SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME 2-MERCAPTOBENZOTHIAZOLE DERIVATIVES

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By

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CERTIFICATE

This is to certify that the thesis entitled, "**Synthesis and Biological Evaluation of Some 2-Mercaptobenzothiazole Derivatives**" is a record research work done by Mr. Md. Afzal Azam at Department of Pharmaceutical Chemistry, J. S. S. College of Pharmacy, Ootacamund, under my supervision during the year 2007-2011 and that this thesis has not previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title. I also certify that the thesis represents independent work done by the candidate and has not formed in part or fully the basis for the award of any other previous research degree. This work has been completed and ready for evaluation in partial fulfillment for the award of Degree of Doctor of Philosophy under The Tamil Nadu Dr. M. G. R. Medical University, Chennai.

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DECLARATION

I hereby declare that the thesis entitled "**Synthesis and Biological evaluation of Some 2-Mercaptobenzothiazole Derivatives**" submitted by me for the award of Degree of Doctor of Philosophy under The Tamil Nadu Dr. M. G. R. Medical University, Chennai is the result of my original and independent work done at J. S. S. College of Pharmacy, Ootacamund during the years 2007-2011 under the supervision of Dr. B. Suresh and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title previously.

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ABSTRACT

Fast and effective relief of pain and inflammation in the human being is continued to be a major challenge for the medicinal chemistry researchers. Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the alleviation of pain and inflammation associated with a number of pathological conditions. However, chronic administration of NSAIDs has been associated with clinically significant complications such as gastrointestinal (GI) symptoms including mucosal damage, bleeding, nausea, heartburn, dyspepsia, abdominal pain and renal toxicity.

Co-administration of multiple drugs for treatment of inflammatory conditions associated with microbial infection is a major risk especially in patients with prior history of peptic ulcer disease, patients with impaired liver or kidney functions and patients taking anticoagulants, corticosteroids, etc. concurrently. A mono therapy of a drug with dual antimicrobial and anti-inflammatory activity would be preferred from the pharmacoeconomic and patient compliance point of view. In view of the above-mentioned facts some novel 2-mercaptobenzothiazoles carrying 1,3,4-oxadiazole (**ODZ_{1-15}** and **OXZ_{1-13}**), acetohydrazide (**ACH_{1-5}**), 1,3,4-thiadiazole (**TDZ_{1-13}**), 1,3,4-triazole (**TRZ_{1-13}**) and 2-pyrazoline (**PYZ_{1-19}** and **PYS_{1-9}**) moieties at the second position were synthesized. This combination was suggested in an attempt to develop hybrid compounds that would act as antimicrobial and analgesic-anti-inflammatory agents with minimal gastrointestinal (GI) side effects.

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity by the cup plate method. The tested compounds **ODZ_{1-4}**, **ODZ_{6}**, **ODZ_{10}**, **ODZ_{11}** and **ODZ_{13}** showed significant inhibitory activity (inhibition zone 24-32 mm) against all the tested bacterial strains whereas compounds **OXZ_{8}**, **TRZ_{1}**, **ATZ_{1}**, **TDZ_{4}**, **TDZ_{8}**, **TDZ_{9}**, **TDZ_{10}**, **PYZ_{8}**, **PYZ_{9}**, **PYZ_{10}** and **PYZ_{14}** indicated specific inhibitory activity (inhibition zone **>** 19 mm) against the Gram-negative bacteria *Pseudomonas aeruginosa*. In the present investigation tested compounds did not posses antifungal activity.

Compounds that showed significant antibacterial activity were evaluated for their *in vivo* analgesic activity using tail immersion method in mice and results were compared with the reference standard drug paracetamol. In case of 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethy)-1,3,4-oxadiazol-2-yl]acetamide the highest activity (76.5% analgesia) was observed at 3 h in derivative **ODZ_{10}.** At first and second hour derivatives **OXZ_{8}** and **OXZ_{11}**

belonging to the 2-{(benzo[d]thiazol-2-ylthio)methyl}-5-(aryloxymethyl)-1,3,4-oxadiazoles series and derivatives **TDZ_1** and **TDZ_9** belonging to the 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl]acetamide series exhibited significant analgesic activity (46.0-76.8%). Among 2-pyrazoline incorporated 2-mercaptobenzothiazoles, derivatives **PYZ_5**, **PYZ_9**, **PYZ_{10}** and **PYZ_{14}** exhibited potent analgesic activity (55.9 to 69.8%) at second and third hour following oral administration.

Compounds were evaluated for their anti-inflammatory activity using the carrageenan induced paw oedema method in rats. At 2 and 3 h compounds **ODZ_4**, **ODZ_6** and **ODZ_{10}** belonging to the 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethy)-1,3,4-oxadiazol-2-yl]acetamide series were effective in inhibiting the paw oedema (58.2-68.2%), when compared with the reference drug diclofenac sodium. In case of 2-{(benzo[d]thiazol-2-ylthio)methyl}-5-(aryloxymethyl)-1,3,4-oxadiazole/triazole and 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl]acetamide series highest activity (81.6%) was found in derivative **TDZ_4** at 1h after carrageenan injection. Among the 2-pyrazoline incorporated 2-mercaptobenzothiazole analogs highest activity (76.9%) was observed at second hour in derivative **PYZ_{14}**. On the other hand, sulfonyl analogs (**PYS_{1-3}**) showed weak anti-inflammatory activity at all time interval.

Compounds that exhibited higher analgesic and anti-inflammatory profiles in the pre-mentioned animal models and promising antibacterial activities were further evaluated for their ulcerogenic potential. The results indicated low severity index of the tested compounds **ODZ_{10}**, **TDZ_4**, **TDZ_9**, **PYZ_9** and **PYZ_{14}** ranging from 2.0±0.3 to 3.2±0.7.

ABBREVIATIONS

CCR	=	CC chemokine receptors
$CDCl_3$	=	Deuterated chloroform
CSF	=	Colony stimulating factor
COX	=	Cyclooxygenase
DHP	=	Dehydropeptidase
ED1	=	Ectodermal Dysplasia 1
DMSO	=	Dimethyl sulfoxide
DMF	=	Dimethylformamide
EETEs	=	Epoxyeicosatetraenoic acids
FDA	=	Food and drug administration
GABA	=	Gamma-Aminobutyric acid
GC/MS	=	Gas chromatography-mass spectrometry
GFAP	=	Glial fibrillary acidic protein
GDNF	=	Glia cell line-derived neurotrophic factor
GI	=	Gastrointestinal
GLT	=	Glutamate transporter
g	=	Gram
GM-CSF	=	Granulocyte-macrophage colony stimulating factor
HETEs	=	Hydroxyeicosatetraenoic acids
HD	=	Huntington´s disease
HPLC	=	High performance liquid chromatography
5-HPETEs	=	Hydroperoxyeicosatetraenoic acids
5-HT	=	5-Hydroxytryptamine
IC_{50}	=	50% Inhibitory concentration
ICE	=	IL-1converting enzyme
IFN	=	Interferon
Ig	=	Immunoglobulin
mL	=	Millilitre
IL	=	Interleukin
IL-1ra	=	IL-1 receptor antagonist
IR	=	Infrared
JNK	=	c-Jun N-terminal kinase
KBr	=	Potassium bromide
kg	=	Kilogram
LTA	=	Lipoteichoic acid
LPS	=	Lipopolysaccharide
LTs	=	Leukotrienes

LPS	=	Lipopolysaccharide
LXs	=	Lipoxins
LPS	=	Lipopolysaccharide
LT	=	Leukotriene
MAPK	=	Mitogen-activated protein kinase
MBT	=	2-Mercaptobenzothiazole
mg	=	Milligram
MMP	=	Matrix metalloproteinase
mol	=	Mole
M.p.	=	Melting point
MS	=	Multiple sclerosis
EI-MS	=	Electron impact mass spectroscopy
ESI-MS	=	Electron spray ionization mass spectroscopy
MHC	=	Major histocompatibility complex
MIP	=	Macrophage inflammatory protein
mRNA	=	Messenger RNA
NF-kB Nf-kB	=	Eukaryotic transcription factor
NF-κB	=	Nuclear factor kappaB
NMR	=	Nuclear magnetic resonance
NO	=	Nitric oxide
NSAID	=	Non-steroidal anti-inflammatory drug
OGD	=	Oxygen-glucose deprivation
PBP	=	Penicillin binding protein
PG	=	Prostaglandin
PMNL	=	Polymorphonuclear leukocytes
QA	=	Quinolinic acid
SVCAM	=	Soluble vascular adhesion molecule
TA	=	Teichoic acid
TGF	=	Transforming growth factors
THF	=	Tetrahydrofuran
TMS	=	Trimethylsilane
TNF	=	Tumor necrosis factor
TXs	=	Thromboxanes
TGF	=	Transforming growth factor
Th	=	T helper cells
TNF	=	Tumor necrosis factor
TLC	=	Thin layer chromatography
TLR	=	Toll like receptor
TNF	=	Tumor necrosis factor
UV	=	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1. Inflammation

Inflammation is a body's normal protective response to tissue injury. It is the body's effort to inactivate or destroy invading microorganisms, remove irritants and set the stage for tissue repair. When healing is complete though, the inflammatory process usually subsides[1] (Mycek *et al.*, 2000). Sometimes however, the defense reactions themselves cause progressive tissue injury as in the case of arthritis requiring anti-inflammatory (or immunosuppressive) drugs to modulate the inflammatory process[1,2] (Mycek *et al.*, 2000; Payan and Katzung, 1995). Also local and systematic inflammations such as those elicited by bacterial infections at mucosal surfaces are often times treated with non-steroidal anti-inflammatory drugs (NSAIDs) in combination with other drugs. However, the use of anti-inflammatory therapy has been questioned on the basis that such treatment may compromise the immune function. It is the body's effort to inactivate or destroy invading organisms, remove irritants and set the stage for tissue repair[3] (Madigan *et al.*, 2000). Characteristics of the inflammatory response include redness, swelling, pain and heat which are localized at the site of infection[4] (Furr, 1992). Even though inflammation is a non-specific immunological reaction, it employs specific cell mediated immunological responses such as the leukocytes and their cytokine secretions, which mediate on inflammatory reactions. Inflammation is triggered by the release of chemical mediators from injured tissues and migrating cells. The specific chemical mediators vary with the type of inflammatory process and include amines e.g. histamines and 5-hydroxytryptamine, lipids e.g. prostaglandins, small peptides such as bradykinin and larger peptides such as interleukin-1, which is a cytokine[1,4] (Furr, 1992; Mycek *et al.*, 2000). Most NSAIDs act by inhibiting the synthesis of prostaglandins. Thus, understanding of NSAIDs requires a comprehension of the actions of prostaglandins.

Prostaglandins (PGs) and related fatty acid derivatives of arachidonic acid are among the most potent naturally occurring autacoids. They are critically important cell regulatory substances[5] (Rang *et al*., 1999). The PGs and several other biologically active lipids and peptidolipid acids are formed from the same precursor (arachidonic acid) through interrelated enzymatic pathways. These other lipids are nearly all carboxylic acids and include the thromboxanes (TXs), the hydroxyeicosatetraenoic acids (HETEs), the leukotrienes (LTs), most recently discovered lipoxins (LXs) and epoxyeicosatetraenoic acids (EETEs). They are generally known as the eicosanoids[6] (Hecker *et al*., 1995). Free arachidonic acid is metabolized mainly by two divergent enzymatic pathways that are variably distributed among different cells: the cyclooxygenase (COX) and the lipoxygenase pathways[1,2,5] (Mycek *et al*., 2000; Payan and Katzung, 1995; Rang *et al*., 1999). The products of the cyclooxygenase pathway are the ring structured eicosanoids (PGs, TXs and Prostacyclines) catalyzed by two enzymes COX-1 and COX-2. The COX-2 appears to be the form of the enzyme associated with cells involved in the inflammatory process. The COX-1 is a constitutive enzyme expressed in most tissues including blood platelets. It is involved in cell-cell signaling and in tissue homeostasis[5] (Rang *et al*., 1999). Clearly, the anti-inflammatory action of NSAIDs is mainly related to their inhibition of COX-2. Most of the undesirable effects of NSAIDs have been suggested to result from their inhibition of the COX-1 enzymatic pathway[2] (Payan and Katzung, 1995). Therefore selective COX-2 inhibitors with better safety profile have been marketed as a new generation of NSAIDs. But careful prospective examination of COX-2 inhibitors has revealed unexpected cardiovascular (CV) adverse effect[7a] (Dogne *et al*., 2005), which led to the withdrawal of COX-inhibitors from the market. However, the relative risk of CV events with traditional NSAIDs has been shown to be low. The CV risk associated with traditional and selective NSAIDs seems to be related to dose and duration of use, and to the potency of COX-2 inhibition[7b] (Garcia *et al*., 2008). NSAID use is not associated with a first occurrence of heart failure but may exacerbate preexisting disease. In patients with preexisting heart failure, NSAID use may induce systemic vasoconstriction, causing an increase in afterload with further reduction in cardiac contractility and cardiac output[7c] (Page *et al*., 2000). Advanced heart failure is associated with increased secretions of antidiuretic hormone, angiotensin II, and norepinephrine. The ensuing renal ischemia may lead to water retention and hyponatremia, resulting in further worsening of heart failure and increased risk for acute renal failure. Acute renal failure may occur with any COX-2-selective or nonselective NSAID.

The lipoxygenase pathway is catalyzed mainly by 5-lipoxygenase enzyme. The products of this pathway are the 5-HPETE (5-hydroperoxyeicosatetraenoic acid) and 12-HPETE, which are

unstable and converted to the HETEs or to leukotrienes or lipoxins depending on the tissues[1] (Mycek *et al*., 2000). Prostaglandins and related compounds are produced in minute quantities by virtually all tissues. They generally act on the tissues in which they are synthesized and are rapidly metabolized into inactive products at their sites of action. Thus, the PGs do not circulate in the blood in significant concentrations[1] (Mycek *et al*., 2000). In general, prostaglandins have a variety of effects on smooth muscles, platelets,

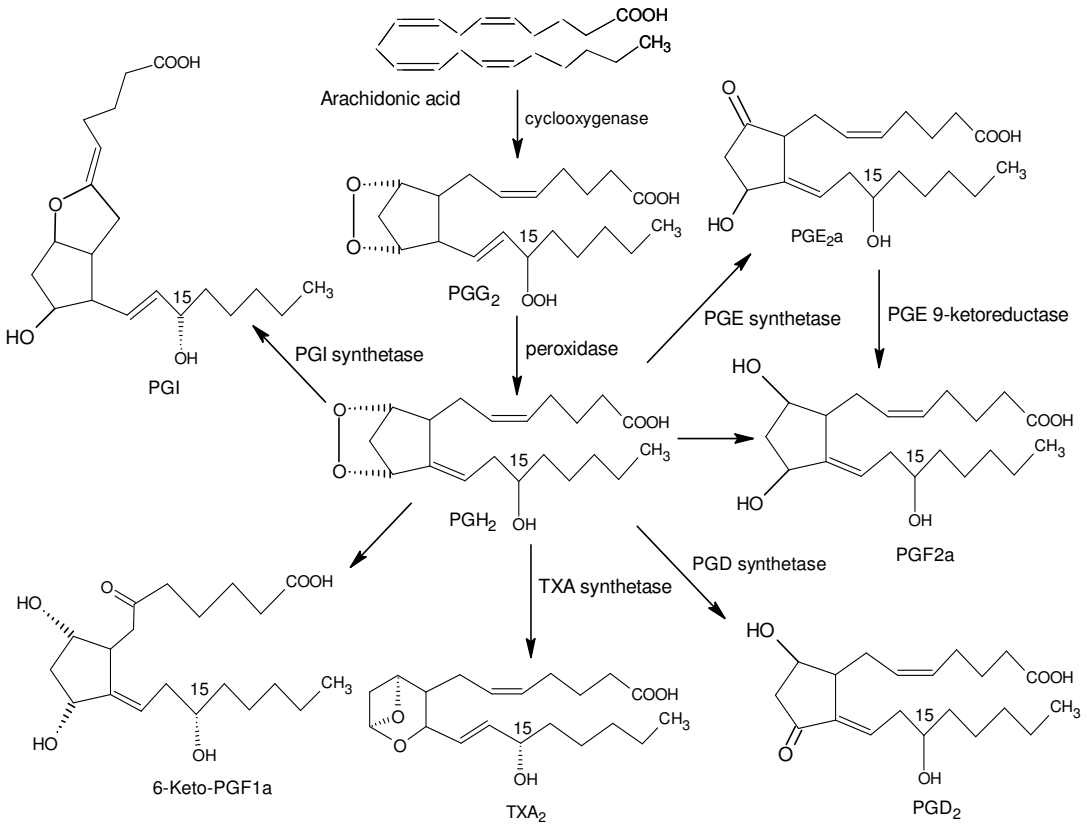


Figure 1. The cyclooxygenase pathway involves the prostaglandin and thromboxane biosynthesis. The cyclooxygenase and endoperoxidase steps are catalyzed by a single enzyme, prostaglandin endoperoxide (PGH) synthetase.

reproductive system, central nervous system and on cells involved in inflammation. The leukotrienes are present in the tissues in many inflammatory conditions. They have a powerful chemotactic effect on eosinophils, neutrophils and macrophages. They promote broncho-constriction and alter vascular permeability. The prostaglandins are not chemo-attractants, but the leukotrienes and some of the HETEs are strong chemoattractants[6] (Hecker *et al*., 1995). The primary inflammatory cytokines are important coplayers when COX-2 is induced in inflammatory cells. They regulate cyclooxygenase and lipoxygenase enzymatic activities[6,8]

(Hecker *et al*., 1995; Rang *et al*., 1999b) and are another group of chemo-attractants produced by leukocytes. Cytokines are known to have anti-proliferative and antimicrobial activities[9] (Salmon *et al*., 1995). For example, infections with Gram-negative bacteria induce inflammatory response that could be local or systemic[10,11] (De Man *et al*., 1988; Gilbert, 1992). The inflammation is elicited by whole bacterium and lipid A moiety of lipopolysaccharide (LPS) on the surfaces of Gram-negative bacteria. The LPS induces the recruitment of a wide range of cytokines, such as the tumour necrosis factor, (TNF), Interleukin-1 (IL-1), IL-6 and colony stimulating factors (CSFs).

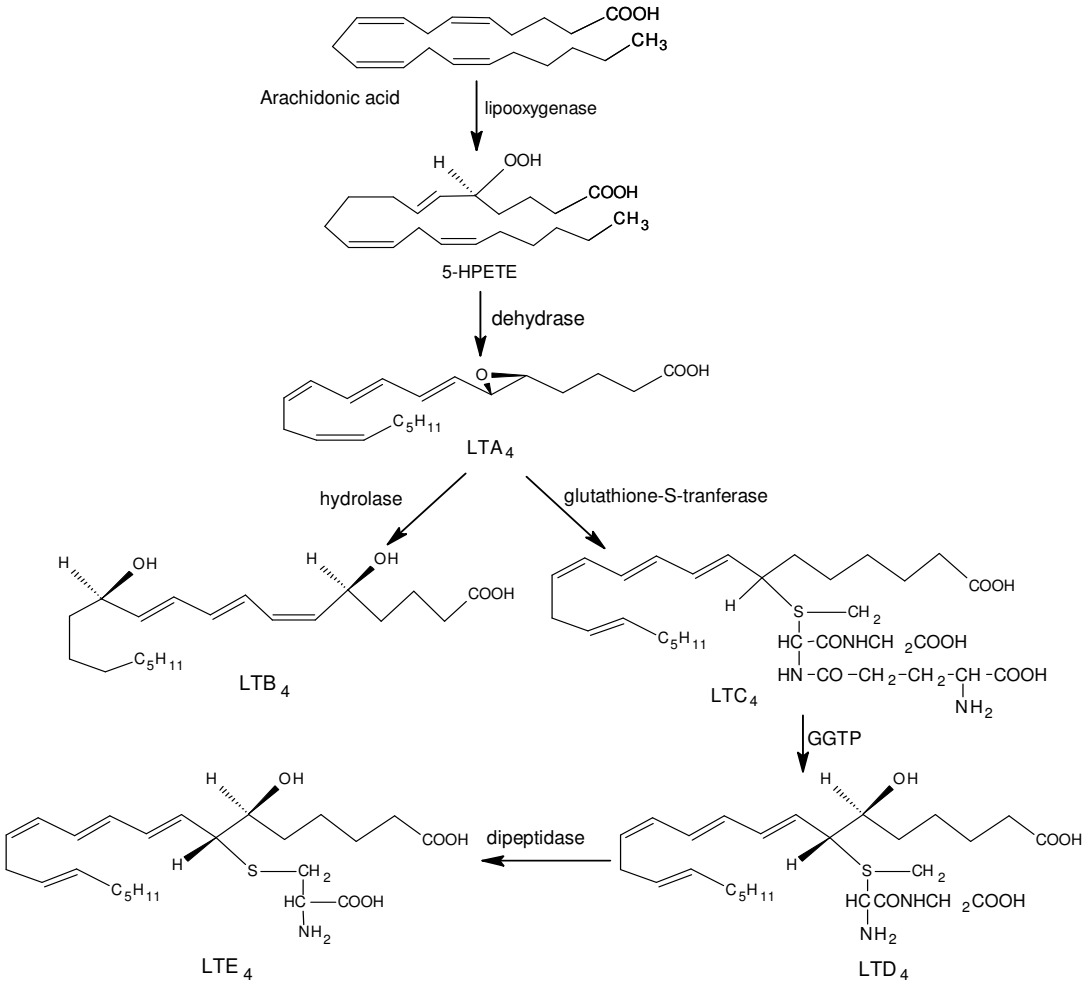


Figure 2. The lipooxygenase pathway involves leukotriene biosynthesis. Both the lipooxygenase and dehydrase reactions are driven by the single enzyme, 5-lipooxygenase (GGTP, gama glutamyl transpeptidase).

