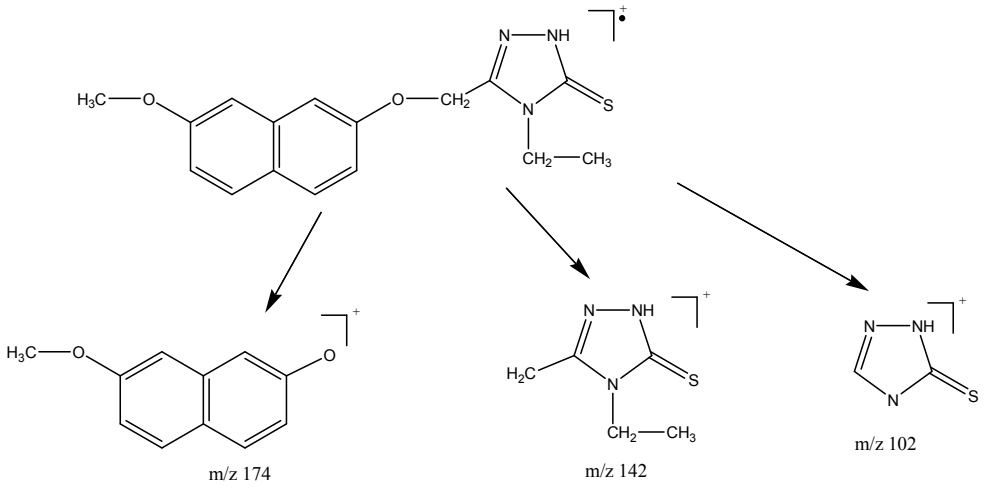


Figure 5.7. The mass spectrum of **Compound 2c**

The mass spectra of 1,2,4-triazole-3-thione derivatives were illustrated with **Compound 3b** (Figure 5.8). The protonated molecular ion $[M+H]^+$ was seen at *m/z*: 316 and molecular ion with sodium $[M+Na]^+$ was observed at *m/z*: 338. The fragmentation pathway for **Compound 3b** proposed to be as follow: the fragments of this compound are 3-methyl-4-ethyl-1,2,4-triazole-5-thione and 7-methoxy-2-naphthyloxy and 1,2,4-triazole-5-thione.