As earlier mentioned, NSAIDs act by blocking prostaglandin and thromboxane synthesis. This is achieved through the inhibition of the cyclooxygenase pathway which is responsible for

their synthesis. With a few exceptions, NSAIDs do not inhibit lipoxygenase activity at concentrations that markedly inhibit cyclooxygenase activity[6] (Hecker *et al.*, 1995). Since the lipoxygenase and cyclooxygenase pathways have the same precursor (arachidonic acid), inhibiting the metabolism of arachidonic acid via the cycloxygenase pathway would cause metabolism to tend more to the lipoxygenase pathway; thus causing more products formation than usual via that pathway. Hence, the use of NSAIDs leads to an increase in the formation of inflammatory leukotrienes.

It has been discovered that even among the cyclooxygenase dependent pathways, inhibiting the synthesis of one derivative may increase the synthesis of an enzymatically related product. This is however not usually desirable as the thromboxanes and leukotrienes are known to be pathologic eicosanoids[6] (Hecker *et al.*, 1995). The activities of prostaglandins and leukotrienes in the body produce somewhat opposite effects. PGE_2 and PGI_2 are the two common prostaglandins synthesized in humans. PGE_2 inhibits the activation and proliferation of B-lymphocytes by T-lymphocytes ($CD4^+$) while leukotriene-B_4 (LTB_4) stimulates it while inhibiting both antigen driven and mitogen-induced B-lymphocyte proliferation and differentiation to plasma cells. This results in the inhibition of immunoglobulin M (IgM) synthesis and enhanced class switch to IgE. PGE_2 and possibly PGI_2 inhibit the expression of IL-1 and its effect on T-lymphocytes, while LTB_4 and LTD_4 increase its expression. PGE_2 and probably PGI_2 inhibit interferon gamma (INT-γ) activity on macrophages, while LTB_4, LTC_4 and LTD_4 stimulate it. Also PGE_2 and possibly PGI_2 inhibit the action of IL-2 from $CD4^+$ lymphocytes to $CD8^+$, LTB_4 does the reverse[6,9] (Hecker *et al.*, 1995; Salmon *et al.*, 1995).

1.2. Antimicrobials as anti-inflammatory agents

Many antibacterial agents have been shown to exert effects on leucocytes, particularly neutrophils and some of these agents have been found to effect experimental inflammation in animals. Although the best investigated family of antibacterial agents during the last decade is the group of macrolides, quinolones and tetracyclines, there also is a growing body of data about the anti-inflammatory and immunomodulatory actions of other antibacterial agents.

Macrolide antibiotics accumulate in inflammatory cells at concentrations upto several hundred-fold higher than those in extracellular fluid[12a] (Labro *et al.*, 2000) enabling phagocytic cells to deliver concentrated active drug to site of infections. The mechanism of intracellular accumulation is not clear, but exhibits characteristics of an (protein mediated) process[12a] (Labro *et al.*, 2000). Concentration occurs in the cytoplasm and azurophilic granules of the neutophils,

thus favouring antibiotic delivery to bacteria phagocytosed by leukocytes. Cytokines stimulate *in vitro* accumulation of macrolides into macrophages, suggesting that at the site of inflammation (infection), cells may accumulate even more macrolide antibiotics than under physiological conditions[12b] (Labro *et al*., 1994). Efflux or relase of macrolides from leukocytes varies among macrolides, being very fast with erythromycin and clarithromycin, but very slow with azithromycin[12c] (Bosnar *et al*., 2005), so that the later agent is retained much longer in the cells. This offers the possibility of both prolonged activity and against invading bacteria and extended modulation of leukocyte function, beyond that which might be observed in short-term cell cultures *in vitro*. Other antibacterials can also accumulate to some degree in the cells, but nowhere near the extent of that of the macrolides. For instance, uptake *via* the nucleoside transport system may explain the approximate 20-fold cellular accumulation of clindamycin into alveolar macrophages[12d] (Hand *et al*., 1984). Apart from erythromycin, the only other antibiotics that showed some selective accumulation (2-5 fold) were the lipid soluble chloramphenicol, rifampin, tetracycline and lincomycin. Neutrophil uptake of the quinolone and ciprofloxacin is also approximately 5-fold that of the extracellular fluid.

In general, macrolides inhibit synthesis of reactive oxygen species and/or secretion of proinflammatory cytokines (*in vitro*) such as interleukin-8 (IL-8) and tumor necrosis factor α (TNF-α) as well as mediators of inflammation such as nitric oxide (NO). They are able to inhibit leukocyte chemotaxis by suppressing synthesis of endogenous chemotactic factors[13a] (Oda *et al*., 1994). Furthermore, the inhibition of bacteria-epithelial cell interaction and modulation of signaling pathways involved in the inflammatory response and direct effects on neutrophils have been observed[13b] (Sharma *et al*., 2007). A significant decrease of pro-inflammatory IL-8 and TNF-α was observed[14] (Pukhalsky *et al*., 2004) in the sputum specimens treated with clarithromycin at a dose of 250 mg every other day orally. Telithromycin belonging to the group of ketolide antibiotics also appears to exert immunomodulatory effects. In lipopolysaccharide (LPS)-induced systemic inflammation in mice, a single dose of 150 mg kg^{-1} telithromycin decreased the LPS-induced mRNA expression and protein synthesis of pro-inflammatory cytokines such as TNF-α, IL-1β and interferon γ (IFN-γ)[15] (Lotter *et al*., 2006). This suggests that non antibacterial compounds can be developed with anti-inflammatory activity, base on the macrolide structure.

Tetracyclines are widely used in the treatment of inflammatory acne. These antibiotics inhibit the proliferation of *Propionibacterium acnes*, but tetracycline also significantly inhibits the release of reactive oxygen species (ROS) from human polymorphonuclear (PMN) leukocytes and reduces the capacity of *Propionibacterium acnes* to produce neutrophil chemotactic factors,

providing evidence that it has anti-inflammatory actions[16] (Jain *et al*., 2002). Tetracyclines have been also used in reactive arthritis. The anti-inflammatory action of tetracyclines seems related to a non-antibacterial mechanism. In addition, the anti-inflammatory action of tetracycline has been proposed to be of benefit to prevent endotoxic shock by blockade of LPS-induced TNF-α and IL-1β secretion[17] (Shapira *et al*., 1996). Anti-inflammatory property of doxycycline and minocycline is investigated[18,19] (Suomalainen *et al*., 1992; Golub *et al*., 1991) and found to suppress microglia activation as assessed by Iba1 (ionized calcium-binding adaptor molecule 1) staining and activation of ED1, a membrane-bound lysosomal glycoprotein, both markers for microglial activation. This effect was accompanied by down-regulation of pro-inflammatory molecules such as NO, IL-1β and TNF-α.

Cefodizime a 2-amino-5-thiazolyl cephalosporin has been investigated *in vitro*, *ex vivo*, and *in vivo* in humans and animals. Cefodizime inhibited the LPS-stimulated release of TNF-α and IL-1 from human monocytes and TNF-α and IL-6 secretion into the bronchoalveolar lavage fluid after intranasal challenge with heat-killed *Pneumococci*[20] (Bergeron *et al*., 1998).

In vivo administration of geldanamycin (an antibacterial rifamycin) attenuates lung inflammation and acute lung injury in animal models, thereby suggesting that geldanamycin also has anti-inflammatory effects. Supporting this *in vivo* effect, geldanamycin inhibits the TNF-α-mediated IL-8 gene expression possibly through inhibition of NF-κB activation. Another explanation might be that geldanamycin inhibits the production of TNF-α, IL-6, and IL-1β in activated macrophages possibly through heat shock protein (HSP) 90 which is also critical in the intracellular signaling pathways promoting inflammatory cytokine production[21] (Wax *et al*., 2003).

Fosfomycin (1-cis-1,2-epoxypropylphosphoric acid) is a broad spectrum bactericidal antibiotic unrelated to any other known antibacterial agent. It significantly reduced volume, protein amounts and cell counts in the exudates obtained from a rat air pouche inflammed with carrageenan when compared to the placebo-treated animals[22] (Morikawa *et al*., 2003). The content of PGE_2, TNF-α, and mRNA for cyclooxygenase-2 were also markedly suppressed in fosfomycin-treated rats. Histological examination showed suppression of the inflammatory response in the pouch tissues from fosfomycin-treated rats.

Fluoroquinolones have also been studied for their anti-inflammatory activity in response to the bacterial infections. The best investigated agent moxifloxacin and alatrofloxacin shown inhibition of inflammatory mediators and of three major signal transduction pathways involved in inflammatory responses[23] (Weiss *et al*., 2004): NFκB, mitogen-activated protein kinase ERK

and c-Jun N-terminal kinase (JNK). In the year 1993 cefaclor was shown to promote phagocytosis of *Escherichia coli* by granulocytes and bactericidal activity of these cells was both increased. In contrast, synthesis of IL-6 and of the pro-inflammatory TNF-α was decreased[24] (Scheffer *et al*., 1993). Biological effects of exogenous endotoxin was investigated[25] (Goscinski *et al*., 2004) in a porcine model by tobramycin or ceftazidime to modulate the inflammatory response and suggested a possible anti-inflammatory effect by both ceftazidime and tobramycin caused by a significantly greater reduction in the IL-6 plasma level response to endotoxin in comparison with the untreated group.

At therapeutic concentrations, coumermycin (gyrase B inhibitors) has been reported to impair chemotaxis, superoxide anion production, and intracellular killing of PMNs. It suppresses the production of proinflammatory cytokines (TNF-α, IL-1 and IL-6) by LPS-stimulated human monocytes[26] (Luhrmann *et al*., 1998). Phenolics (such as thymol and eugenol) have been used as disinfectants in medicine and dentistry for centuries. However, determining their modes of action as dual function (antibacterial and anti-inflammatory) agents are more recent[27] (Dewhirst *et al*. 1980). Anti-inflammatory effects of 2,4,4'-tricloro-2'-hydroxy diphenyl ether (triclosan) were assessed in laboratory, *in situ* and long term clinical studies to ascertain mechanism(s) of action. Triclosan inhibited purified enzymes from the cyclooxygenase and lipooxygenase pathways at 8 and 1 μg mL^{-1}, respectively. Further, triclosan reduced[28] (Mustafa *et al*., 2000) the formation of IL-1β, IFN-γ and microsomal prostaglandin E synthase-1 expression in human gingival fibroblasts. Fusafungine, a peptide antibiotic effective on a range of Gram-positive bacteria and certain types of Gram-negative cocci in the airways. Human studies and in a rabbit model of sinus inflammation indicated its anti-inflammatory effects[29a] (German-Fattal *et al*., 2004) attributed to an increase in NK cell activity, inhibition of proinflammatory cytokines such as IL-1, TNF-α and down regulation of intracellular adhesion molecules. Tauroline (taurolidone, bis (1,1-dioxoperhydro-(1,2,4,thiazinyl-4)-methane), a stable non-antibiotic antimicrobial agent irreversibly binds and inactivates LPS[29b] (Bedrosian *et al*., 1991). The anti-inflammatory property of tauroline is attributed to its effects at blocking LPS induced production of IL-1 and TNF.

In addition to the modulatory effects of antibacterial agents on the processes leading to the development of the anti-inflammatory response, there is also evidence for their ability to facilitate the resolution of inflammation through stimulation of apoptosis (programmed cell death). Indeed, therapeutic induction of apoptosis as a means to resolve chronic inflammation is gaining increasing interest. Neutrophils are normally extremely short lived, with a circulating half time of only 6-7 h. This means that normal individuals make (and destroy) about 50 billion

neutrophils per day, and many more in inflammatory states[12a] (Labro *et al.*, 2000). Importantly, phagocytosis of bacteria also induces apoptosis in neutrophils and this is accompanied by specific gene-mediated attenuation of many functional aspects of these cells. In contrast to necrotic neutrophils apoptotic neutrophils are ingested by macrophages[30] (Ren *et. al.*, 2003). Thus, granulocyte-induced tissue injury and chronic inflammation may result not only from excessive leukocyte recruitment but also inhibition of normal apoptosis based clearance mechanisms.

1.3. Chemistry of 2-mercaptobenzothiazole

2-Mercaptobenzothiazole (MBT) is an interesting bicyclic heteroatomic molecule. As regards to more fundamental aspects, one of the most interesting properties of MBT is existence of thiol (**1**) and thione (**2**) tautomeric forms (**Figure 3**).

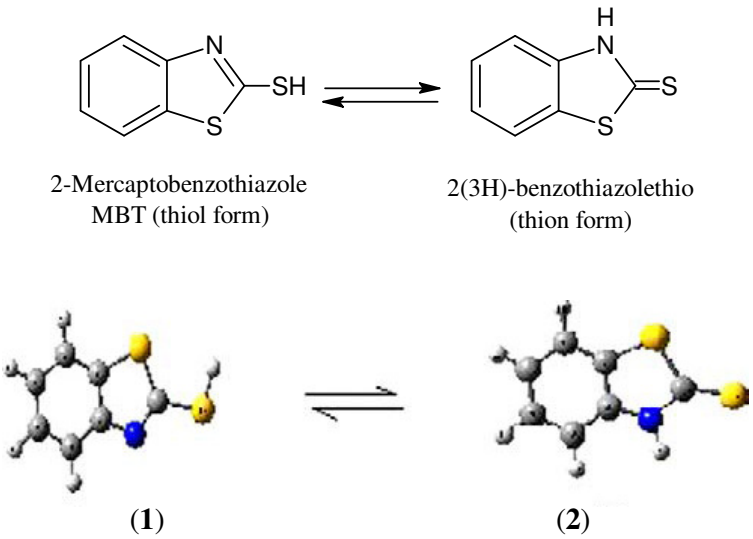


Figure 3. Optimized geometry of 2-MBT monomer in thiol (**1**) and thion (**2**) forms

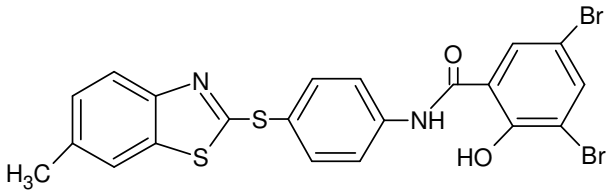
The first preparation of MBT was reported[31] (Hofmann, 1887) in the 19th century. The classical synthesis reported by Hofmann involves the condensation of o-amidothiophenol and carbon disulfide. Further research on this compound and its possible derivatives was desirable when its value as an accelerator of rubber vulcanization was discovered. The preparation of MBT from aniline, sulfur and carbon disulfide described by Sebrell and Boord[32] (1923) not only offered a commercial preparation but made it possible to prepare several derivatives, using substituted aniline according to the same method. However the process is unsuitable for the synthesis of 6-halogenomercaptobenzothiazoles, since only undefinable products are formed in

the reaction with 2,4-dichloronitrobenzene. For laboratory use it seemed very desirable to develop a simple method of preparing MBT which could be extended to several new derivatives. This problem was alleviated by Teppema and Sebrell[33] (1927) who prepared it in one step from o-nitrochlorophenol, a comparatively cheap material. This method is capable of extension to the preparation of other derivatives such as the halogen, amido and carboxyl derivatives from the corresponding o-nitrochlorophenol derivatives. The synthetic potential of such reactions has subsequently been evaluated by Dunbrook and Zimmermann[34] (1934) who obtained MBT in good yield (87.5-90%) by heating o-nitrochlorobenzene with carbon disulfide in the presence of sodium polysulfide. The reaction was completed in a shorter time and the optimum yield of MBT was somewhat higher than those of reported by Teppema and Sebrell[33] (1927). Since then various processes for the preparation of MBT are described in the literature, the preparation of unsubstituted MBT, in particular, being the subject of a large number of applications and publications[35-37] (Magill *et al.,*1934; Bronx *et al.,*1963; Handte *et al.,*1984). A method for preparing MBT distinctly different from processes previously recorded was described by Zhu *et al.*[38,39] (2004 and 2005) using aromatic nucleophilic substitution reactions of ortho-haloanilines with potassium/sodium *O*-ethyl xanthate at relatively mild temperatures (95-120 °C) with good yields. However, the yields of MBT were not satisfied when ortho-chloroanilines rather than ortho-fluoroanilines were used as substrate. In addition, reaction time was very long, and it was difficult to isolate and purify the products.

In search of a rapid and efficient method for the synthesis of MBT under milder conditions, Huang *et al.*[40] (2007) for the first time prepared substituted MBT under microwave irradiation by allowing the ortho-haloanilines to react with potassium *O*-ethyl dithiocarbonate on a solid support. 2-Mercaptobenzothiazole with different substituent in the aryl ring was obtained in high yields (45-87%). Alternative cyclization procedure have been developed by Narkhede *et al.*[41] (2007) who prepared S-alkyl, S-acyl and dimeric derivatives of MBT under microwave irradiation in higher yields (77-87%) using silica gel, alumina and a new solid support, fly ash, a waste generated at thermal power stations.

Very little use has been made of nucleophilic and electrophilic aromatic substitutions in the 2-mercaptobenzothiazole series; hence the influence of the thiazole ring on such processes is not well established. The nitration products of MBT have been prepared and studied by Teppema *et al.*[42] (1927). The position of the nitro group in nitro-2-mercaptobenzothiazole has been proved to be in the benzene ring in position 6. The chlorination of the MBT in aqueous acetic acid is proved to be an exothermic reaction[43] (Findlay *et al*., 1966) and the best yield (47%) of 2-chlorobenzothiazole was obtained when temperature of the reaction mixture was maintained at

45 °C. The mechanism of MBT S-alkylation together with complications concerning N-alkyl compound formation has been discussed[44-50] (D'Amico, 1953; D'Amico, 1957; Morgan, 1958; D'Amico, 1961; Easmon *et al.*, 1997; Diekman and Park, 1973; Rocklin *et al.*, 1965). Ligand-assisted Cu(I)-catalyzed sequential intra- and intermolecular *S*-arylations leading to the direct synthesis of arylthiobenzothiazoles in one pot from dithiocarbamates was developed[51] (Murru *et al.*, 2009) and this strategies was finally applied to the synthesis of cathespin-D inhibitor analog (**3**) as demonstrating its potential utility.



Cathepsin-D inhibitor analog

(**3**)

Owing to the importance of MBT as an accelerator for the vulcanization of rubber with sulfur has stimulated many workers to prepare and extensively evaluate its derivatives. Among the processes reported for the preparation of benzothiazole-2-sulfenamides[52] (Ebelke, 1944) is one involving an intermediate benzothiazole-2-sulfenyl chloride[53] (Messer, 1941). These compounds have found wide application as accelerators in the rubber processing industry. Alternate methods for the direct preparation of sulfenamides have also been described[54-56](Hanalick, 1942; Cooper, 1944; Smith, 1951). The work of D'Amico *et al.*[57,58] (1963 and 1965) is important in connection with the synthesis of benzothiazolesulfenamides which avoided the use of an amine as a reactant and also furnishes the desired compound in higher yields (76-90%). In 1948, Allen *et al.*[59] described the synthesis of sulfamyl-2-mercaptobenzothiazoles by heating together a mixture of N-(4′-substituted phenyl)-3-nitro-4-chlorobenzenesulfonamide, sodium sulfide and sulfur in water on a steam bath. In another report Hendry[60] (1961) proposed a single step process for preparing secondary aminothiazoledisulfides by the reaction of MBT with a secondary amine, chlorine and a sulfur halide in high yield (>83%) and found to be useful as delayed action and non-scorching accelerators in rubber compounding. A detailed study[61] (Halasa *et al.*, 1971) has been made of the conditions necessary for the Michael and Mannich reactions with MBT. Also there are reports[62-67] (Katritzky *et al.*, 1987; Chevrie *et al.*, 2003; Huang *et al.*, 2000; Nunno *et al.*, 2000; Rodriguez *et al.*, 2000; Tsubouchi *et al.*, 1995) of using MBT derivatives as useful synthones for various chemical reactions leading to the synthesis of various biologically active molecules.

1.4. Quantification and identification of 2-mercaptobenzothiazole (MBT)

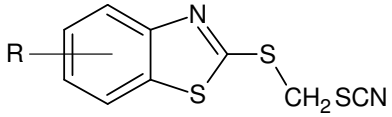
The structural characterization of MBT by infrared (IR) spectra was performed by Chesick *et al.* [68] (1971), Dong *et al.*[69] (1995) and Mohamed *et al.*[70] (2008). Bohlig *et al.*[71] (1999) studied the IR and Raman spectra of this molecule along with semiempirical calculation for normal mode frequencies in its monomeric thiol form (I). In 2006, Rai *et al.*[72] have performed the vibrational analysis of this molecule in detail, using IR and Raman spectroscopy and density function theoretical (DFT) calculations for the monomeric thiol and thione form of this molecule. The structural identification of mercaptobenzothiazoles by NMR, MS and IR spectroscopy was performed by Fiehn *et al.*[73] (1997) in addition to HPLC/UV and GC/MS for their analysis. The GC-MS was also developed by Wilson *et al.*[74] (1991) to identify some benzothiazoles in urine extracts. During the last decade, Niessen *et al.*[75] (1993), Sandhyarani and Pradeep[76] (2000), Chipinda *et al.*[77] (2007) and others explored new chromatography-mass spectrometry (LC-MS) techniques for their ability to detect and identify MBT and its derived products. Recently Leerdam *et al.*[78] (2009) developed a sensitive method for the trace determination of MBT by LC-MS. It was analyzed using thin layer chromatography (TLC) in 1988 by Larsen *et al*.[79], also by Fukuoka and Tanaka[80] in 1987. TLC has been also used by Jung *et al.*[81] (1988) and Dhindsa *et al.*[82] (1990).

1.5. Biologically active 2-mercaptobenzothiazole derivatives

MBT and its derivatives are manufactured worldwide for a wide variety of applications. It also plays a role in analysis as a reagent for cadmium as well as for the determination of copper, lead, bismuth, silver, mercury, thallium, gold, platinum and iridium[83] (Ullmann's Encyclopedia of Industrial Chemistry, 1995). S-Alkyl and S-acyl derivatives of MBT were reported to possess antifungal and antibacterial activities[84,85] (Budjakova *et al.*, 1994; Sidoova *et al.*, 1997) and also found to be useful in the leather industry[86] (Lee *et al.*, 1997). 2-(Thiocyanomethylthio)benzothiazole is a potential contact fungicide[87] (Muthusubramanian *et al.*, 2001) for several economically important crops such as barley, cotton, corn and wheat. 2,2′-Dithiobis(benzothiazole) is used as a fungicide, insecticide, sensitizer and anti-scorching agent in vulcanization of rubber[88] (Ramadas *et al.*, 1999).

In the year 1979 Rada *et al.*[89] screened a series of benzothiazole derivatives for their virus inhibitory activity and found antiviral activity for MBT with two out of three viruses tested. Kuchta *et al.*[90] (1989) studied the anti-*Candida* activity of MBT, as they assumed yeast and yeast-like organisms to be the most sensitive. The antifungal effects of MBT were also tested

towards *Aspergillus niger* with a suspension of spore-free mycelium homogenate as inoculums. After 5 days of cultivation, 33 mg L^{-1} MBT was the lower limit for 100% growth inhibition. Similar results, although obtained in other conditions, were reported by [91] (Foltinova *et al.,* 1978) for the fungus *Trichophyton rubrum*, but for complete growth inhibition of *Microsporum gypseum* and *Epidermophyton floccosum* MBT concentrations had to exceed 50 mg L^{-1}. The results of Owens[92] (1969) suggested that the thiol group of MBT is essential for toxicity, since benzothiazole (BT) was not an active fungicide. A similar suggestion can be made from the experiments of Dimond and Horsfall[93] (1963), where the presence of zinc destroyed the fungicidal activity of MBT. Antifungal activity of S-thiocyanomethyl compounds of 2-mercaptobenzothiazoles (**4**), which in turn was prepared by

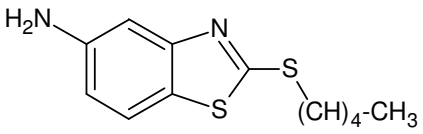


R=H,Cl,Br,NO_2,NH_2,OH,CH_3,C_2H_5

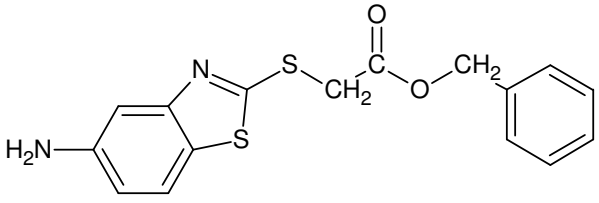
(**4**)

reacting a metal salt of MBT or substituted mercaptobenzothiazoles with chloromethyl thiocyanate in an alcoholic solution is described[94] (Buckman *et al*., 1970). A significant inhibitory activity against *Aspergillus niger*, *Penicillium roquefortl* and *Chaetomium globosum* (MIC 75, 50 and 50 ppm, respectively) was observed for the compound 2-(thiocyanomethylthio) benzothiazole (**4**:R=H) after twenty eight days incubation. On the other hand 5-chloro and 6-nitro analogs exhibited potent inhibitory activity against *Aspergillus niger* and *Chaetomium globosum* (MIC 5 and 7 ppm, respectively after 14 days incubation.

The effects of 6-amino-2-n-pentylthiobenzothiazole (APB) (**5**) on ergosterol biosynthesis in *Candida albicans* and *Saccharomyces cerevisiae* were studied[95-97] (Kutcha *et al.*, 1992; Bujdakova *et al.*, 1994; Kuchta *et al.*, 1995). APB markedly blocks formation of ergosterol in *Saccharomyces cerevisiae* and accumulation of squalene, lanosterol, 4,4-dimethylzymosterol, and 4-methylfecosterol were observed. In another report Bujdakova *et al.*[98] (1993) described



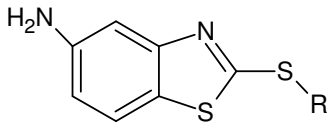
(**5**)



(**6**)

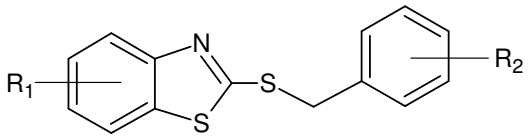
the anti-*Candida* activity of 6-amino-2-n-pentylthiobenzothiazole (APB) (**5**) and benzylester of 6-amino-(2-benzothiazolylthio)acetic acid (**6**) and compared to that of MBT. They assumed yeast and yeast-like organisms to be the most sensitive. For all 15 *Candida* strains tested, MBT brought about 50% growth inhibition at concentrations varying between 1 and 78 mg L^{-1}. Compounds (**5**) and (**6**) exhibited good activity against the *Candida albicans* yeast form, similar to MBT.

In 1997, Sidoova *et al.*[99] described the synthesis and antimicrobial activity of 2-alkenylthio-6-aminobenzothiazoles (**7**). The authors disclosed that the compounds have shown antibacterial activity against *Staphylococcus aureus* and anticandidous activity against *Candida albicans*. Compounds bearing $-(CH_2)_3-CH=CH_2$ or $-CH_2CH=CH-C_2H_5$ groups at second position of the MBT ring showed maximum inhibitory effect (MIC 15.6 μg mL^{-1}) against *Candida albicans*.



R= $-CH_2CH_2CH=CH_2$, $-CH_2CH=CHCH_3$, $-(CH_2)_3CH=CH_2$, $CH_2CH=CHC_2H_5$, $-CH_2CH=C(CH_3)_2$, $-(CH_2)_4CH=CH_2$

(**7**)



R_1=4-F, 6,7-difluoro, 4-Cl-6-CF_3
R_2=2-F, 2,6-difluoro, 3,4-difluoro

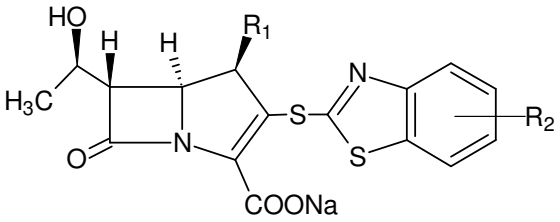
(**8**)

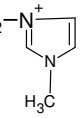
Huang *et al.*[100] (2006) has prepared a series of polyfluorinated 2-benzylthiobenzothiazoles (**8**) and tested them for their antifungal activities. Most of the synthesized compounds showed significant fungicidal activity against *Rizoctonia solani*, *Botrytis cinereapers* and *Dothiorella gregaria* at 50 μg mL^{-1} concentration.

Not only does MBT exert adverse effects on viruses, yeasts and fungi, it also acts on bacteria. For this reason, the compound was under study as a potential nitrification inhibitor in soils[101] (Bremner and Krogmeier, 1989). Another example of the antibacterial activity of MBT is given by Foltinova *et al.*[91] (1978). Growth inhibition of *Staphylococcus aureus*, *Bacillus subtilis* and *Esherichia coli* was 50% at about 42 mg L^{-1} and 100% at about 135 mg L^{-1}. Aktulga[102] (1972) considered MBT to be strongly inhibitory towards *Corynebacterium diphtheriae* and considerably less effective against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* In the experiments of Reemtsma *et al.*[103] (1995), 2-mercaptobenzothiazole concentrations of 7 mg L^{-1}, at maximum, were found to be too low to

obtain 50% growth inhibition of *Vibro fischeri.* Extensive studies[104] (De Wever *et al.*, 1994) indicated clearly that MBT in particular inhibited the growth of bacteria and yeast, but its effects were bacteriostatic rather than bactericidal and were different for different species. In some reports, the effect of MBT on specific bacterial enzymes is studied. Shuto *et al.*[105] (1989), in their search for new herbicides, focused on inhibitors of tryptophan synthase. Tests with the well characterized tryptophan synthase from *Escherichia coli* showed a considerable inhibitory activity for MBT. MBT also seems to be an excellent inhibitor of lactate metabolism in *Desulfovibrio desulfuricans.* Possibly, the sulfhydryl group of MBT complexes with an essential divalent cation, such as iron, which is assumed to be present in the lactate dehydrogenase complex[106] (Czechowski and Rossmoore, 1981). Similar mechanisms are presented by other authors to explain enzyme inactivation by MBT[107-109] (Pierpoint, 1966; Palmer and Roberts 1967; Johnson *et al.*, 1970)

2-Mercaptobenzothiazole derivatives have been pointed to as promising antibacterial agents. For example benzothiazol moiety linked by sulfur to the 3-position of carbapenems (**9**) displayed significant potency against methicillin-resistant *Staphylococcus aureus* (MRSA)[110] (Waddell *et al.*, 1995), leading to the hypothesis that MBT moiety is a "binding element". It was observed that introduction of a 1-β-methyl group at position 1 enhanced the DHP-1 stability of compounds at least six-fold more compared to the 1-β-hydrogen analogs. A parallel increase in chemical stability is also observed. Further, the introduction of a 1-β-methyl group appeared to enhance potency against MRSA.

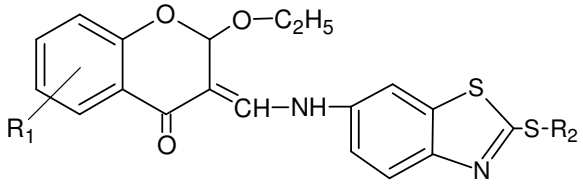


R_1=H,CH_3; R_2=H,5-Cl,5-CH_2OH,5-CF_3,6-CN,
6-NO_2,6-OC_2H_5,6-NH_2,6-Cl, 5-CH_2–N$^+$ 

(**9**)

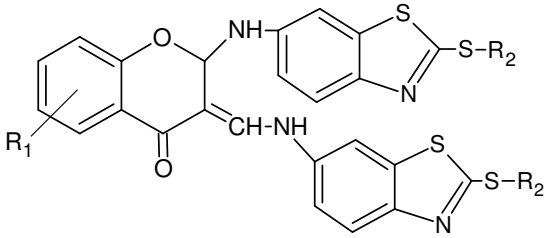
The synthesis and antimicrobial activity of a series of MBT derivatives (**10**) and (**11**) bearing chroman-4-one moiety is described by El-Shaaer *et al.*[111] (1998). Authors disclosed that derivatives (**10**) bearing 6-Cl or 6,7-dimethyl substituents in the benzothiazole ring exhibited

significant *in vitro* activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and against *Mycobacterium tuberculosis*. The same compounds showed potent activity against *Candida albicans* and *Saccharomyces cerevisiae*.



R_1=6-Cl,6,7-$(CH_3)_2$,6-CH_3
R_2=H,2,4-dinitrophenyl, CH_3

(**10**)

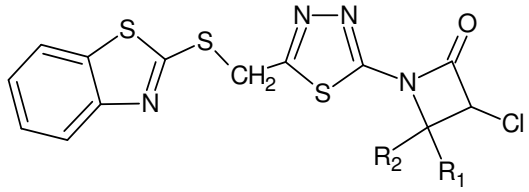


R_1=6-Cl,6,7-$(CH_3)_2$,6-CH_3
R_2=H,2,4-dinitrophenyl, CH_3

(**11**)

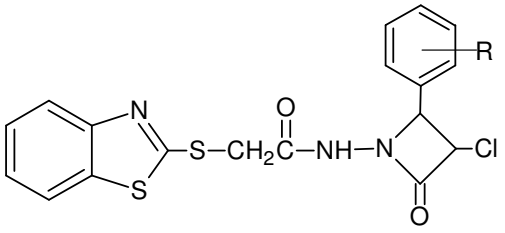
In search of an ideal antibacterial agent, derivatives of MBT possessing both 1,3,4-thiadiazole and 3-chloro-2-azetidinone moieties (**12**) have also been prepared[112,113] (Guru *et al.*, 2001; Srivastava *et al.*, 2004). The investigators disclosed that derivatives having phenyl or 4-chlorophenyl substituents at fourth position of the azetidinone nucleus as the most potent compound against both Gram-positive and Gram-negative bacterial strains.

In a preliminary study Desai *et al.*[114] (2005) described the synthesis and *in vitro* antimicrobial activity of mercaptobenzothiazoles derivatives (**13**) bearing substituted azetidin-2-one moiety at second position of the MBT nucleus. The authors found that compound having 2-chlorophenyl group at fourth position of the azetidinone nucleus was most active against the tested strains of bacteria and fungi.



R_1=H,CH_3,C_6H_5
R_2=C_6H_5,2-Cl.C_6H_5,3-Cl.C_6H_5,4-Cl.C_6H_5,CH_3,2-NO_2.C_6H_5
CH=CH.C_6H_5,4-OCH_3.C_6H_4,4-Cl.C_6H_5,4-N$(CH_3)_2$.C_6H_5
2-Br.C_6H_5,3-Br.C_6H_5,4-Br.C_6H_5,2,4-di-NO_2.C_6H_5

(**12**)

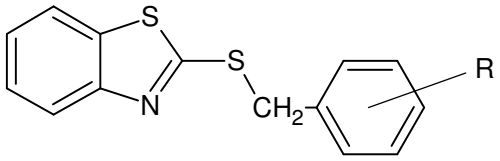


R=4-NO_2,3,4,5-(OCH_3),2-OH, 3-OH,4-OH,
2-OCH_3,4-OCH_3,2-Cl,3-Cl,4-Cl

(**13**)

2-Benzylsulfanyl derivatives of MBT (**14**) are also effective in the inhibition (*in vitro*) of *Mycobacterium tuberculosis* and non-tuberculous mycobacteria. Compounds bearing two nitro

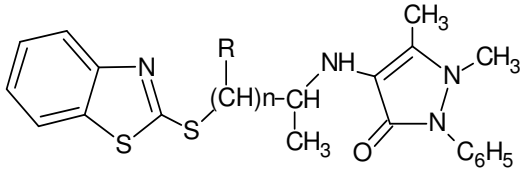
groups or a thioamide group exhibited appreciable activity particularly against non-tuberculous stains[115,116] (Koci *et al.*, 2002; Kumar *et al.*, 2005).



R= H,4-NO_2,3-NO_2,2-NO_2,3,5-di-NO_2,2,4-di-NO_2
2-Br,2-NO_2,4-I,4-Br, 2-Br-5-I,2-Br-3-CH_3,2,5-di-Br
4-CN,3-CN,4-$CSNH_2$,3-$CSNH_2$

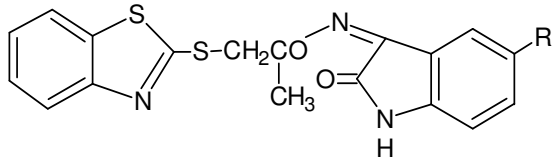
(**14**)

Incorporation of 3-pyrazolinone (**15**) and 2-indolinone (**16**) moieties at the second position of MBT nucleus resulted in a marked increase in antibacterial activity. Particularly, 2-indolinone derivatives bearing nitro or fluoro groups were found to be more potent[117] (Karali *et al.*, 2004) against *Staphyloccocus epidermidis*.



R=H,CH_3,C_6H_5,C_2H_5

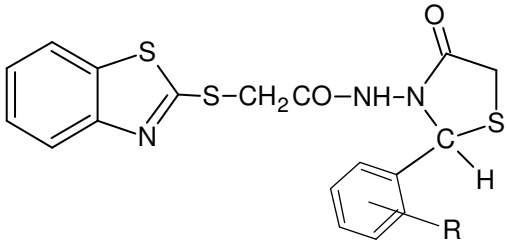
(**15**)



R=H,CH_3,F,Br,NO_2

(**16**)

In 2006, Desai *et al.*[118] described the synthesis and antimicrobial activity of MBT carrying 4-oxo-thiazolidine moiety (**17**). *In vitro* screening results indicated significant inhibitory activity of derivatives having 4-NO_2, 2-OH, 4-OCH_3 or 4-Cl groups in the phenyl ring at second position

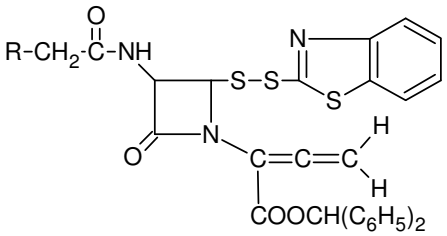


R=4-NO_2,3,4,5-$(OCH)_3$,2-OH,3-OH,4-OH,2-OCH_3,
4-OCH_3,2-Cl,3-Cl,4-Cl

(**17**)

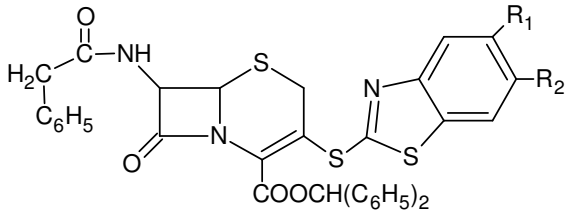
of the thiazolidine ring against *Staphyloccocus aureus* and *Bacillus subtilis*. The same compounds also showed significant inhibitory activity against *Candida albicans*, *Candida krusei* and *Candida parapsilosis.*

Kant *et al.*[119] (1994) proposed an efficient approach to the synthesis of 3-substituted cephems bearing carbon-based substituents of choice at the C(3) position from inexpensive penicillins. The strategy involves the synthesis of an allenylazetidinone (**18**) from penicillin sulfoxide followed by the addition of an organocuprate at low temperature. The chemistry has been applied to the synthesis of a variety of 3-substituted cephems bearing MBT moiety (**19**). Allenylazetidinone (**18**) was also used to prepare precursors to synthesize important antibiotics, i.e. cefadroxil, cefixime and cefzil.



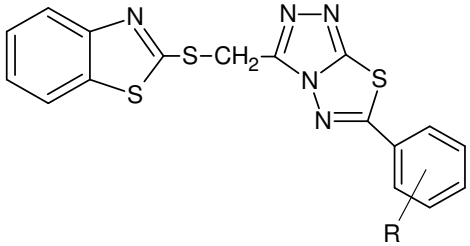
R=C_6H_5,C_6H_5O

(**18**)



(**19**)

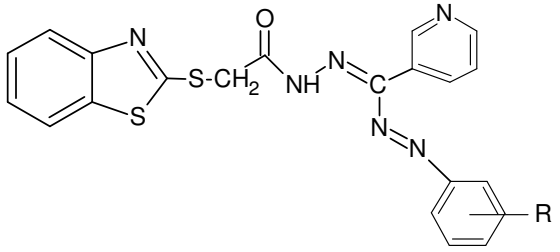
2-Mercaptobenzothiazole has been pointed to as promising anthelmintic agents. For example derivatives of 2-mercaptobenzothiazole containing 2-[(6-phenyl/substituted phenyl [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl] (**20**) and [substituted phenyldiazenyl] (pyridine-3-yl)methylidene]acetohydrazide (**21**) moieties were found to be effective against *Hymenolepis nana* infection in mice[120,121] (Hussain *et al.*, 1992 and 1993).



R=H,3-NO_2,4-NO_2,4-Cl,2-Cl,

R=H,3-NO_2,4-NO_2,4-Cl,2-Cl

(**20**)

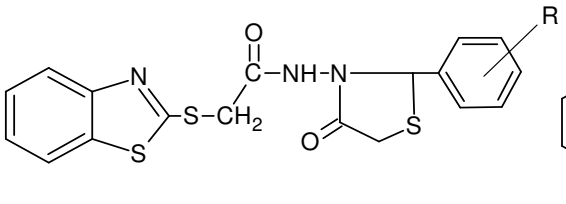


R=4-Br,4-NO_2,4-CH_3,4-OCH_3,3-NO_2

(**21**)

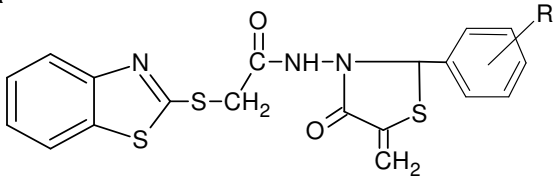
Compounds containing MBT have also been reported to possess analgesic and anti-inflammatory activities. For example derivatives (**22**) and (**23**) containing 4-oxothiazolidine and

their 5-arylidenes moieties at the second position of MBT nucleus showed moderate to weak anti-inflammatory activity in carrageenan induced rat paw oedema method[122] (Srivastava *et al.*, 2004). Moreover, compounds possessing 3-Cl.C_6H_5, 2-Br.C_6H_5, 3-Br.C_6H_5, 4-Br.C_6H_5 substituents at second position of the 4-oxothiazolidine ring exhibited significant antibacterial and antifungal activities. Few compounds were also reported to have anthelmintic activity against *Ancyclostoma ceylanicum* and *Hymenolepsis nana* at a dose of 250 mg mL^{-1}.



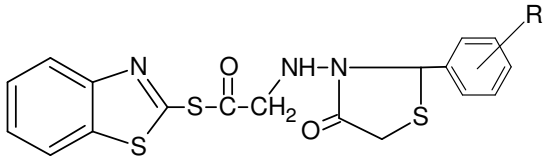
R=4-NO_2,3,4,5-$(OCH)_3$,2-OH,3-OH,4-OH,
2-OCH_3,4-OCH_3,2-Cl,3-Cl,4-Cl

(**22**)



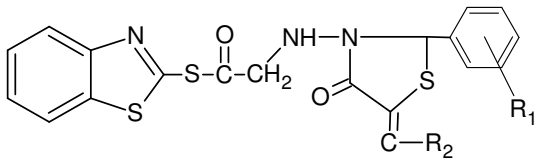
(**23**)

A novel series of compound bearing 4-oxothiazolidine (**24**) and their 5-arylidenes (**25**) moieties at second position of the 2-mercaptobenzothiazole (**1**) nucleus has been synthesized and evaluated for their antimicrobial and anti-inflammatory activities[123] (Yadav *et al.* 2005). There was a marked increase in the antibacterial and antifungal activities but no appreciable increase in the anti-inflammatory activity was observed.



R=2-NO_2,3-NO_2,3-Cl,4-Cl,2-Br,3-Br,
4-Br,2-OH,3-OH,4-OH

(**24**)

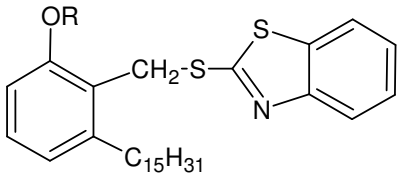


R_1=R_2=2-NO_2,3-NO_2,3-Cl,4-Cl,2-Br,3-Br,2-OH,3-OH

(**25**)

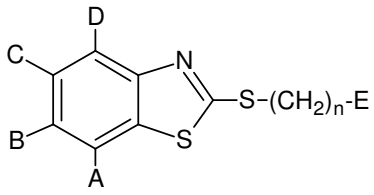
In search of ideal anti-inflammatory agent, which devoid of typical side effects common to all anti-inflammatory agents, Paramashivappa *et al.*[124] (2003) have synthesized 2-{[2-alkoxy-6-pentadecylphenyl) methyl]thio]-1H-benzothiazoles (**26**) using anacardic acid and investigated their ability to inhibit human cyclooxygenase enzyme (COX-2). Compound bearing OCH_3 group at the second position of the phenyl ring was found to be 470-fold selective towards COX-2 compared to COX-1.