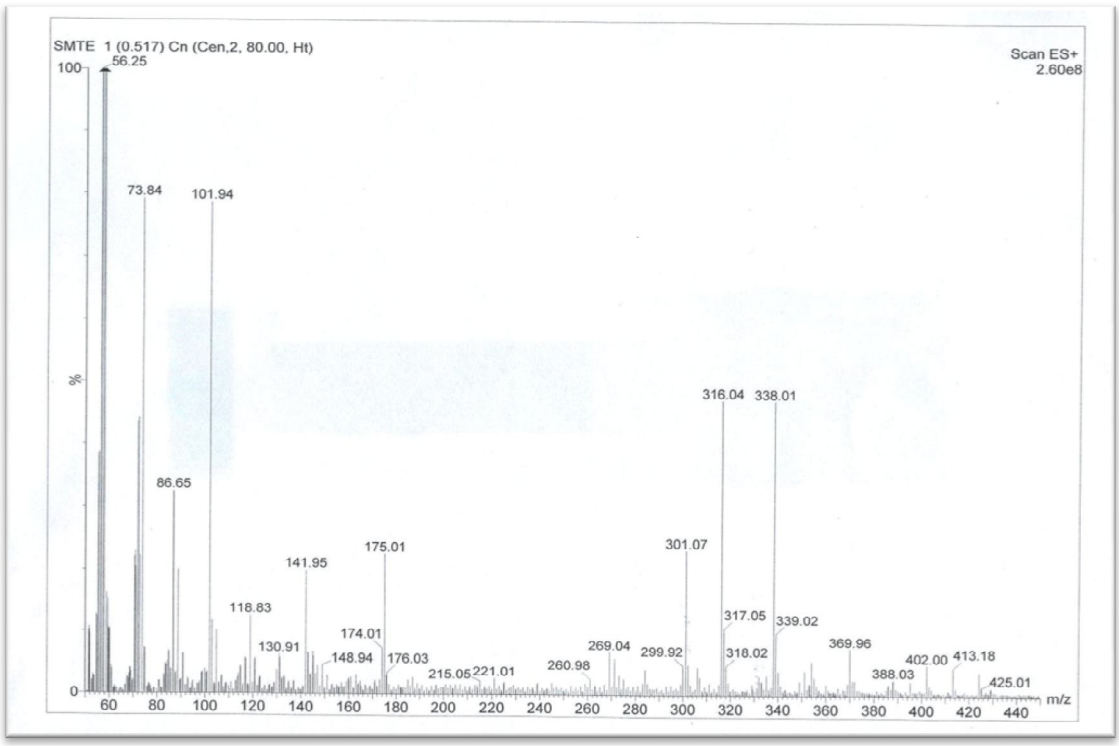


Figure 5.8. The mass spectrum of **Compound 3b**

5-((7-Methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazole derivatives and 3-((7-methoxy-2-naphthyloxy)methyl)-4-subtituted-1,2,4-triazole-5-thione derivatives (**Compounds 2a-d** and **3a-d**) were evaluated for their ability to inhibit the COX-1 and COX-2 enzymatic activity using COX inhibitor screening assay kit. Indomethacin and NS-398 were used as standard inhibitor compounds.

The resulting data of this experiment showed that the target compounds did not have remarkable inhibitory effects on COX-1 and COX-2 enzymes compared to standard. However, in the series, **Compound 2b** (COX-1 IC_{50} >250 μM; COX-2 IC_{50}: 150.2μM) possess better selectivity and activity on COX-2, while **Compound 3b** (COX-1 IC_{50}: 45.6 μM; COX-2 IC_{50}: 176.5μM) possess better inhibitory activity on COX-1 than rest of compounds.

However, none of the synthesized compounds possessed better inhibtory activity on COX-1 and COX-2 than standard compounds (indomethacin and NS-398). Among the compounds screened for their inhibitory activity, **Compound 2b** was found to have more selective inhibition effect on COX-2 enzyme than the rest of compounds. Also, **Compounds 3b and 3c** were found to possess selective inhibition effects on COX-1 enzyme than the other compounds.

With the aim of getting insights into the structural basis for its activity, **Compound 2a** was docked into the active site of COX-2 enzyme by using MOE software program. Docking studies showed that the naphthyl ring of the compounds fitted into the hydrophobic cavity formed Val349, Tyr355, Leu359 and Leu531. A hydrogen bond occurred between C=O moiety of Leu352 and methylene group of **Compound 2b**. Although thiadiazole moiety did not fill the adjunct pocket, but thiadiazole ring formed an arene-cation interaction with His90. The selectivity of this compound may be due to the presence of this interaction.

6. RESULT AND SUGGESTIONS

In this study, the target compounds 5-((7-methoxy-2-naphthoxy)methyl)-2-substitutedamino-1,3,4-thiadiazole **(compounds 2a-d)** and 3-((7-methoxy-2-naphthoxy)methyl)-4-substituted-1,2,4-triazole-5-thione **(compounds 3a-d)** derivatives which have been expected to have ability to inhibit COX-2 and COX-1 enzymes were synthesized. The characterization of compounds were elucidated by FT-IR, ^{1}H-NMR, ^{13}C-NMR, MS spectral and elemental analysis.

5-((7-Methoxy-2-naphthyloxy)methyl)-2-substitutedamino-1,3,4-thiadiazole derivatives and 3-((7-methoxy-2-naphthyloxy)methyl)-4-subtituted-1,2,4-triazole-5-thione derivatives **(Compounds 2a-d** and **3a-d)** were screened for their ability to inhibit COX-2 and COX-1 enzymatic activity using a COX inhibitor screening assay kit. The potency (IC_{50} values) of test compounds was determined and compared to that of the reference molecules NS-398 (selective COX-2 inhibitor) and indomethacin (selective COX-1 inhibitor). Additionally, to understand the the interaction of **Compound 2b**, which showed more selectivity on COX-2, with COX-2 enzyme a *docking* study was carried out.

The inhibitory effects of target compounds on COX-1 and COX-2 enzymes were lower than standards (NS-398 and indomethacin). However, among the compounds screened for COX-1 and COX-2 inhibition activity, **Compound 2b** was shown a selective inhibitory effect on COX-2 enzyme than the rest of compounds. Also, **Compounds 3b** and **3d** were shown selective inhibitory effect on COX-1 enzyme then the other compounds.

Based on *Docking* studies, in the 1,3,4-thiadiazole derivatives, ethyl substitution to 2-amino group increase the inhibitory activity and COX-2 selectivity.

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CURICULLUM VITAE

Was born in 1981, Baghlan-Afghanistan. Primary, secondery and high school in Kabul and graduated from Faculty of Pharmacy, Kabul University in 2003. From 2005 to 2010 worked as an assistant in Faculty of Pharmacy, Kabul University.