Anderson *et al.*[125] (1989) examined a series of compounds (**27**) as inhibitors of leukotriene biosynthesis and/or as inhibitors of the action of lipoxygenase and/or as inhibitors of slow reacting substance of anaphylaxis (SRS-A) and thus its smooth muscle contracting and secretory effects. The compounds 2-(5-hexenylthio)-6-benzothiazolol maleic acid salt and 6-{[6-(3-carboxy-1-oxopropyl)amino]-2-benzothiazolyl]thio]hexanoic acid methyl ester caused marked inhibition of SRS-A. Some of the compounds showed significant inhibition of leukotriene and lipoxygenase.



R=CH_3,C_2H_5

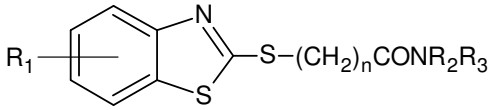
(**26**)



n=4-11; A=H or OH; B=H,OH,OCH_3;
C=H,OH,$NHCO(CH_2)_2COOH$,tetrahydropyran-2-yl-oxy;
D=H,OH,OCH_3;E=H,Cl,Br,CN,$OCOCH_3$,$CH=CH_2$
$COOCH_3$,$CH=CH_2$,$C(CH_3)_3$

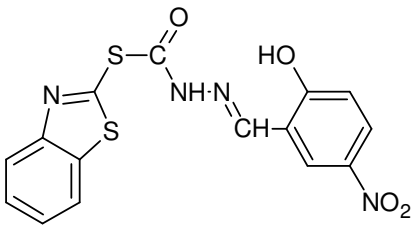
(**27**)

In 1992, Greco *et al.*[126] have synthesized a series of N-[(2-benzothiazolylthio)alkyl]-N'-hydroxyurea derivatives (**28**) and evaluated for biological activity as inhibitors of 5-lipoxygenase both *in vivo* and *in vitro*. Results indicated that the hydroxyl group of the hydroxyurea must be unsubstituted. It is also evident that the N′-methyl substituent imparts superior activity *in vivo*; this correlation is not as pronounced *in vitro*.



n=1 or 2
R_1=H,6-OC_2H_5,5-Cl,7-Cl,6-$CH(CH_3)_2$
R_2=H,OH,OCH_3,
R_3=H,CH_3,$CH(CH_3)_2$

(**28**)



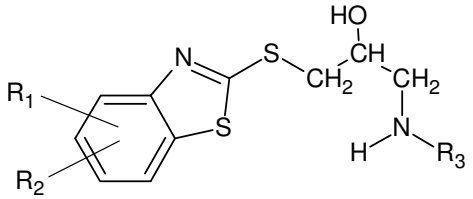
(**29**)

In search for compounds that can selectively inhibit neutrophil activation by inflammatory mediators Han *et al.*[127] (2006) identified compound (**29**) by means of high throughput, which inhibited the respiratory burst (RB) of human neutrophils in response to soluble inflammatory mediators, such as TNF and formylated methionyl-leucyl-phenylalanine (fMLF), but not in

response to phobol myristate acetate (PMA) and did not suppress the antibacterial activity of neutrophils.

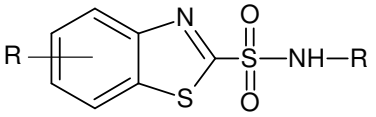
In continuation of work seeking novel propanolamines which have excellent antihypertensive activity without showing any β-adrenergic receptor blocking activity, Hibino *et al.*[128] (1976) investigated the antihypertensive activity of the analogs of 2-mercaptobenzothiazole (**30**). Most of the compounds exhibited significant antihypertensive activity.

In the year 1973 Korman[129] prepared a number of aryl-substituted benzothiazole-2-sulfonamides (**31**). All the synthesized compounds showed potent carbonic anhydrase inhibitor activity. One of these, the 6-ethoxybenzothiazole-2-sulfonamide, produced a clinically useful diuresis.



R_1=H,Cl,NO_2,COOH,$COOCH_3$,$NHCOCH_3$
R_2=H,CH_3,NH_2,NO_2
R_3=$CH(CH_3)_2$,$CH_2CH_2(CH_3)_2$,$CH(CH_3).C_6H_5$

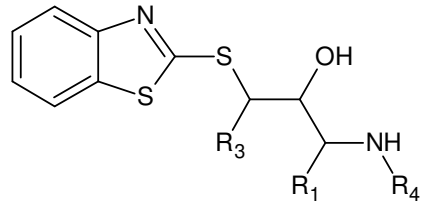
(**30**)



R=H,4-CH_3,6-CH_3,5-Cl,4-OCH_3,
6-OC_2H_5,6-$NHCOCH_3$

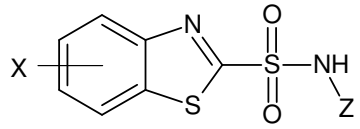
(**31**)

Analogs of 2-mercaptobenzothiazole (**32**) containing substituted hydroxypropyl carbamate group at the second position have been synthesized by Fitzpatrick *et al.*[130] (2004 and 2005). Compounds were evaluated for the inhibition of transient lower oesophageal phincter relaxations and for the treatment of gastro-oesophageal reflux disease and found to have high affinity and potencies for the $GABA_B$ receptors as revealed by low IC_{50} and EC_{50} in the ^3H GABA radioligand binding and ileum assay, respectively.



R_2=H,C_1-C_7 alkyl,C_1-C_7 alkoxy,C_1-C_7 thioalkoxy,
mercapto,aryl, heteroaryl
R_3=t-butyl,phthalimide

(**32**)



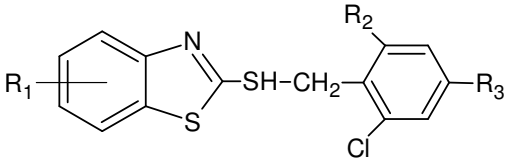
Z=H,$COCH_3$,CH_3,C_3H_7,5-Cl,4-OCH_3,
X=6-OC_2H_5,6-OH,6-Cl,6-Br,6-F,
6-NH_2,6-$OCOCH_3$

(**33**)

Schoenwald and Barfknecht[131] (1990) described synthesis of analogs of benzothiazole-2-sulfonamides (**33**) as carbonic anhydrase inhibitors. Compounds were tested topically for their

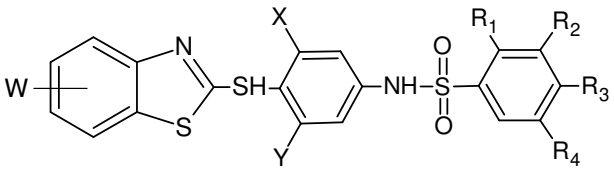
ability to reduce intraocular eye pressure in glaucoma. Among these, 6-chlorobenzothiazole-2-sulfonamide was found to be highly effective.

McGee *et al.*[132] (2004) have focused on the identification of 2-mercaptobenzothiazole analogs (**34**) and (**35**) as peroxisome proliferator-activated receptors (PPARγ) activators and compounds were found to be useful agents for the treatment of obesity and related disorders associated with undesirable adipocytes maturation.



R_1=5-CH_3,7-CH_3,5-Cl,6-Cl,
7-Cl,8-Cl,7-SO_2CH_3
R_2=H,Cl
R_3=NH_2,NO_2,CH_3,Cl,

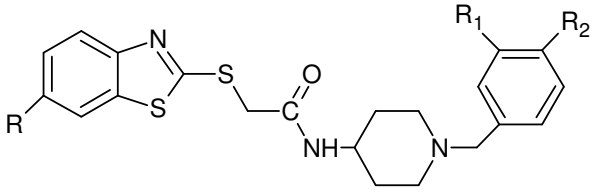
(**34**)



X=Cl,F; Y=H,Cl;R_1=H,Cl,CH_3; R_2=H,Cl
R_3=H,Cl,I,CH_3,t-Bu,CF_3; R_4=H,CH_3,
W=6-Cl,4-CH_3,6-SO_2CH_3

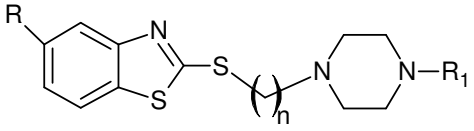
(**35**)

To identify an orally active non-peptide antagonist that is selective for the CCR3 receptors, Naya *et al.*[133,134] (2001 and 2003) identified 2-(benzothiazolylthio)acetamide derivative (**36:** R=R_1= R_2=H) as lead compound which on further derivatization led to the identification of potent and selective antagonists. The 7-acetamidobenzothiazole derivative (**36:**R=-$NHCOCH_3$, R_1=R_2=Cl) with an IC_{50} of 1.5 nM and a 3600-fold selectivity over that of CCR1 receptor was found to be the best compound of this series.



R=H,OC_2H_5,$CONH_2$,NH_2,$NHCOCH_3$,$NHCOC_6H_5$
$CONHCH_3$,$CON(CH_3)_2$,$CONHCH_2C_6H_5$
R_1=R_2=H,Cl

(**36**)



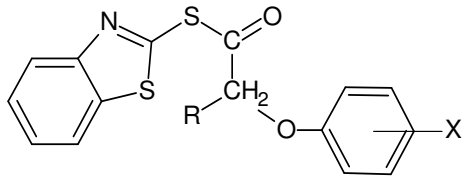
n= 0,1,2,4
R=H,Cl
R_1=2-$CH_3OC_6H_5$, 2-$NO_2C_6H_5$,
pyridin-2-yl,pyrimidin-2-yl

(**37**)

A series of new compounds (**37**) containing a benzothiazole nucleus linked to an arylpiperazine by different thioalkyl chains was prepared by Siracusa *et al.*[135] (2008). They were tested in radioligand binding experiments to evaluate their affinity for 5-HT_{1A} and 5-HT_{2A} serotonergic, α_1 adrenergic, D_1, and D_2 dopaminergic receptors. Many of tested compounds

showed an interesting binding profile; in particular, compound bearing 4-(2-methoxyphenyl) piperazine linked to the 2-mercaptobenzothiazole nucleus by methylene group displayed very high 5-HT_{1A} receptor affinity and selectivity over all the other investigated receptors.

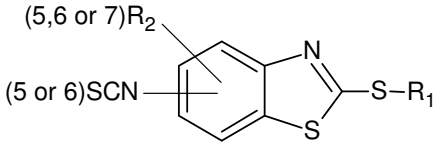
Khare *et al.*[136] (1996) synthesized substituted phenoxyacetyl as well as propionyl-2-mercaptobenzothiazoles (**38**) and tested their anthelmintic, analgesic and antimicrobial activities. Authors disclosed marked antimicrobial activities of di/trihalophenoxyacetyl-2-mercaptobenzothiazoles, whereas 2,4,6-trichloro/2,4,6-tribromophenoxypropionyl-2-mercapto-benzothiazoles showed significant analgesic and anthelmintic activities.



R=H or CH_3
X=2-NO_2,3-NO_2,4-NO_2,2,4,6-$(NO_2)_3$,2-Cl
3-Cl,4-Cl,2,4-di-Cl,2,4,6-tri-Cl,2,4,6-tri-Br

(**38**)

In 1976, Gallay *et al.*[137] has prepared a series of 2-alkylthio-5/6-isothiocyanobenzothiazoles (**39**), as antimicrobial and anthelmintic agents. The compounds were tested in mice infested by *Hymenolepsis nana* or *Nematospiroides dubius*, in rats infested by *Fasciola hepatica* and in fowl infested with *Ascaridia galli*. Same compounds were also tested for their antimicrobial activities against different strains of Gram-positive and Gram-negative bacteria and against ten strains of fungi. Some of the tested compounds exhibited significant anthelmintic and antimicrobial activities.

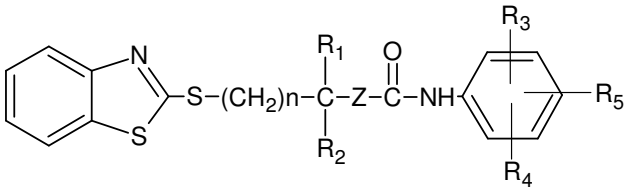


R_1=H,CH_3,C_2H_5,$(CH_2)_2CH_3$,$CH(CH_3)_2$,
$(CH_2)_3CH_3$,cyclopentyl,cyclohexyl
R_2=H,CH_3,C_2H_5,$CH=CH_2$,$COCH_3$,CH_2-C_6H_5,

(**39**)

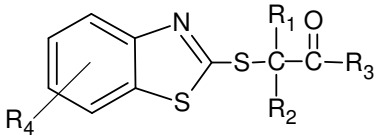
Shibuya *et al.*[138,139] (2001 and 2004) synthesized a series of pharmacological active anilides (**40**), which were found acyl coenzyme A cholesterol acyltransferase (ACAT) inhibitor and therefore useful as anti-hyperlipidemic agent. Serum cholesterol and triglyceride lowering

activities are also reported[140] (Doll *et al.,* 1981) to be associated with 2-mercaptobenzothiazole anlogs (**41**). In addition, a shift in the ratio of α-lipoproteins and β-lipoproteins in the direction of increasing α-lipoproteins was observed.



n=0-15; Z=NH or single bond;
R_1,R_2=H or CH_3;
R_3,R_4,R_5=2-$CH(CH_3)_2$,3-OCH_3,H, 4-OCH_3
6-$CH(CH_3)_2$,2-F,4-F,6-CH_3,5-OCH_3

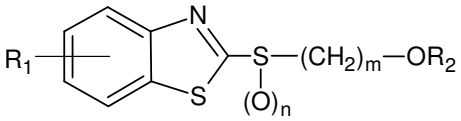
(**40**)



R_1=H,CH_3
R_2=C_6H_5,tolyl,xylyl,pyridyl
R_3=OH,OCH_3,OC_2H_5,$NHCH_2CH_2OH$
R_4=H,Cl,Br,OH,OC_2H_5

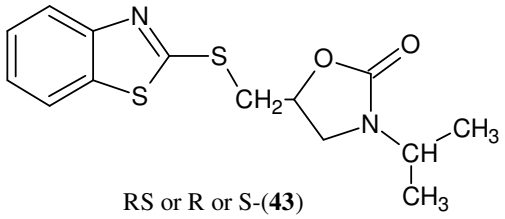
(**41**)

Machinami *et al.*[141] (1994) described the synthesis and antiulcer activity of 5-chloro-2-[(2-alkoxyethyl)thio/sulfanyl/sulfonyl]benzothiazoles (**42**). Test compound 5-chloro-2-[(2-ethoxyethyl)sulfanyl]benzothiazole exhibited potent ulceration inhibitory ratio in the submerged restraint stress, histamine-induced and aspirin induced ulcer tests (93.0, 50.0 and 85.0%, respectively) compared to the standard drug omeprazole (99.0% in each case).



n=0-2;m=2-3;R_1=H,5-Cl,6-OC_2H_5;
R_2=H,C_6H_5

(**42**)

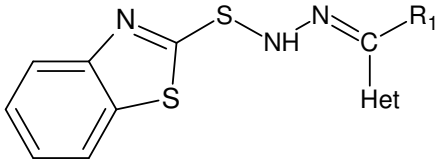


RS or R or S-(**43**)

Selective monoamine oxidase (MAO) inhibitors have been developed to treat a variety of neurological disorders. Lamanna *et al.*[142] (2004) has focused on the incorporation of 3-isopropyloxazolidin-2-one moiety at second position of the 2-mercaptobenzothiazole through methylene group and these enantiomerically pure and/or racemic form molecules were biologically tested for monoamine oxidase-A (MAO-A) and MAO-B activities by bovine mitochondria as enzyme source and kynuraminc as substrate. Compounds, (R,S)-**43**, (S)-**43** and (R)-**43** were found to be equipotent with no MAO-A/MAO-B selectivity.

In 2006, Hofmann *et al.*[143] described the synthesis and *in vitro* anti-tumor activities of 2-benzothiazolyl hydrazones (**44**) against 12 different tumor cells. Compounds exhibited excellent

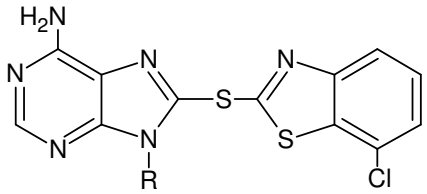
anti-tumor activities against human cancer cells. Furthermore, some of the synthesized compounds have been shown to be capable of inducing apoptosis.



R_1=H,CH_3,CH_2CH_3,$(CH_2)_2CH_3$,$CH(CH_3)_2$, $C(CH_3)_3$,C_6H_5,$CH_2C_6H_5$,cyclopropyl, cyclohexyl

Het=2-pyridinyl,diazinyl,2-quinolinyl, 3-isoquinolinyl,quinoxalinyl

(**44**)

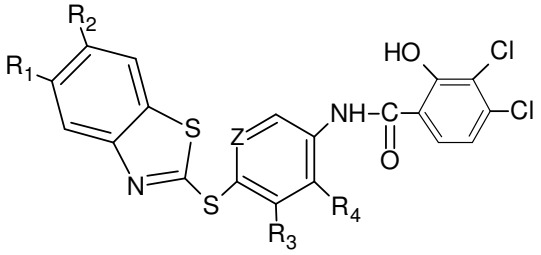


R=CH_2CH_3,$(CH_2)_2CH_3$,$(CH_2)_3CH_3$,$(CH_2)_2OC(O)CH_3$ $(CH_2)_4CN$,$(CH_2)_2OH$,$(CH_2)_3OH$,$(CH_2)_4OH$ CH_2CH_2-morpholine,CH_2CH_2NH-cyclopropane

(**45**)

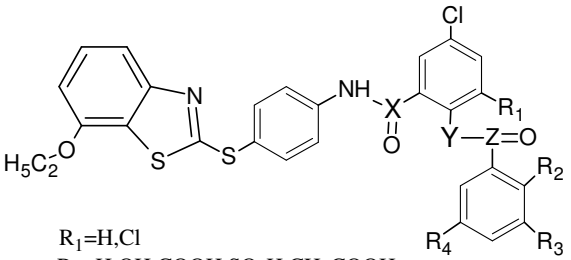
Zhang *et al.*[144] (2006) reported the discovery of benzothiazolothiopurines (**45**) as potent heat shock protein 90 inhibitors (Hsp90). The benzothiazole moiety was found to be exceptionally sensitive to substitutions on the aromatic ring with a 7′-substituent essential for activity. Some of these compounds exhibited low nanomolar inhibition activity in a Her-2 degradation assay (28-150 nM), good aqueous solubility, and oral bioavailability profiles in mice. *In vivo* efficacy experiments demonstrated that compounds of this class inhibit tumor growth in an N87 human colon cancer xenograft model via oral administration. The parent compound, which carries no substituent on the benzothiazole ring, induces Her-2 degradation in MCF-7 cells with an IC_{50} = 5000 nM. Introduction of a chlorine atom at the 7′-position leads to a nearly 30-fold potency gain (IC_{50} = 180 nM).

Cathepsin D, a lysosomal aspartyl protease, has been implicated in the pathology of Alzheimer's disease as well as breast and ovarian cancer. Impressed by these facts Dumas *et al.*[145] (1999) synthesized and screened 2-mercaptobenzothiazole analogs (**46**) as cathepsin D inhibitor. It was observed that the heteroatom linker between the two rings can be either sulfur or oxygen, while substitution of the middle ring resulted in slight increase in activity when a lipophilic substituent (chlorine, methyl, trifluoromethyl) is added ortho to the heteroatom linker. The overall potency of these analogs seems to track with the lipophilicity of the side-chain. The sulfonamide-sulfonate (**47**) was an unexpected finding. The best compound of this new series was found to be the amide-sulfonamide bearing X=C, Y=NH, R_2=OH, R_1, R_3 and R_4=Cl groups (IC_{50} = 250 nM, n = 5).



R_1=H,OCH_3,OC_2H_5,OCF_3; R_2=H,Cl,OCH_3
R_3=H,Cl,CH_3,CF_3,CN,OCH_3; Z=CH,Cl,N

(**46**)

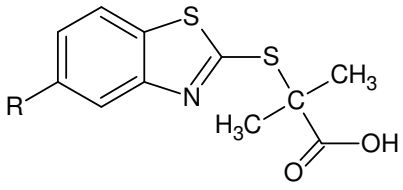


R_1=H,Cl
R_2=H,OH,COOH,SO_3H,CH_2COOH
R_3=H,Cl,CH_3,CF_3,CN,OCH_3
R_4=H,Cl
X or Z=SO,C
Y=O,NH,CH_2

(**47**)

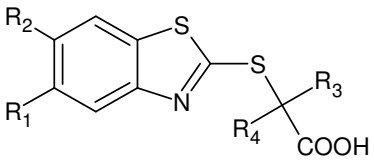
Benzothiazole analogues of clofibric acid (**48)** were synthesized and screened for their antiplatelet activity by Ammazzalorso *et al.*[146] (2005). 5-Chloro analog showed the best antiaggregating properties, revealing a valuable dose dependent pattern.

In 2009, Giampietro *et al.*[147] have also synthesized a series of 2-benzothiazolylthioalkanoic acids (**49**) through systematic structural modifications of clofibric acid and evaluated for human peroxisome proliferator-activated receptor α (PPARα) transactivation activity, with the aim of obtaining new hypolipidemic compounds. Overall, the potencies of some newly designed agonists were slightly higher than those of typical fibrates, such as clofibrate. While unsubstituted derivative proved inactive, the overall effect of the introduction of substituents in the 5 and 6 positions improved PPARα agonistic activity. Among the series the 5-bromine derivative (R_1=Br, R_2 =H, R_3 and R_4 =CH_3) with an EC_{50} value of 2.5 μM and was found to be 10-20 times more effective than other compounds of the same series.



R=H,Cl

(**48**)

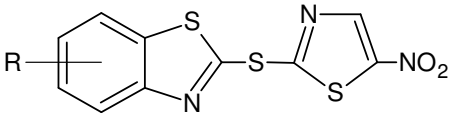


R_1=H,Cl,CN,Br,CF_3,OCH_3,
R_2=H,Cl,CN,Br,CF_3,OC_2H_5; R_3=H,CH_3
R_4=H,$(CH_2)_2CH_3$,$(CH_2)_5CH_3$,C_6H_5

(**49**)

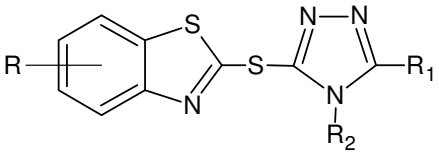
It is well known that up-regulation of c-Jun N-terminal kinases (JNKs, a family of serine/threonine protein kinases) activity is associated with a number of disease states such as

type-2 diabetes, obesity, cancer, inflammation and stroke[148,149] (Manning *et al.*, 2002 and 2003). In consideration of the above fact De *et al.*[150] (2009) described the synthesis and JNK inhibition activity of series of 2-thioetherbenzothiazoles (**50**) and (**51**). Compound bearing 2-nitrothiazole moiety at the second position of the MBT nucleus showed promising result with an IC_{50} of 1.8 µM in the kinase assay in a substrate competitive manner.

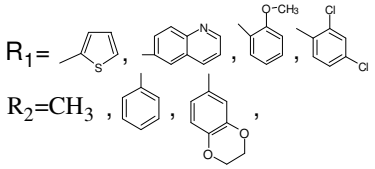


R=H,6-OCH_3,6-Cl, 7-Cl,7-OC_2H_5,SO_3H

(**50**)



R=H,6-OCH_3,6-Cl, 7-Cl,7-OC_2H_5,SO_3H

R_1= 

R_2=CH_3 ,

(**51**)

CHAPTER 2

OBJECTIVE AND PLAN OF WORK

2.1. AIMS AND OBJECTIVES

A major mechanism of action of NSAIDs is lowering prostaglandin (PG) production through the inhibition of cyclo-oxygenase (COX) enzyme that catalyses the conversion of arachidonic acid into PG[151] (Hamberg *et al*., 1974). Because PG has dual function; mediation of inflammation[152,153] (Song *et al*., 1999; Kalgutkar *et al*., 2000) and cytoprotection[154] (Sondhi *et al*., 2002) in the stomach and intestine, long term usage of NSAIDs to relieve the symptoms of inflammation and pain results in gastrointestinal (GI) disorders[154] (Sondhi *et al*., 2002), and renal toxicity[155,156] (Allison *et al*., 1992; Flower, 2003).

It is known that bacterial infections often produce pain and inflammation. In normal practice, chemotherapeutic, analgesic, and anti-inflammatory drugs are prescribed simultaneously. However, the use of anti-inflammatory therapy has been questioned on the basis that such treatment may lessen immunological response to bacterial infections[157-159] (File, 2003; Linder *et al*., 1990; Stevens, 1995) and as such while confounding the progression of disease by suppressing inflammation, fever and pain, they could actually be enhancing the progression of bacterial infection. Moreover, this also increases the risk for developing NSAIDs-related complications especially in elderly, patients with prior history of peptic ulcer disease and patients with impaired liver or kidney functions and patients taking anticoagulants, corticosteroids, etc. concurrently. Hence, there is a pressing need for drugs having both antimicrobial and analgesic-anti-inflammatory activities with minimum adverse effects.

A literature survey revealed that substances possesing 1,3,4-oxadiazole[160-163] (Karthikeyan *et al*., 2008; Islam *et al*., 2008; Amir *et al*., 2004; Padmavathi *et al*., 2009), acetohydrazide[173-175] (Ersan *et al*., 1998; Ahmad *et al*., 2009; Verma *et al*., 1984), 1,3,4-thiadiazole[164-167] (Kadi *et al*., 2010; Farshori *et al*., 2010; Kumar *et al*., 2008; Schenone *et al*., 2006), 1,3,4-triazole[168,169] (Holla *et al*., 1992; Mullican *et al*., 1993) or 2-pyrazoline[170-172] (Azarifar *et al*., 2002; Khode *et al*., 2009; Bansal *et al*., 2001) moieties have occupied a unique position in the design and

synthesis of novel biologically active agents with remarkable antimicrobial, analgesic and anti-inflammatory activities. In addition, 2-mercaptobenzothiazoles are known to possess antiallergic[133,134] (Naya *et al*., 2001 and 2002), antimicrobial[113,114] (Srivastava *et al*., 2004; Desai *et al*., 2005) and anti-inflammatory[122-124] (Srivastava *et al*., 2004; Yadav *et al*., 2005; Paramshivappa *et al*., 2003) properties. The use of 2-mercaptobenzothiazole as a scaffold in medicinal chemistry establishes this moiety as an important structural class. Based on the above observations it appeared of interest to link the 2-mercaptobenzothiazole nucleus at the second position to some 1,3,4-oxadiazole, acetohydrazide, 1,3,4-thiadiazole, 1,3,4-triazole and 2-pyrazoline ring systems to bring them in the same matrix to serve as a new scaffold. This combination was suggested in an attempt to investigate the influence of such hybridization and structure variation on the anticipated antimicrobial, analgesic and anti-inflammatory activities, hoping to add some synergistic biological significance to the target molecules. In the present investigation it is proposed to synthesize, characterize and evaluate the antimicrobial, analgesic, anti-inflammatory and ulcerogenic effects of 1,3,4-oxadiazole, acetohydrazide, 1,3,4-thiadiazole, 1,3,4-triazole and 2-pyrazoline incorporated 2-mercaptobenzothiazoles.

2.2. PLAN OF WORK

Stage I: (a) Synthesis of the following compounds;

- Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethy)-1,3,4-oxadiazol-2-yl]acetamides (**ODZ_{1-15}**) according to **Scheme 1**.
- Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-n'-[(aryloxy)acetyl] acetohydrazides (**ACH_{1-5}**) according to **Scheme 1**.
- Synthesis of 2-{(benzo[d]thiazol-2-ylthio)methyl}-5-(aryloxymethyl)-1,3,4-oxadiazoles (**OXZ_{1-13}**) according to **Scheme 2**.
- Synthesis of 3-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-5-(aryloxymethyl)-4H-1,2,4-triazol-4-amines (**TRZ_{1-7}**) according to **Scheme 2**.
- Synthesis of 5-(aryloxymethyl)-1,3,4-thiadiazol-2-amines (**ATZ_{1-13}**) according to **Scheme 3**.
- Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl]acetamides (**TDZ_{1-13}**) according to **Scheme 3**.

- Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-disubstituted-4,5-dihy- dro-1H-pyrazol-1-yl)ethanones (**PYZ_{1-19}**) according to **Scheme 4**.
- Synthesis of 2-(1,3-benzothiazol-2-ylsulfonyl)-1-(3,5-disubstituted-4,5-dihy- dro-1H-pyrazol-1-yl)ethanones (**PYS_{1-9}**) according to **Scheme 4**.

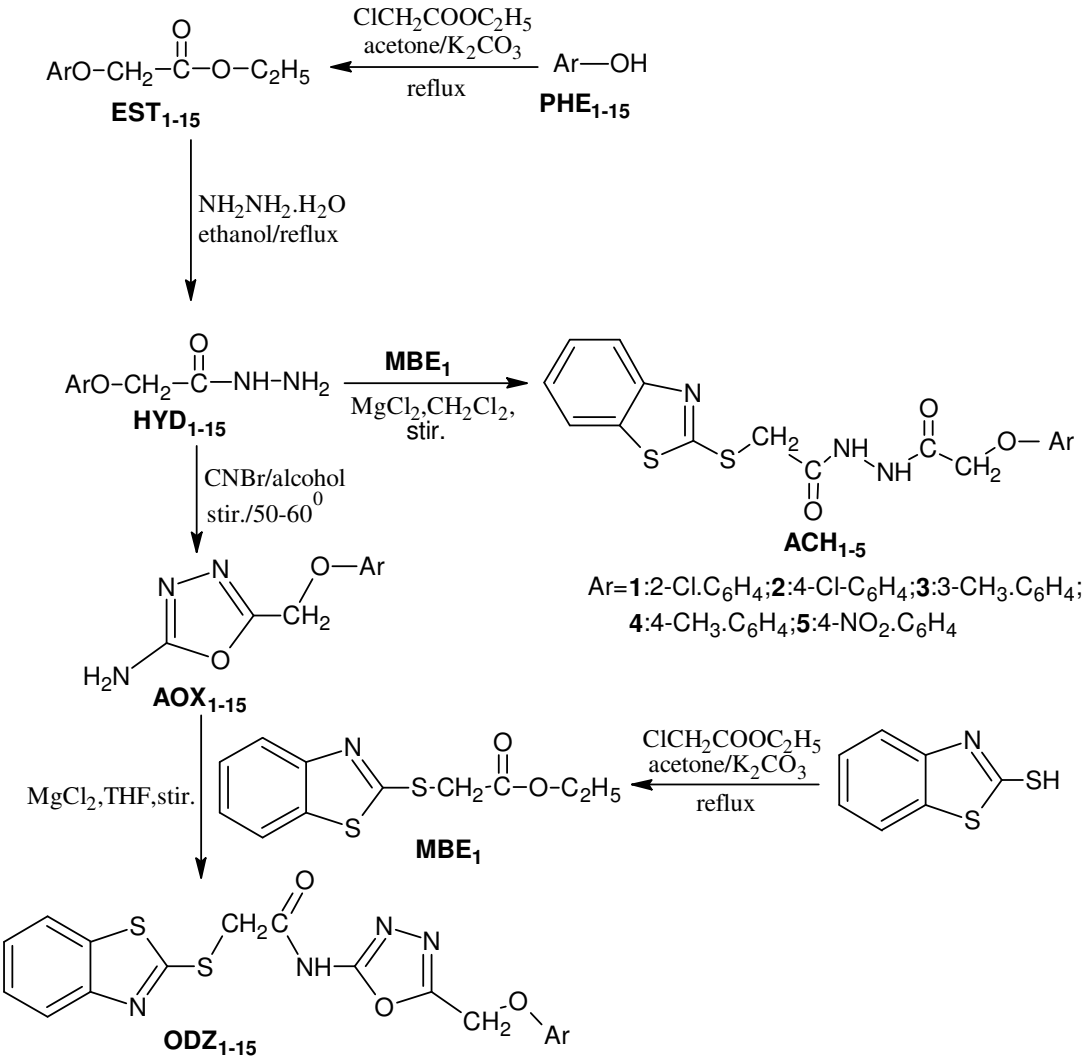
(b) Characterization of the synthesized compounds by;

- Infrared spectroscopy (IR)
- ^{1}H NMR spectroscopy
- ^{13}C NMR spectroscopy
- Mass spectroscopy
- Elemental analysis

Stage II. Evaluation of synthesized compounds for;

- *In vitro* antibacterial and antifungal activity
- Acute oral toxicity study
- *In vivo* analgesic activity
- *In vivo* anti-inflammatory activity
- *In vivo* ulcerogenic activity

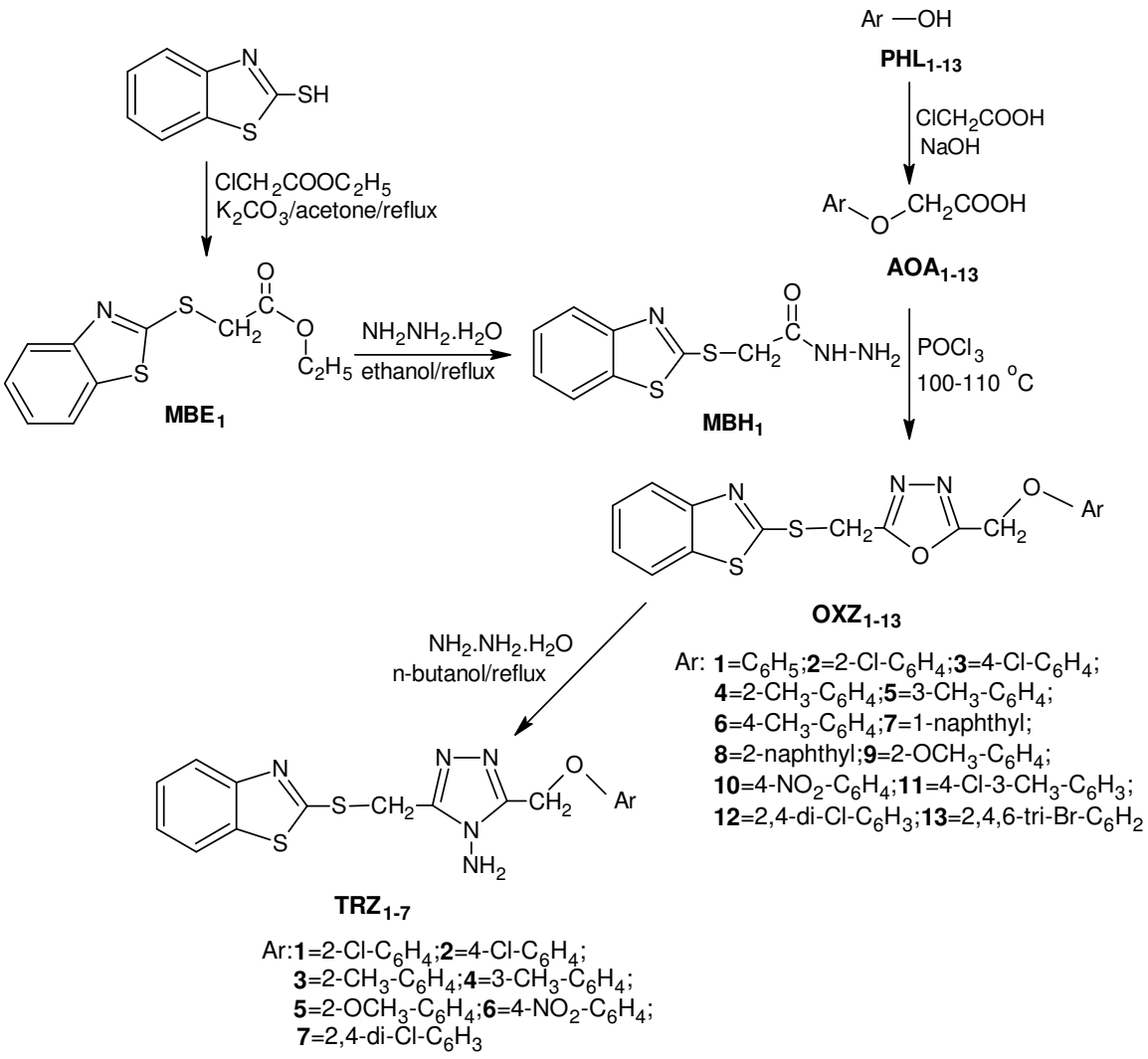
Schemes



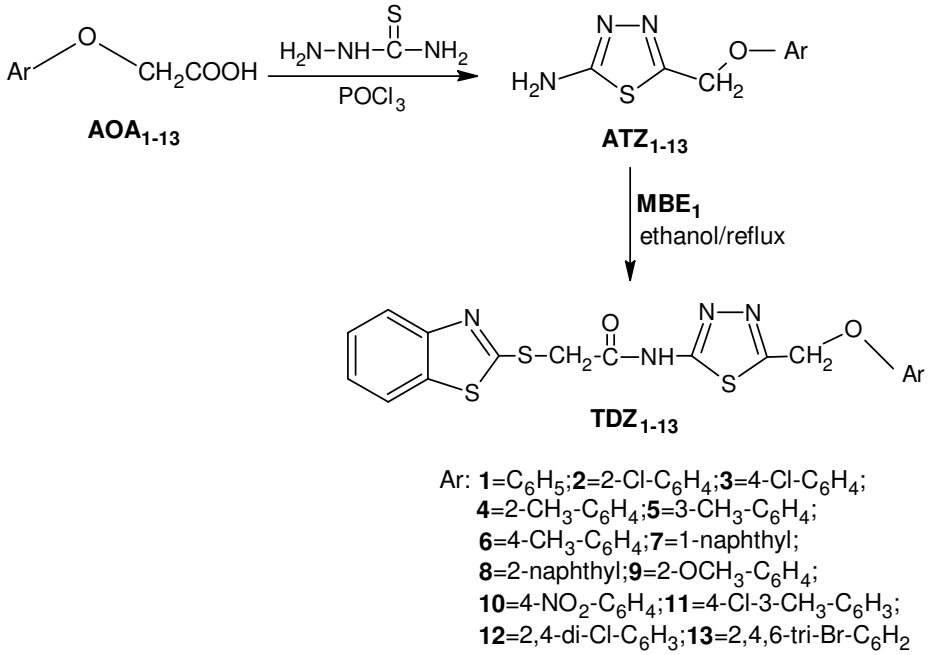
Ar=**1**:2-Cl.C_6H_4;**2**:4-Cl-C_6H_4;**3**:3-CH_3.C_6H_4;
4:4-CH_3.C_6H_4;**5**:4-NO_2.C_6H_4

Ar=**1**:2-Cl.C_6H_4; **2**:4-Cl.C_6H_4;**3**: 2,4,6-tri-Br.C_6H_2;
4:3-CH_3.C_6H_5;**5**:4-CH_3.C_6H_4;**6**:1-naphthyl; **7**:2-naphthyl;
8:4-Cl-2-CH_3.C_6H_3**;9**:2-NO_2.C_6H_4;**10**:4-NO_2.C_6H_4;
11:2,4-di-Cl-C_6H_3;**12**:2,6-di-Cl-C_6H_3;**13**:2-$NHCOCH_3$-4-Cl
-C_6H_3**;14**:2-$NHCOCH_3$-4-$CH_3C_6H_3$;**15**:4-$NHCOCH_3$.C_6H_4

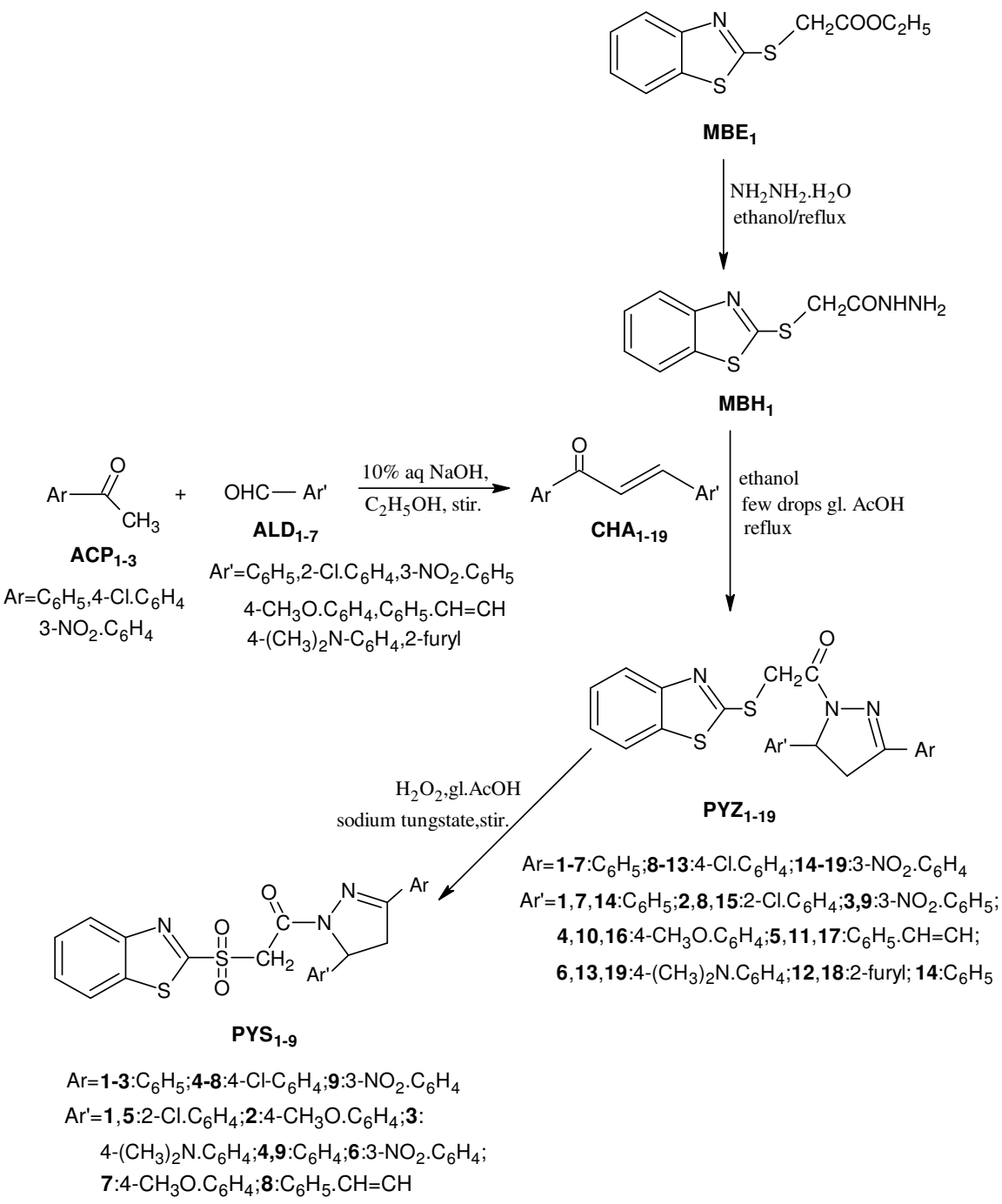
Scheme 1. Synthesis of some Novel 1,3,4-Oxadiazole and Acetohydrazide Incorporated 2-Mercaptobenzothiazoles



Scheme 2. Synthesis of some Novel 1,3,4-Oxadiazole and 1,2,4-Triazole Incorporated 2-Mercaptobenzothiazoles



Scheme 3. Synthesis of some Novel 1,3,4-Thiadiazole Incorporated 2-Mercaptobenzothiazoles



Scheme 4. Synthesis of some Novel 2-Pyrazoline Incorporated 2-Mercaptobenzothiazoles

CHAPTER 3

EXPERIMENTAL

3.1. Synthesis general procedure

2-Mercaptobenzothiazole (A. R. grade), ethyl chloroacetate, cyanogen bromide, hydrazine hydrate (99%), phosphorous oxychloride, chloroacetic acid and magnesium chloride were procured from S. D. Fine Chemicals Pvt. Ltd., Mumbai and used without further purification. Required aldehydes, acetophenones and phenols were procured from either S. D. Fine Chemicals Pvt. Ltd., Mumbai or Sisco research Laboratories Pvt. Ltd., Mumbai and used without further purification. All solvents were purified and dried using the standard methods.

Melting points were determined in open glass capillaries and were uncorrected. The reaction progress was routinely monitored by thin layer chromatography (TLC) on silica gel plates. The IR spectra were recorded on KBr disks, using a Shimadzu 8400S FT-IR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were registered on a Bruker AV-III 400 spectrometer operating at 400 MHz for ^{1}H and 100.63 MHz for ^{13}C, using DMSO-d_6 or $CDCl_3$ as the solvent. Chemical shifts are reported in ppm, using the solvent or TMS as internal standard. LC-MS and EI-MS were obtained on Shimadzu 2010A and Jeol GC Mate II instruments, respectively. Elemental analyses were carried out on a Flash EA 1112 series instrument.

Ethyl (benzothiazol-2-ylthio)acetate (**MBE_1**) was prepared according to the reported method[117] (Karali *et al.*, 2004), and condensed with hydrazine hydrate (99%) in absolute ethanol to obtain benzothiazol-2-ylthio acetic acid hydrazide (**MBH_1**)[118] (Desai *et al.*, 2006).

3.1.1. Synthesis of some Novel 1,3,4-Oxadiazole and acetohydrazide Incorporated 2-Mercaptobenzothiazoles

2-Aryloxyacetohydrazides **$HYD_{1\text{-}15}$** were prepared by refluxing corresponding esters with hydrazine hydrate (99%) in absolute ethanol in accordance with the method described in the literature[168] (Holla *et al.*, 1992). Hydrazides **$HYD_{1\text{-}15}$** on reactions with cyanogen bromide in

absolute ethanol and subsequent neutralization with sodium bicarbonate solution gave the corresponding 5-(aryloxy)methyl-1,3,4-oxadiazol-2-amines (**AOX_{1-15}**)[162] (Amir *et al.*, 2004).

3.1.1.1 Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethy)-1,3,4-oxadiazol-2-yl]acetamides (**ODZ_{1-15}**)

General procedure. To a solution of ethyl (benzothiazol-2-ylthio)acetate (**MBE_1**) (0.50 mol) in THF (50 mL), $MgCl_2$ (0.5 eqiv., 0.25 mol) was added. The slurry obtained was stirred for 5 min, followed by the addition of appropriate 5-(aryloxymethyl)-1,3,4-oxadiazol-2-amines (**AOX_{1-15}**) (0.50 mol) over 10 min. Stirring was continued for 17-20 h at room temperature. The reaction was monitored by TLC. Excess of solvent was distilled off and water (50 mL) was added to the reaction mixture. The solid separated was filtered, washed several times with water and recrystallized from appropriate solvent to give the title compounds **ODZ_{1-15}** (**Scheme 1**). Physical data of compounds **ODZ_{1-15}** are presented in **Table 1**.

2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(2-chlorophenyl)methyl]-1,3,4-oxadiazol-2-yl} acetamide (**ODZ_1**)

IR (KBr, cm^{-1}): 3311, 3119 (NH), 3022 (CH aromatic), 2928 (CH_2), 1654 (C=O), 1616 (C=N), 1600 (C=C), 1527 (amide II), 1294 (C-O-C oxadiazole), 1246, 1024 (C-O-C), 759 (C-S-C), 742 (Ar-Cl). ^{1}H NMR (DMSO-d_6): δ 7.88-7.72 (m, 4H, ArH), 7.62 (s, 1H, NH, D_2O exchangeable), 7.52-7.33 (m, 4H, ArH), 4.90 (s, 2H, OCH_2), 3.81 (s, 2H, SCH_2). ESI-MS *m/z*: 433 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{13}ClN_4O_3S_2$: C, 49.88; H, 3.00; N, 12.93. Found: C, 49.91; H, 3.02; N, 12.95%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(4-chlorophenyl)methyl]-1,3,4-oxadiazol-2-yl} acetamide (**ODZ_2**)

IR (KBr, cm^{-1}): 3288, 3122 (NH), 2851 (CH_2), 1660 (C=O), 1618 (C=N), 1558 (amide II), 1257 (C-O-C oxadiazole), 1210, 1199 (C-O-C), 746 (C-S-C), 680 (Ar-Cl). ^{1}H NMR (DMSO-d_6): δ 7.92-7.75 (m, 4H, ArH), 7.67 (s, 1H, NH, D_2O exchangeable), 7.56-7.38 (m, 4H, ArH), 4.87 (s, 2H, OCH_2), 3.78 (s, 2H, SCH_2). ESI-MS *m/z*: 433 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{13}ClN_4O_3S_2$: C, 49.88; H, 3.00; N, 12.93. Found: C, 49.86; H, 3.05; N, 12.90%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(2,4,6-tribromophenyl)methyl]-1,3,4-oxadiazol-2-yl} acetamide (**ODZ_3**)

IR (KBr, cm^{-1}): 3329, 3105 (NH), 3061 (ArH), 2930 (CH_2), 1657 (C=O), 1620 (C=N), 1604 (C=C aromatic), 1555 (amide II), 1267 (C-O-C oxadiazole), 1207, 1079 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 7.95-7.77 (m, 4H, ArH), 7.71 (s, 1H, NH, D_2O exchangeable), 7.41-7.29 (m, 2H,

ArH), 4.92 (s, 2H, OCH_2), 3.84 (s, 2H, SCH_2). ESI-MS *m/z*: 635 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{11}Br_3N_4O_3S_2$: C, 34.01; H, 1.73; N, 8.81. Found: C, 34.04; H, 1.70; N, 8.79%.

***2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(3-methylphenoxy)methyl]-1,3,4-oxadiazol-2-yl} acetamide* (ODZ$_4$)**

IR (KBr, cm^{-1}): 3358, 3145 (NH), 3063 (CH aromatic), 2920 (CH_2), 2868 (CH_3) 1695 (C=O), 1658, 1587 (C=C aromatic), 1523 (amide II), 1263 (C-O-C oxadiazole), 1232, 1033 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 7.93-7.72 (m, 4H, ArH), 7.59 (s, 1H, NH, D_2O exchangeable), 7.47-7.23 (m, 4H, ArH), 4.92 (s, 2H, OCH_2), 3.88 (s, 2H, SCH_2), 2.12 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 168.08 (C=O), 166.29 (C=N benzothiazole), 165.59 (C=N oxadiazole), 163.58 (C=N oxadiazole), 156.92, 152.35, 134.75, 132.82, 130.13, 129.29, 128.18, 126.32, 122.85, 121.79, 121.09, 120.72, 65.59, 60.87, 35.59. EI-MS Calcd. for $C_{19}H_{16}N_4O_3S_2$ (412.4853): *m/z* 414.5344 ($M^{+\cdot}$+2H), 396, 253, 224, 209, 181, 168, 137, 123, 109, 91, 70.

***2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(4-methylphenoxy)methyl]-1,3,4-oxadiazol-2-yl} acetamide* (ODZ$_5$)**

IR (KBr, cm^{-1}): 3292, 3126 (NH), 3037 (CH aromatic), 2980 (CH_3), 2922 (CH_2), 1692 (C=O), 1660 (C=N), 1585 (C=C aromatic), 1510 (amide II), 1267 (C-O-C oxadiazole), 1247, 1031 (C-O-C). ^{1}H NMR ($CDCl_3$): δ 8.17-7.84 (m, 4H, ArH), 7.70 (s, 1H, NH, D_2O exchangeable), 7.57-7.41 (m, 4H, ArH), 5.21 (s, 2H, OCH_2), 3.85 (s, 2H, SCH_2), 2.14 (s, 3H, CH_3). ESI-MS: *m/z* 411 ($M^{+\cdot}$-1). Anal. Calcd. for $C_{19}H_{16}N_4O_3S_2$: C, 55.33; H, 3.88; N, 13.59. Found: C, 55.35; H, 3.86; N, 13.61%.

***2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-yl} acetamide* (ODZ$_6$)**

IR (KBr, cm^{-1}): 3277, 3160 (NH), 3095 (CH aromatic), 1678 (C=O), 1658, (C=N), 1581 (C=C aromatic), 1508 (amide II), 1256 (C-O-C oxadiazole), 1226, 1057 (C-O-C). ^{1}H NMR ($CDCl_3$): δ 7.99-7.79 (m, 4H, ArH), 7.67-7.31 (m, 7H, ArH), 7.03 (s, 1H, NH, D_2O, exchangeable), 5.13 (s, 2H, OCH_2), 3.79 (s, 2H, SCH_2). ESI-MS: *m/z* 448 ($M^{+\cdot}$). Anal. Calcd. for $C_{22}H_{16}N_4O_3S_2$: C, 58.92; H, 3.57; N, 12.50. Found: C, 58.94; H, 3.59; N, 12.53%.

***2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(naphthalen-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl} acetamide* (ODZ$_7$)**

IR (KBr, cm^{-1}): 3308, 3142 (NH), 3021 (CH aromatic), 2935 (CH_2), 1654 (C=O), 1627 (C=N), 1599 (C=C aromatic), 1508 (amide II), 1298 (C-O-C oxadiazole), 1170, 1031 (C-O-C), 750 (C-S-C). ^{1}H NMR ($CDCl_3$): δ 8.12-7.52 (m, 4H, ArH), 7.77 (s, 1H, NH, D_2O exchangeable), 7.50-7.22 (m, 7H, ArH), 5.50 (s, 2H, OCH_2), 4.58 (s, 2H, SCH_2). ^{13}C NMR

(DMSO-d_6): δ 169.30 (C=O), 167.09 (C=N benzothiazole), 160.86 (C=N oxadiazole), 159.70 (C=N oxadiazole), 157.31, 150.21, 134.39, 130.85, 129.86, 129.70, 128.56, 128.36, 126.32, 124.50, 121.78, 121.09, 113.27, 111.72, 111.51, 111.41, 66.32, 60.16. ESI-MS *m/z*: 451 ($M^{+\cdot}$+2). Anal. Calcd. for $C_{22}H_{16}N_4O_3S_2$: C, 58.92; H, 3.57; N, 12.50. Found: C, 58.89; H, 3.61; N, 12.55%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(4-chloro-2-methylphenoxy)methyl]-1,3,4-oxadiazol-2-yl}acetamide (**ODZ_8**)

IR (KBr, cm^{-1}): 3465, 3120 (NH), 3072 (CH aromatic), 2970 (CH_3), 2922 (CH_2), 1701 (C=O), 1658 (C=N), 1585 (C=C aromatic), 1523 (amide II), 1264 (C-O-C oxadiazole), 1244, 1033 (C-O-C), 729 (C-Cl). ^{1}H NMR ($CDCl_3$): δ 8.13-7.87 (m, 4H, ArH), 7.81-7.71 (m, 3H, ArH), 7.18 (s, 1H, NH, D_2O exchangeable), 5.19 (s, 2H, OCH_2), 3.81 (s, 2H, SCH_2), 2.31 (s, 3H, CH_3). ESI-MS: *m/z* 449 ($M^{+\cdot}$+2). Anal. Calcd. for $C_{19}H_{15}ClN_4O_3S_2$: C, 51.00; H, 3.35; N, 12.52. Found: C, 51.03; H, 3.32; N, 12.55%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(2-nitrophenoxy)methyl]-1,3,4-oxadiazol-2-yl} acetamide (**ODZ_9**)

IR (KBr, cm^{-1}): 3470, 3113 (NH), 3086 (CH aromatic), 2935 (CH_2), 1685 (C=O), 1608 (C=N), 1591 (C=C aromatic), 1514, 1348 (NO_2), 1251 (C-O-C oxadiazole), 1222, 1016 (C-O-C), 750 (C-S-C). ^{1}H NMR ($CDCl_3$): δ 7.97-7.78 (m, 4H, ArH), 7.67-7.51 (m, 4H, ArH), 7.11 (s, 1H, NH, D_2O exchangeable), 4.97 (s, 2H, OCH_2), 3.78 (s, 2H, SCH_2). ESI-MS: *m/z* 443 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{13}N_5O_5S_2$: C, 48.75; H, 2.93; N, 15.80. Found: C, 48.72; H, 2.90; N 15.83%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(4-nitrophenoxy)methyl]-1,3,4-oxadiazol-2-yl} acetamide (**ODZ_{10}**)

IR (KBr, cm^{-1}): 3470, 3285 (NH), 3049, 3034 (CH aromatic), 1655 (C=O), 1658 (C=N), 1591 (C=C aromatic), 1541 (amide II), 1516, 1348 (NO_2), 1255 (C-O-C oxadiazole), 1220, 1016 (C-O-C). ^{1}H NMR ($CDCl_3$): δ 8.02-7.81 (m, 4H, ArH), 7.70 (s, 1H, NH, D_2O exchangeable), 7.68-7.12 (m, 4H, ArH), 5.02 (s, 2H, OCH_2), 3.80 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ 168.14 (C=O), 166.33 (C=N benzothiazole), 165.47 (C=N oxadiazole), 162.96 (C=N oxadiazole), 160.21, 153.53, 139.68, 135.22, 130.94, 128.69, 127.78, 125.54, 122.65, 121.48, 64.13, 62.25. ESI-MS: *m/z* 442 ($M^{+\cdot}$-1). Anal. Calcd. for $C_{18}H_{13}N_5O_5S_2$: C, 48.75; H, 2.93; N, 15.80. Found: C, 48.70; H, 2.97; N, 15.85%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(2,4-dichlorophenoxy)methyl]-1,3,4-oxadiazol-2-yl} acetamide (**ODZ_{11}**)

IR (KBr, cm^{-1}): 3317, 3115 (NH), 3078 (CH aromatic), 2922 (CH_2), 1692 (C=O), 1660 (C=N), 1540 (amide II), 1257 (C-O-C oxadiazole), 1211, 1008 (C-O-C), 675 (C-Cl). ^{1}H NMR ($CDCl_3$): δ 8.02-7.81 (m, 4H, ArH), 7.76 (s, 1H, NH, D_2O exchangeable), 7.69-7.12 (m, 3H, ArH), 5.02 (s, 2H, OCH_2), 3.80 (s, 2H, SCH_2). ESI-MS: *m/z* 466 ($M^{+\cdot}$-1). Anal. Calcd. for $C_{18}H_{12}Cl_2N_4O_3S_2$: C, 46.25; H, 2.56; N, 11.99. Found: C, 46.27; H, 2.59; N, 11.96%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(2,6-dichlorophenoxy)methyl]-1,3,4-oxadiazol-2-yl} acetamide (**ODZ_{12}**)

IR (KBr, cm^{-1}): 3309, 3167 (NH), 2918 (CH_2), 1668 (C=O), 1642 (C=N), 1504 (amide II), 1256 (C-O-C oxadiazole), 1247, 1037 (C-O-C), 678 (C-Cl). ^{1}H NMR ($CDCl_3$): δ 7.97-7.78 (m, 4H, ArH), 7.67-7.51 (m, 3H, ArH), 7.11 (s, 1H, NH, D_2O exchangeable), 4.97 (s, 2H, OCH_2), 3.78 (s, 2H, SCH_2). ESI-MS: *m/z* 467 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{12}Cl_2N_4O_3S_2$: C, 46.25; H, 2.56; N, 11.99. Found: C, 46.23; H, 2.61; N, 12.03%.

N-(5-{[2-(acetylamino)-4-chlorophenoxy]methyl}-1,3,4-oxadiazol-2-yl)-2-(1,3-benzothiazol-2-ylsulfanyl)acetamide (**ODZ_{13}**)

IR (KBr, cm^{-1}): 3342, 3122 (NH), 3012 (CH aromatic), 1678 (C=O), 1654 (C=N), 1599 (C=C), 1521 (amide II), 1269 (C-O-C oxadiazole), 1195, 1012 (C-O-C), 756 (C-S-C), 744 (Ar-Cl). ^{1}H NMR ($CDCl_3$): δ 8.12-7.80 (m, 4H, ArH), 7.76-7.35 (m, 3H, ArH), 7.22 (s, 1H, NH, D_2O exchangeable), 6.88 (s, 1H, NH, D_2O exchangeable), 5.14 (s, 2H, OCH_2), 3.76 (s, 2H, SCH_2), 2.39 (s, 3H, $COCH_3$). ESI-MS: *m/z* 491 ($M^{+\cdot}$+1). Anal. Calcd. for $C_{20}H_{16}ClN_5O_4S_2$: C, 48.97; H, 3.26; N, 14.28. Found: C, 48.94; H, 3.29; N, 14.25%.

N-(5-{[(4-methyl-2-acetylamino)phenoxy]methyl}-1,3,4-oxadiazol-2-yl)-2-(1,3-benzothiazol-2-ylsulfanyl)acetamide (**ODZ_{14}**)

IR (KBr, cm^{-1}): 3425, 3151 (NH), 3001 (CH aromatic), 2980 (CH_3), 2922 (CH_2), 1687 (C=O), 1662 (C=N), 1537 (amide II), 1257 (C-O-C oxadiazole), 1205, 1033 (C-O-C). ^{1}H NMR ($CDCl_3$): δ 7.99-7.78 (m, 4H, ArH), 7.75-7.25 (m, 3H, ArH), 7.13 (s, 1H, NH, D_2O exchangeable), 6.02 (s, 1H, NH, D_2O exchangeable), 5.21 (s, 2H, OCH_2), 3.69 (s, 2H, SCH_2), 2.29 (s, 3H, $COCH_3$), 1.98 (s, 3H, CH_3). ESI-MS: *m/z* 469 ($M^{+\cdot}$). Anal. Calcd. for $C_{21}H_{19}N_5O_4S_2$: C, 53.73; H, 4.05; N, 14.92. Found: C, 53.76; H, 4.01; N, 14.97%.

N-(5-{[4-(acetylamino)phenoxy]methyl}-1,3,4-oxadiazol-2-yl)-2-(1,3-benzothiazol-2-ylsulfanyl)acetamide (**ODZ_{15}**)

IR (KBr, cm^{-1}): 3427, 3196 (NH), 3061 (CH aromatic), 1669 (C=O), 1660 (C=N), 1602 (C=C aromatic), 1508 (amide II), 1264 (C-O-C oxadiazole), 1210, 1018 (C-O-C). ^{1}H NMR ($CDCl_3$): δ 7.96-7.76 (m, 4H, ArH), 7.72-7.36 (m, 4H, ArH), 7.21 (s, 1H, NH, D_2O exchangeable), 6.45 (s, 1H, NH, D_2O exchangeable), 4.98 (s, 2H, OCH_2), 3.59 (s, 2H, SCH_2),

2.17 (s, 3H, $COCH_3$). ESI-MS: *m/z* 455 ($M^{+\cdot}$). Anal. Calcd. for $C_{20}H_{17}N_5O_4S_2$: C, 52.74; H, 3.73; N, 15.38. Found: C, 52.71; H, 3.77; N, 15.41%.

3.1.1.2. Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-n'-[(aryloxy)acetyl] acetohydrazides (**$ACH_{1\text{-}5}$**)

General procedure. To a solution of appropriate 2-aryloxyacetohydrazides (**$HYD_{1\text{-}15}$**) (0.50 mol) in dichloromethane (50 mL), $MgCl_2$ (0.5 eqiv., 0.25 mol) was added. The slurry obtained was stirred for 5 min, followed by the addition of ethyl (benzothiazol-2-ylthio)acetate (**MBE_1**) (0.50 mol) over 10 min. Stirring was continued for 16-18 h at room temperature. The reaction was monitored by TLC. Excess of solvent was distilled off and water (50 mL) was added to the reaction mixture. The solid thus separated was filtered, washed several times with water and recrystallized from ethanol to give the title compounds **$ACH_{1\text{-}5}$** (**Scheme 1**). Physical data of compounds **$ACH_{1\text{-}5}$** are presented in **Table 2**.

2-(1,3-benzothiazol-2-ylsulfanyl)-N'-[(2-chlorophenoxy)acetyl]acetohydrazide (**ACH_1**)

IR (KBr, cm^{-1}): 3304, 3105 (NH.NH), 3059 (ArH), 2864 (CH_2), 1681, 1639 (C=O), 1618 (C=N), 1597 (C=C aromatic), 1244, 1018 (C-O-C), 753 (C-S-C), 742 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.89 (s, 2H, NHNH, D_2O exchangeable), 7.95-7.75 (m, 4H, ArH), 7.64-7.25 (m, 4H, ArH), 5.43 (s, 2H, OCH_2), 4.53 (s, 2H, SCH_2). ESI-MS: *m/z*: 408 ($M^{+\cdot}$), 279, 223, 209, 186, 181, 137, 126, 118, 112, 102, 83, 56, 42. Anal. Calcd. for $C_{17}H_{14}ClN_3O_3S_2$: C, 50.06; H, 3.46; N, 10.30. Found: C, 50.02; H, 3.49; N, 10.35%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N'-[(4-chlorophenoxy)acetyl]acetohydrazide (**ACH_2**)

IR (KBr, cm^{-1}): 3313, 3200 (NHNH), 3036 (ArH), 2916 (CH_2), 1664, 1627 (C=O), 1616 (C=N), 1600 (C=C), 1255, 1037 (C-O-C), 829 (p-substituted benzene), 750 (C-S-C), 732 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.97 (s, 2H, NHNH, D_2O exchangeable), 7.98-7.70 (m, 4H, ArH), 7.65-7.34 (m, 4H, ArH), 5.49 (s, 2H, OCH_2), 4.48 (s, 2H, SCH_2). ESI-MS: *m/z*: 408 ($M^{+\cdot}$). Anal. Calcd. for $C_{17}H_{14}ClN_3O_3S_2$: C, 50.06; H, 3.46; N, 10.30. Found: C, 50.09; H, 3.43; N, 10.33%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N'-[(3-methylphenoxy)acetyl]acetohydrazide (**ACH_3**)

IR (KBr, cm^{-1}): 3306, 3126 (NHNH), 3067 (ArH), 2965 (CH_3), 2926 (CH_2), 1685, 1645 (C=O), 1618 (C=N), 1602 (C=C), 1243, 1027 (C-O-C), 758 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.77 (s, 2H, NHNH, D_2O exchangeable), 7.94-7.87 (m, 4H, ArH), 7.72-7.04 (m, 4H, ArH), 5.51 (s, 2H, OCH_2), 4.44 (s, 2H, SCH_2), 2.32 (s, 3H, CH_3). ESI-MS: *m/z*: 387 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{17}N_3O_3S_2$: C, 55.80; H, 4.42; N, 10.84. Found: C, 55.84; H, 4.40; N, 10.80%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N'-[(4-methylphenoxy)acetyl]acetohydrazide (**ACH_4**)

IR (KBr, cm^{-1}): 3309, 3100 (NHNH), 3043 (ArH), 2989 (CH_3), 2912 (CH_2), 1666, 1647 (C=O), 1620 (C=N), 1597 (C=C), 1240, 1037 (C-O-C), 817 (p-substituted benzene), 769 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.27 (s, 2H, NHNH, D_2O exchangeable), 7.87-7.79 (m, 4H, ArH), 7.75-7.13 (m, 4H, ArH), 5.56 (s, 2H, OCH_2), 4.84 (s, 2H, SCH_2), 2.26 (s, 3H, CH_3). ESI-MS: *m/z*: 387 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{17}N_3O_3S_2$: C, 55.80; H, 4.42; N, 10.84. Found: C, 55.77; H, 4.46; N, 10.82%.

2-(1,3-benzothiazol-2-ylsulfanyl)-N'-[(4-nitrophenoxy)acetyl]acetohydrazide (**ACH_5**)

IR (KBr, cm^{-1}): 3304, 3169 (NHNH), 3095 (ArH), 2926 (CH_2), 1681, 1627 (C=O), 1611 (C=N), 1598 (C=C aromatic), 1541, 1344 (NO_2), 1213, 1012 (C-O-C), 729 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 9.07 (s, 2H, NHNH, D_2O exchangeable), 7.90-7.78 (m, 4H, ArH), 7.50-7.28 (m, 4H, ArH), 5.52 (s, 2H, OCH_2), 4.58 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ 168.29 (C=O), 167.91 (C=O), 166.99 (O-Ar), 165.29 (C=N benzothiazole), 152.32, 151.28, 134.85, 126.31, 124.50, 121.79, 121.07, 118.41, 117.83, 68.89, 60.80. ESI-MS: *m/z*: 418 ($M^{+\cdot}$). Anal. Calcd. for $C_{17}H_{14}N_4O_5S_2$: C, 48.80; H, 3.37; N, 13.39. Found: C, 48.78; H, 3.34; N, 13.42%.

3.1.2. Synthesis of some Novel 1,3,4-Oxadiazole, 1,2,4-Triazole and 1,3,4-Thiadiazole Incorporated 2-Mercaptobenzothiazoles

Aryloxyacetic acid derivatives (**AOA_{1-13}**) were prepared by reactions of corresponding phenols (**PHL_{1-13}**) with chloroacetic acid in basic medium[181] (Furniss *et al*., 2005).

3.1.2.1. Synthesis of 2-{(benzo[d]thiazol-2-ylthio)methyl}-5-(aryloxymethyl)-1,3,4-oxadiazoles (**OXZ_{1-13}**)

General procedure. To a mixture of appropriate aryloxyacetic acids (**AOA_{1-13}**) (0.01 mol) and benzothiazol-2-ylthio acetic acid hydrazide (**MBH_1**) (0.01 mol), phosphorous oxychloride (5 mL) was added and the reaction mixture was heated under reflux for 5 to 6 h. After completion of the reaction the content of the flask was cooled and poured onto crushed ice. It was then neutralized by 5% sodium bicarbonate solution and the separated solid was filtered, washed several times with water, dried and recrystallized from appropriate solvent to give the title compounds **OXZ_{1-13}** (**Schme 2**). Physical data of compounds **OXZ_{1-13}** are presented in **Table 7**.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(phenyloxymethyl)-1,3,4-oxadiazole (**OXZ_1**)

IR (KBr, cm^{-1}): 3061 (CH aromatic), 2848 (CH_2), 1599 (C=N), 1583 (C=C aromatic), 1276 (C-O-C oxadiazole), 1219, 1080 (C-O-C), 752 (C-S-C), 727, 690 (monosubstituted benzene). ^{1}H NMR ($CDCl_3$): δ 7.99-7.68 (m , 4H, ArH), 7.54-6.98 (m, 5H, ArH), 5.62 (s, 2H, OCH_2), 3.50 (s,

2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ166.33 (C=N benzothiazole), 164.84 (C=N oxadiazole), 159.92 (C=N oxadiazole), 157.75 (O-Ar), 153.80, 136.12, 131.66, 129.63, 125.64, 121.73, 121.12, 120.92, 114.32, 60.98, 55.73. EI-MS Calcd. for $C_{17}H_{13}N_3O_2S_2$ (355.4340): m/z 356.4742 ($M^{+\cdot}$+H), 281, 223, 206, 186, 159, 148, 133, 122, 107, 95, 76, 68.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2-chlorophenyloxymethyl)-1,3,4-oxadiazole (**OXZ_2**)

IR (KBr, cm^{-1}): 3063 (CH aromatic), 2926, 2852 (CH_2), 1599 (C=N), 1587 (C=C aromatic), 1276 (C-O-C oxadiazole), 1228, 1058 (C-O-C), 750 (C-S-C). ^1H NMR (DMSO-d_6): δ 8.43-7.99 (m, 4H, ArH), 7.82-6.88 (m, 4H, ArH), 5.13 (s, 2H, OCH_2), 3.46 (s, 2H, SCH_2). ESI-MS: *m/z* 388 ($M^{+\cdot}$-2). Anal. Calcd. for $C_{17}H_{12}ClN_3O_2S_2$: C, 52.30; H, 3.33; N, 10.76. Found: C, 52.27; H, 3.35; N, 10.72

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(4-chlorophenyloxymethyl)-1,3,4-oxadiazole (**OXZ_3**)

IR (KBr, cm^{-1}): 3063 (CH aromatic), 2928 (CH_2), 1605 (C=N), 1600 (C=C aromatic), 1301 (C-O-C oxadiazole), 1240, 1092 (C-O-C), 825 (para-substituted benzene), 754 (C-S-C). ^1H NMR (DMSO-d_6): δ8.38-7.92 (m, 4H, ArH), 7.79-6.82 (m, 4H, ArH), 5.21 (s, 2H, OCH_2), 3.32 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ167.91 (C=N benzothiazole), 166.99 (C=N oxadiazole), 165.29 (C=N oxadiazole), 152.32 (O-Ar), 151.28, 134.85, 126.31, 124.50, 121.79, 121.07, 118.41, 117.83, 114.19, 60.80, 52.54. ESI-MS: *m/z* 388 ($M^{+\cdot}$-2). Anal. Calcd. for $C_{17}H_{12}ClN_3O_2S_2$: C, 52.30; H, 3.33; N, 10.76. Found: C, 52.36; H, 3.29; N, 10.75%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2-methylphenyloxymethyl)-1,3,4-oxadiazole (**OXZ_4**)

IR (KBr, cm^{-1}): 3057 (CH aromatic), 2970 (CH_3), 2833 (CH_2), 1660 (C=N), 1599 (C=C aromatic), 1249 (C-O-C oxadiazole), 1219, 1026 (C-O-C), 754 (C-S-C). ^1H NMR (DMSO-d_6): δ 8.53-7.90 (m, 4H, ArH), 7.86-6.78 (m, 4H, ArH), 4.68 (s, 2H, OCH_2), 3.17 (s, 2H, SCH_2), 2.18 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ166.23, 164.54, 160.27, 157.41, 153.25, 136.16, 131.25, 129.54, 129.21, 125.44, 124.24, 121.80, 121.55, 121.26, 114.35, 60.64, 55.57, 21.48. ESI-MS: *m/z* 369 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{15}N_3O_2S_2$: C, 58.53; H, 4.06; N, 11.38. Found: C, 58.57; H, 4.02; N, 11.34%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(3-methylphenyloxymethyl)-1,3,4-oxadiazole (**OXZ_5**)

IR (KBr, cm^{-1}): 3053 (CH aromatic), 2912 (CH_2), 2864 (CH_3), 1602 (C=N), 1585 (C=C aromatic), 1249 (C-O-C oxadiazole), 1157, 1082 (C-O-C), 756 (C-S-C). ^1H NMR (DMSO-d_6): δ 8.23-7.89 (m, 4H, ArH), 7.79-6.98 (m, 4H, ArH), 4.73 (s, 2H, OCH_2), 3.31 (s, 2H, SCH_2), 1.98 (s, 3H, CH_3). ESI-MS: *m/z* 368 ($M^{+\cdot}$-1). Anal. Calcd. for $C_{18}H_{15}N_3O_2S_2$: C, 58.53; H, 4.06; N, 11.38. Found: C, 58.56; H, 4.02; N, 11.36%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(4-methylphenyloxymethyl)-1,3,4-oxadiazole (**OXZ_6**)

IR (KBr, cm^{-1}): 3063 (CH aromatic), 2918 (CH_2), 2868 (CH_3), 1660 (C=N), 1598 (C=C aromatic), 1311 (C-O-C oxadiazole), 1238, 1078 (C-O-C), 815 (para-substituted benzene), 756 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.17-7.83 (m, 4H, ArH), 7.74-6.97 (m, 4H, ArH), 4.82 (s, 2H, OCH_2), 3.22 (s, 2H, SCH_2), 1.85 (s, 3H, CH_3). ESI-MS: *m/z* 370 ($M^{+\cdot}$+1). Anal. Calcd. for $C_{18}H_{15}N_3O_2S_2$: C, 58.53; H, 4.06; N, 11.38. Found: C, 58.57; H, 4.12; N, 11.35%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(naphthalen-1-yloxymethyl)-1,3,4-oxadiazole (**OXZ_7**)

IR (KBr, cm^{-1}): 3024 (CH aromatic), 2978 (CH_3), 2931 (CH_2), 1627 (C=N), 1595 (C=C aromatic), 1263 (C-O-C oxadiazole), 1234, 1087 (C-O-C), 756 (C-S-C). ^{1}H NMR ($CDCl_3$): δ 7.80-7.68 (m, 4H, ArH), 7.46-7.21 (m, 7H, ArH), 4.79 (s, 2H, OCH_2), 4.21 (s, 2H, SCH_2). ESI-MS: *m/z* 406 ($M^{+\cdot}$+1). Anal. Calcd. for $C_{21}H_{15}N_3O_2S_2$: C, 62.22; H, 3.70; N, 10.37. Found: C, 62.27; H, 3.67; N, 10.33%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(naphthalen-2-yloxymethyl)-1,3,4-oxadiazole (**OXZ_8**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2926 (CH_2), 1627 (C=N), 1599 (C=C aromatic), 1254 (C-O-C oxadiazole), 1215, 1087 (C-O-C), 752 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.23-7.85 (m, 4H, ArH), 7.73-6.82 (m, 7H, ArH), 4.53 (s, 2H, OCH_2), 3.35 (s, 2H, SCH_2). ESI-MS: *m/z* 405 ($M^{+\cdot}$). Anal. Calcd. for $C_{21}H_{15}N_3O_2S_2$: C, 62.22; H, 3.70; N, 10.37. Found: C, 62.26; H, 3.74; N, 10.34%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2-methoxyphenyloxymethyl)-1,3,4-oxadiazole (**OXZ_9**)

IR (KBr, cm^{-1}): 3057 (CH aromatic), 2970 (CH_3), 2833 (CH_2), 1660 (C=N), 1599 (C=C aromatic), 1250 (C-O-C oxadiazole), 1219, 1109 (C-O-C), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.30-7.94 (m, 4H, ArH), 7.81-7.23 (m, 4H, ArH), 4.62 (s, 2H, OCH_2), 3.68 (s, 3H, OCH_3), 3.26 (s, 2H, SCH_2). ESI-MS: *m/z* 385 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{15}N_3O_3S_2$: C, 56.10; H, 3.89; N, 10.90. Found: C, 56.15; H, 3.92; N, 10.95%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(4-nitrophenyloxymethyl)-1,3,4-oxadiazole (**OXZ_{10}**)

IR (KBr, cm^{-1}): 3074 (CH aromatic), 2927 (CH_2), 1660 (C=N), 1591 (C=C aromatic), 1514, 1342 (NO_2), 1300 (C-O-C oxadiazole), 1253, 1111 (C-O-C), 846 (para-substituted benzene), 750 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.15-7.98 (m, 4H, ArH), 7.78-7.33 (m, 4H, ArH), 4.63 (s, 2H, OCH_2), 3.29 (s, 2H, SCH_2). ESI-MS: *m/z* 400 ($M^{+\cdot}$). Anal. Calcd. for $C_{17}H_{12}N_4O_4S_2$: C, 51.00; H, 3.00; N, 14.00. Found: C, 50.88; H, 3.04; N, 13.87%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(4-chloro-3-methylphenyloxymethyl)-1,3,4-oxadiazole (**OXZ_{11}**)

IR (KBr, cm^{-1}): 3053 (CH aromatic), 2949 (CH_3), 2924 (CH_2), 1656 (C=N), 1597 (C=C aromatic), 1305 (C-O-C oxadiazole), 1238, 1166 (C-O-C), 1039 (C-Cl), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.98-7.86 (m, 4H, ArH), 7.76-6.98 (m, 3H, ArH), 4.52 (s, 2H, OCH_2), 3.33 (s,

2H, SCH_2), 1.87 (s, 3H, CH_3). ESI-MS: *m/z* 404 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{14}ClN_3O_2S_2$: C, 53.46; H, 3.46; N, 10.39. Found: C, 53.43; H, 3.44; N, 10.42%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2,4-dichlorophenyloxymethyl)-1,3,4-oxadiazole (**OXZ_{12}**)

IR (KBr, cm^{-1}): 3058 (CH aromatic), 2925 (CH_2), 1660 (C=N), 1597 (C=C aromatic), 1288 (C-O-C oxadiazole), 1246, 1101 (C-O-C), 1060 (C-Cl), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.32-7.85 (m, 4H, ArH), 7.79-7.23 (m, 3H, ArH), 5.12 (s, 2H, OCH_2), 3.34 (s, 2H, SCH_2). ESI-MS: *m/z* 424 ($M^{+\cdot}$). Anal. Calcd. for $C_{17}H_{11}Cl_2N_3O_2S_2$: C, 48.11; H, 2.59; N, 9.90. Found: C, 48.13; H, 2.55; N, 9.84%.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2,4,6-tribromophenyloxymethyl)-1,3,4-oxadiazole (**OXZ_{13}**)

IR (KBr, cm^{-1}): 3066 (CH aromatic), 2924 (CH_2), 1654 (C=N), 1597 (C=C aromatic), 1309 (C-O-C oxadiazole), 1240, 1099 (C-O-C), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.17-7.89 (m, 4H, ArH), 7.83-7.65 (m, 2H, ArH), 4.78 (s, 2H, OCH_2), 3.42 (s, 2H, SCH_2). ESI-MS: *m/z* 593 ($M^{+\cdot}$+1). Anal. Calcd. for $C_{17}H_{10}Br_3N_3O_2S_2$: C, 34.45; H, 1.68; N, 7.09. Found: C, 34.47; H, 1.64; N, 7.07%.

3.1.2.2. Synthesis of 3-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-5-(aryloxymethyl)-4H-1,2,4-triazol-4-amines (**TRZ_{1-7}**)

General procedure. To a solution of approriate 2-{(benzo[d]thiazol-2-ylthio)methyl}-5-(aryloxymethyl)-1,3,4-oxadiazoles (**OXZ_{1-13}**) (0.05 mol) in n-butanol (25 mL), hydrazine hydrate (99%, 0.15 mol) was added and the reaction mixture was heated under reflux for 5 to 6 h. Then, potassium hydroxide (0.10 mol) was added to the reaction mixture and the precipitate formed was filtered. The solid obtained was acidified with Conc. HCl to pH 3, washed several times with water and dried. The resultant solid was recrystallized from appropriate solvent to give the title compounds **TRZ_{1-7}** (**Schme 2**). Physical data of compounds **TRZ_{1-7}** are presented in **Table 8**.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(2-chlorophenoxy)methyl]-4H-1,2,4-triazol-4-amine (**TRZ_1**)

IR (KBr, cm^{-1}): 3167, 3149 (NH_2), 3061 (CH aromatic), 2922 (CH_2), 1665 (C=N), 1599 (C=C aromatic), 1236, 1016 (C-O-C), 1080 (C-Cl), 752 (C-S-C). ^{1}H NMR (DMSO-d_6): δ8.23-7.98 (m, 4H, ArH), 7.35-6.88 (m, 4H, ArH), 5.53 (s, 2H, NH_2, D_2O exchangeable), 5.12 (s, 2H, OCH_2), 3.48 (s, 2H, SCH_2). ESI-MS: *m/z* 403 ($M^{+\cdot}$-1), 276, 262, 222, 219, 206, 180, 166, 134,

128, 112, 97, 82, 78, 58. Anal. Calcd. for $C_{17}H_{14}ClN_5OS_2$: C, 50.49; H, 3.46; N, 17.32. Found: C, 50.52; H, 3.42; N, 17.36%.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(4-chlorophenoxy)methyl]-4H-1,2,4-triazol-4-amine (**TRZ_2**)

IR (KBr, cm^{-1}): 3196, 3157 (NH_2), 3064 (CH aromatic), 2924 (CH_2), 1647 (C=N), 1600 (C=C aromatic), 1242, 1050 (C-O-C), 1005 (C-Cl), 756 (C-S-C). ^1H NMR (DMSO-d_6): δ7.46-7.20 (m, 4H, ArH), 7.09-6.91 (m, 4H, ArH), 5.46 (s, 2H, NH_2, D_2O exchangeable), 4.89 (s, 2H, OCH_2), 4.67 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ166.17 (C=N benzothiazole), 160.52 (C=N triazole), 154.16 (C=N triazole), 152.88, 134.02, 127.51, 126.54, 125.98, 125.57, 124.85, 121.23, 120.83, 106.27, 66.97, 64.80. ESI-MS: *m/z* 422 ($M^{+\cdot}$+H_2O). Anal. Calcd. for $C_{17}H_{14}ClN_5OS_2$: C, 50.49; H, 3.46; N, 17.32. Found: C, 50.51; H, 3.43; N, 17.35%.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(2-methylphenoxy)methyl]-4H-1,2,4-triazol-4-amine (**TRZ_3**)

IR (KBr, cm^{-1}): 3186, 3165 (NH_2), 3061 (CH aromatic), 2933 (CH_2), 1645 (C=N), 1596 (C=C aromatic), 1251, 1020 (C-O-C), 1080 (C-Cl), 752 (C-S-C). ^1H NMR (DMSO-d_6): δ8.08-7.97 (m, 4H, ArH), 7.83-6.68 (m, 4H, ArH), 5.49 (s, 2H, NH_2, D_2O exchangeable), 4.98 (s, 2H, OCH_2), 3.51 (s, 2H, SCH_2), 2.38 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 166.32 (C=N benzothiazole), 160.51 (C=N triazole), 155.84 (C=N triazole), 154.23, 153.54, 136.12, 131.52, 129.61, 125.60, 122.12, 121.55, 121.11, 114.23, 60.82, 55.58, 34.54. EI-MS Calcd. for $C_{18}H_{17}N_5OS_2$ (383.4904): *m/z* 384.5242 ($M^{+\cdot}$+H), 365, 349, 282, 267, 233, 206, 178, 165, 148, 133, 107, 95, 76, 68.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(3-methylphenoxy)methyl]-4H-1,2,4-triazol-4-amine (**TRZ_4**)

IR (KBr, cm^{-1}): 3200, 3173 (NH_2), 3061 (CH aromatic), 2956 (CH_3), 2926 (CH_2), 1652 (C=N), 1602 (C=C aromatic), 1249, 1018 (C-O-C), 815, 852 (meta-substituted benzene), 756 (C-S-C). ^1H NMR (DMSO-d_6): δ7.98-7.88 (m, 4H, ArH), 7.78-6.86 (m, 4H, ArH), 5.64 (s, 2H, NH_2, D_2O exchangeable), 4.86 (s, 2H, OCH_2), 3.47 (s, 2H, SCH_2), 2.33 (s, 3H, CH_3). ESI-MS: *m/z* 383 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{17}N_5OS_2$: C, 56.39; H, 4.43; N, 18.27. Found: C, 56.34; H, 4.39; N, 18.22%.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(2-methoxyphenoxy)methyl]-4H-1,2,4-triazol-4-amine (**TRZ_5**)

IR (KBr, cm^{-1}): 3186, 3165 (NH_2), 3061 (CH aromatic), 2958 (CH_3), 2933 (CH_2), 1645 (C=N), 1596 (C=C aromatic), 1251, 1215, 1020 (C-O-C), 752 (C-S-C). ^1H NMR (DMSO-d_6): δ

8.12-7.92 (m, 4H, ArH), 7.82-7.23 (m, 4H, ArH), 5.71 (s, 2H, NH_2, D_2O exchangeable), 4.92 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.52 (s, 2H, SCH_2). ESI-MS: *m/z* 440 ($M^{+\cdot}$+CH_3CN). Anal. Calcd. for $C_{18}H_{17}N_5O_2S_2$: C, 54.13; H, 4.26; N, 17.54. Found: C, 54.17; H, 4.21; N, 17.59%.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(4-nitrophenoxy)methyl]-4H-1,2,4-triazol-4-amine (**TRZ_6**)

IR (KBr, cm^{-1}): 3186, 3167 (NH_2), 3064 (CH aromatic), 2920 (CH_2), 1665 (C=N), 1598 (C=C aromatic), 1494, 1315 (NO_2), 1236, 1020 (C-O-C), 831 (para-substituted benzene), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ7.98-7.85 (m, 4H, ArH), 7.78-7.65 (m, 4H, ArH), 6.13 (s, 2H, NH_2, D_2O exchangeable), 5.22 (s, 2H, OCH_2), 3.61 (s, 2H, SCH_2). ESI-MS: *m/z* 368 ($M^{+\cdot}$-NO_2). Anal. Calcd. for $C_{17}H_{14}N_6O_3S_2$: C, 49.27; H, 3.38; N 20.28. Found: C, 49.31; H, 3.36; N, 20.25%.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(2,4-dichlorophenoxy)methyl]-4H-1,2,4-triazol-4-amine (**TRZ_7**)

IR (KBr, cm^{-1}): 3196, 3167 (NH_2), 3063 (CH aromatic), 2924 (CH_2), 1652 (C=N), 1605 (C=C aromatic), 1247, 1016 (C-O-C), 1058 (C-Cl), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ8.05-7.79 (m, 4H, ArH), 7.86-7.62 (m, 3H, ArH), 5.82 (s, 2H, NH_2, D_2O exchangeable), 5.17 (s, 2H, OCH_2), 3.72 (s, 2H, SCH_2). EI-MS Calcd. for $C_{17}H_{13}Cl_2N_5OS_2$ (438.3540): *m/z* 439.3206 $(M^{+\cdot}+H)^{+\cdot}$, 406, 280, 223, 206, 181, 160, 148, 125, 97, 81, 62.

3.1.2.3. Synthesis of 5-(aryloxymethyl)-1,3,4-thiadiazol-2-amines (**ATZ_{1-13}**)

General procedure. A mixture of appropriate aryloxyacetic acids (**AOA_{1-13}**) (0.01 mol), thiosemicarbazide (0.01 mol) and phosphorous oxychloride (5 mL) was gently refluxed for 30 min. After cooling water (10 mL) was added and the reaction mixture was further refluxed for 4 h and filtered. The filtrate was made alkaline with 10% potassium hydroxide solution; the formed precipitate was filtered, washed several times with water, dried and recrystallized from appropriate solvent to give compounds **ATZ_{1-13}** (**Scheme 3**). Physical data of compounds **ATZ_{1-13}** are presented in **Table 9**.

5-(Phenoxymethyl)-1,3,4-thiadiazol-2-amine (**ATZ_1**)

IR (KBr, cm^{-1}): 3275, 3269 (NH_2), 3099 (CH aromatic), 2916 (CH_2), 1635 (C=N), 1599 (C=C aromatic), 1247, 1043 (C-O-C), 840 (C-S-C), 750, 688 (mono-substituted benzene). ^{1}H NMR (DMSO-d_6): δ7.37-6.98 (m, 5H, ArH), 7.24 (s, 2H, NH_2, D_2O exchangeable), 5.23 (s, 2H, OCH_2). ESI-MS: *m/z* 207 ($M^{+\cdot}$). Anal. Calcd. for $C_9H_9N_3OS$: C, 52.17; H, 4.34; N, 20.28. Found: C, 52.14; H, 4.38; N, 20.28%.

5-[(2-Chlorophenoxy)methyl]-1,3,4-thiadiazol-2-amine (**ATZ_2**)

IR (KBr, cm^{-1}): 3258, 3250 (NH_2), 3097 (CH aromatic), 2929 (CH_2), 1641 (C=N), 1593 (C=C aromatic), 1247, 1062 (C-O-C), 842 (C-S-C), 744 (ortho-substituted benzene), 690 (C-Cl). ^1H NMR (DMSO-d_6): δ 7.42-7.12 (m, 4H, ArH), 7.22 (s, 2H, NH_2, D_2O exchangeable), 5.17 (s, 2H, OCH_2). ESI-MS: *m/z* 242 ($M^{+\cdot}$). Anal. Calcd. for $C_9H_8ClN_3OS$: C, 44.62; H, 3.30; N, 17.35. Found: C, 44.59; H, 3.34; N, 17.39%.

5-[(4-Chlorophenoxy)methyl]-1,3,4-thiadiazol-2-amine (**ATZ_3**)

IR (KBr, cm^{-1}): 3313, 3290 (NH_2), 3099 (CH aromatic), 2926 (CH_2), 1620 (C=N), 1597 (C=C aromatic), 1244, 1039 (C-O-C), 821 (para-substituted benzene), 804 (C-S-C), 660 (C-Cl). ^1H NMR (DMSO-d_6): δ 7.35-7.00 (m, 4H, ArH), 7.28 (s, 2H, NH_2, D_2O exchangeable), 5.28 (s, 2H, OCH_2). ESI-MS: *m/z* 243 ($M^{+\cdot}$+1). Anal. Calcd. for $C_9H_8ClN_3OS$: C, 44.62; H, 3.30; N, 17.35. Found: C, 44.65; H, 3.34; N, 17.32%.

5-[(2-Methylphenoxy)methyl]-1,3,4-thiadiazol-2-amine (**ATZ_4**)

IR (KBr, cm^{-1}): 3252, 3246 (NH_2), 3099 (CH aromatic), 2974 (CH_3), 2918 (CH_2), 1641 (C=N), 1593 (C=C aromatic), 1247, 1037 (C-O-C), 846 (C-S-C), 746 (otho-substituted benzene). ^1H NMR (DMSO-d_6): δ 7.52-7.14 (m, 4H, ArH), 7.26 (s, 2H, NH_2, D_2O exchangeable), 5.12 (s, 2H, OCH_2), 2.22 (s, 3H, CH_3). ESI-MS: *m/z* 223 ($M^{+\cdot}$+2). Anal. Calcd. for $C_{10}H_{11}N_3OS$: C, 54.29; H, 4.97; N, 19.00. Found: C, 54.33; H, 4.95; N, 18.97%.

5-[(3-Methylphenoxy)methyl]-1,3,4-thiadiazol-2-amine (**ATZ_5**)

IR (KBr, cm^{-1}): 3287, 3248 (NH_2), 3093 (CH aromatic), 2969 (CH_3), 2932 (CH_2), 1647 (C=N), 1599 (C=C aromatic), 1259, 1019 (C-O-C), 849 (C-S-C). ^1H NMR (DMSO-d_6): δ 7.48-6.98 (m, 4H, ArH), 7.21 (s, 2H, NH_2, D_2O exchangeable), 4.98 (s, 2H, OCH_2), 2.31 (s, 3H, CH_3). ESI-MS: *m/z* 221($M^{+\cdot}$). Anal. Calcd. for $C_{10}H_{11}N_3OS$: C, 54.29; H, 4.97; N, 19.00. Found: C, 54.33; H, 4.95; N, 19.06%.

5-[(4-Methylphenoxy)methyl]-1,3,4-thiadiazol-2-amine (**ATZ_6**)

IR (KBr, cm^{-1}): 3272, 3256 (NH_2), 3085 (CH aromatic), 2958 (CH_3), 2926 (CH_2), 1653 (C=N), 1596 (C=C aromatic), 1267, 1034 (C-O-C), 846 (C-S-C), 815 (para-substituted benzene). ^1H NMR (DMSO-d_6): δ 7.56-6.92 (m, 4H, ArH), 7.31 (s, 2H, NH_2, D_2O exchangeable), 5.08 (s, 2H, OCH_2), 2.37 (s, 3H, CH_3). ESI-MS: *m/z* 221($M^{+\cdot}$). Anal. Calcd. for $C_{10}H_{11}N_3OS$: C, 54.29; H, 4.97; N, 19.00. Found: C, 54.33; H, 4.95; N, 18.96%.

5-[(Naphthalen-1-yloxy)methyl]-1,3,4-thiadiazol-2-amine (**ATZ_7**)

IR (KBr, cm^{-1}): 3265, 3240 (NH_2), 3088 (CH aromatic), 2925 (CH_2), 1635 (C=N), 1599 (C=C aromatic), 1252, 1025 (C-O-C), 849 (C-S-C). ESI-MS: *m/z* 257 ($M^{+\cdot}$). Anal. Calcd. for $C_{13}H_{11}N_3OS$: C, 60.70; H, 4.28; N, 16.34. Found: C, 60.75; H, 4.18; N, 16.31%.

***5-[(Naphthalen-2-yloxy)methyl]-1,3,4-thiadiazol-2-amine* (ATZ$_8$)**

IR (KBr, cm^{-1}): 3276, 3257 (NH_2), 3093 (CH aromatic), 2927 (CH_2), 1646 (C=N), 1597 (C=C aromatic), 1258, 1032 (C-O-C), 849 (C-S-C). ESI-MS: *m/z* 257 ($M^{+\cdot}$). Anal. Calcd. for $C_{13}H_{11}N_3OS$: C, 60.70; H, 4.28; N, 16.34. Found: C, 60.75; H, 4.25; N, 16.37%.

5-[(2-Methoxyphenoxy)methyl]-1,3,4-thiadiazol-2-amine (ATZ$_9$)

IR (KBr, cm^{-1}): 3277, 3268 (NH_2), 3090 (CH aromatic), 2968 (CH_3), 2928 (CH_2), 1632 (C=N), 1598 (C=C aromatic), 1258, 1026 (C-O-C), 844 (C-S-C). ESI-MS: *m/z* 237 ($M^{+\cdot}$). Anal. Calcd. for $C_{10}H_{11}N_3O_2S$: C, 50.63; H, 4.64; N, 17.72. Found: C, 50.68; H, 4.61; N, 17.75%.

***5-[(4-Nitrophenoxy)methyl]-1,3,4-thiadiazol-2-amine* (ATZ$_{10}$)**

IR (KBr, cm^{-1}): 3298, 3266 (NH_2), 3087 (CH aromatic), 2928 (CH_2), 1637 (C=N), 1595 (C=C aromatic), 1525, 1310 (NO_2), 1254, 1018 (C-O-C), 850 (C-S-C), 830 (para-substituted benzene). ESI-MS: *m/z* 252 ($M^{+\cdot}$). Anal. Calcd. for $C_9H_8N_4O_3S$: C, 42.85; H, 3.17; N, 22.22. Found: C, 42.81; H, 3.20; N, 22.26%.

***5-[(4-Chloro-3-methylphenoxy)methyl]-1,3,4-thiadiazol-2-amine* (ATZ$_{11}$)**

IR (KBr, cm^{-1}): 3274, 3268 (NH_2), 3095 (CH aromatic), 2969 (CH_3), 2958 (CH_2), 1646 (C=N), 1598 (C=C aromatic), 1249, 1029 (C-O-C), 849 (C-S-C), 665 (C-Cl). ^{1}H NMR (DMSO-d_6): δ8.09 (s, 2H, NH_2), 7.20-7.08 (m, 3H, ArH), 5.20 (s, 2H, CH_2), 2.08 (s, 3H, CH_3). ESI-MS: *m/z* 256 ($M^{+\cdot}$). Anal. Calcd. for $C_{10}H_{10}ClN_3OS$: C, 46.87; H, 3.90; N, 16.40. Found: C, 46.85; H, 4.02; N, 16.37%.

***5-[(2,4-Dichlorophenoxy)methyl]-1,3,4-thiadiazol-2-amine* (ATZ$_{12}$)**

IR (KBr, cm^{-1}): 3287, 3259 (NH_2), 3078 (CH aromatic), 2929 (CH_2), 1649 (C=N), 1598 (C=C aromatic), 1253, 1028 (C-O-C), 849 (C-S-C), 755, 665 (C-Cl). ESI-MS: *m/z* 277 ($M^{+\cdot}$). Anal. Calcd. for $C_9H_7Cl_2N_3OS$: C, 38.98; H, 2.52; N, 15.16. Found: C, 39.02; H, 2.56; N, 15.13%.

***5-[(2,4,6-Tribromophenoxy)methyl]-1,3,4-thiadiazol-2-amine* (ATZ$_{13}$)**

IR (KBr, cm^{-1}): 3272, 3266 (NH_2), 3095 (CH aromatic), 2929 (CH_2), 1649 (C=N), 1602 (C=C aromatic), 1247, 1037 (C-O-C), 847 (C-S-C). ESI-MS: *m/z* 444 ($M^{+\cdot}$). Anal. Calcd. for $C_9H_6Br_3N_3OS$: C, 24.32; H, 1.34; N, 9.45. Found: C, 24.36; H, 1.31; N, 9.48%.

3.1.2.4. General procedure for synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl]acetamides (TDZ$_{1-13}$)

To a solution of appropriate 5-(aryloxymethyl)-1,3,4-thiadiazol-2-amines (**ATZ$_{1-13}$**) (0.05 mol) in absolute ethanol (50 mL), ethyl (benzothiazol-2-ylthio)acetate (**MBE$_1$**) (0.05 mol) was added and the reaction mixture was refluxed for 16 to18 h, distilled in vacuum and cooled. The

separated solid was filtered, dried and recrystallized from appropriate solvent to give the title compounds **TDZ_{1-13}** (**Scheme 3**). Physical data of compounds **TDZ_{1-13}** are presented in **Table 10**.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-[5-(phenoxymethyl)-1,3,4-thiadiazol-2-yl]acetamide (**TDZ_1**)

IR (KBr, cm^{-1}): 3262, 3100 (NH), 3065 (CH aromatic), 2916 (CH_2), 1635 (C=O), 1605 (C=N), 1589 (C=C aromatic), 1508 (amide II), 1247, 1043 (C-O-C), 750 (C-S-C). ^1H NMR (DMSO-d_6): δ 7.33-7.27 (m, 4H, ArH), 7.25 (s, 1H, CONH, D_2O exchangeable), 7.04-6.94 (m, 5H, ArH), 5.26 (s, 2H, OCH_2), 4.12 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ 169.07 (C=O), 166.82 (C=N thiadiazole), 166.14 (C=N benzothiazole), 162.61 (C=N thiadiazole), 159.62, 153.72, 136.12, 131.58, 129.86, 125.62, 124.32, 121.23, 121.05, 114.52, 60.68, 55.61. ESI-MS: *m/z* 414 ($M^{+\cdot}$), 321, 281, 267, 250, 208, 180, 165, 151, 133, 107, 93, 58. Anal. Calcd. for $C_{18}H_{14}N_4O_2S_3$: C, 52.17; H, 3.38; N, 13.52. Found: C, 52.14; H, 3.40; N, 13.56%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2-chlorophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_2**)

IR (KBr, cm^{-1}): 3252, 3097 (NH), 3062 (CH aromatic), 2926 (CH_2), 1641 (C=O), 1608 (C=N), 1593 (C=C aromatic), 1508 (amide II), 1247, 1062 (C-O-C), 744 (C-S-C). ^1H NMR (DMSO-d_6): δ 7.52 -7.43 (m, 4H, ArH), 7.24 (s, 1H, CONH, D_2O exchangeable), 7.03-6.95 (m, 4H, ArH), 5.33 (s, 2H, OCH_2), 4.20 (s, 2H, SCH_2). ESI-MS: *m/z* 449 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{13}ClN_4O_2S_3$: C, 48.10; H, 2.89; N, 12.47. Found: C, 48.15; H, 2.92; N, 12.43%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(4-chlorophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_3**)

IR (KBr, cm^{-1}): 3282, 3097 (NH), 3041 (CH aromatic), 2926 (CH_2), 1620 (C=O), 1602 (C=N), 1597 (C=C aromatic), 1525 (amide II), 1244, 1039 (C-O-C), 821 (para-substituted benzene), 680 (C-S-C). ^1H NMR (DMSO-d_6): δ 7.58-7.47 (m, 4H, ArH), 7.26 (s, 1H, CONH, D_2O exchangeable), 7.12-6.93 (m, 4H, ArH), 5.22 (s, 2H, OCH_2), 4.32 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ 169.18 (C=O), 166.78 (C=N thiadiazole), 166.22 (C=N benzothiazole), 162.46 (C=N thiadiazole), 160.38, 153.62, 136.16, 133.21, 131.47, 129.27, 125.53, 121.33, 121.12, 114.33, 61.23, 55.78. ESI-MS: *m/z* 449 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{13}ClN_4O_2S_3$: C, 48.10; H, 2.89; N, 12.47. Found: C, 48.11; H, 2.85; N, 12.49%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2-methylphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_4**)

IR (KBr, cm^{-1}): 3252, 3097 (NH), 3034 (CH aromatic), 2976 (CH_3), 2918 (CH_2), 1641 (C=O), 1604 (C=N), 1593 (C=C aromatic), 1506 (amide II), 1247, 1037 (C-O-C), 746 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.82-7.53 (m, 4H, ArH), 7.42 (s, 1H, CONH, D_2O exchangeable), 7.39-6.97 (m, 4H, ArH), 4.86 (s, 2H, OCH_2), 4.20 (s, 2H, SCH_2), 1.82 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 169.12 (C=O), 167.24 (C=N thiadiazole), 166.21 (C=N benzothiazole), 162.65 (C=N thiadiazole), 159.73, 153.69, 137.78, 135.92, 131.51, 130.24, 126.59, 125.54, 121.21, 121.08, 120.70, 114.15, 61.32, 55.69, 21.47. ESI-MS: *m/z* 428 (M^+). Anal. Calcd. for $C_{19}H_{16}N_4O_2S_3$: C, 53.27; H, 3.73; N, 13.08. Found: C, 53.25; H, 3.78; N, 13.12%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(3-methylphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_5**)

IR (KBr, cm^{-1}): 3267, 3100 (NH), 3062 (CH aromatic), 2972 (CH_3), 2924 (CH_2), 1637 (C=O), 1602 (C=N), 1593 (C=C aromatic), 1521 (amide II), 1261, 1045 (C-O-C), 860, 775 (meta-substituted benzene), 742 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.86-7.72 (m, 4H, ArH), 7.51 (s, 1H, CONH, D_2O exchangeable), 7.33-6.86 (m, 4H, ArH), 4.98 (s, 2H, OCH_2), 4.15 (s, 2H, SCH_2), 1.76 (s, 3H, CH_3). ESI-MS: *m/z* 428 (M^+). Anal. Calcd. for $C_{19}H_{16}N_4O_2S_3$: C, 53.27; H, 3.73; N, 13.08. Found: C, 53.25; H, 3.76; N, 13.12%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(4-methylphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_6**)

IR (KBr, cm^{-1}): 3290, 3100 (NH), 3028 (CH aromatic), 2989 (CH_3), 2922, (CH_2), 1633 (C=O), 1612 (C=N), 1587 (C=C aromatic), 1514 (amide II), 1215, 1041 (C-O-C), 810 (para-substituted benzene), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.92-7.86 (m, 4H, ArH), 7.48 (s, 1H, CONH, D_2O exchangeable), 7.42-6.88 (m, 4H, ArH), 5.03 (s, 2H, OCH_2), 4.26 (s, 2H, SCH_2), 1.80 (s, 3H, CH_3). ESI-MS: *m/z* 428 (M^+). Anal. Calcd. for $C_{19}H_{16}N_4O_2S_3$: C, 53.27; H, 3.73; N, 13.08. Found: C, 53.29; H, 3.72; N, 13.17%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(naphthalen-1-yloxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_7**)

IR (KBr, cm^{-1}): 3235, 3098 (NH), 3063 (CH aromatic), 2976 (CH_3), 2918 (CH_2), 1639 (C=O), 1606 (C=N), 1595 (C=C aromatic), 1508 (amide II), 1271, 1107 (C-O-C), 742 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.12-7.88 (m, 4H, ArH), 7.56-7.14 (m, 7H, ArH), 7.29 (s, 1H, NH, D_2O exchangeable), 5.48 (s, 2H, OCH_2), 4.35 (s, 2H, SCH_2). ESI-MS: *m/z* 465 (M^++1). Anal. Calcd. for $C_{22}H_{16}N_4O_2S_3$: C, 56.89; H, 3.44; N, 12.06. Found: C, 56.86; H, 3.41; N, 12.02%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(naphthalen-2-yloxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_8**)

IR (KBr, cm^{-1}): 3258, 3105 (NH), 3060 (CH aromatic), 2918 (CH_2), 1638 (C=O), 1603 (C=N), 1590 (C=C aromatic), 1510 (amide II), 1243, 1040 (C-O-C), 748 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.96-7.65 (m, 4H, ArH), 7.58-7.45 (m, 7H, ArH), 7.39 (s, 1H, CONH, D_2O exchangeable), 5.11 (s, 2H, OCH_2), 4.23 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ169.08 (C=O), 167.52 (C=N thiadiazole), 166.32 (C=N benzothiazole), 163.12 (C=N thiadiazole), 157.63, 153.72, 136.26, 134.59, 131.48, 130.68, 129.42, 127.61, 126.69, 126.32, 125.63, 123.55, 121.23, 121.11, 118.71, 105.81, 62.22, 55.46. ESI-MS: *m/z* 464 ($M^{+\cdot}$). Anal. Calcd. for $C_{22}H_{16}N_4O_2S_3$: C, 56.89; H, 3.44; N, 12.06. Found: C, 56.86; H, 3.49; N, 12.03%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2-methoxyphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_9**)

IR (KBr, cm^{-1}): 3250, 3109 (NH), 3010 (CH aromatic), 2933 (CH_2), 1630 (C=O), 1602 (C=N aromatic), 1593 (C=C aromatic), 1506 (amide II), 1255, 1033 (C-O-C), 771 (ortho-substituted benzene), 745 (C-S-C). ^{1}H NMR (DMSO-d_6): δ7.67-7.55 (m, 4H, ArH), 7.42 (s, 1H, CONH, D_2O exchangeable), 7.24-6.98 (m, 4H, ArH), 5.16 (s, 2H, OCH_2), 4.24 (s, 2H, SCH_2), 3.76 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6): δ 169.70 (C=O), 167.32 (C=N thiadiazole), 166.27 (C=N benzothiazole), 162.58 (C=N thiadiazole), 154.10, 146.23, 145.62, 136.12, 131.48, 125.57, 121.86, 121.73, 121.21, 121.02, 106.42, 115.18, 61.74, 55.45, 54.16. ESI-MS: *m/z* 444 ($M^{+\cdot}$). Anal. Calcd. for $C_{19}H_{16}N_4O_3S_3$: C, 51.35; H, 3.60; N, 12.61. Found: C, 51.39; H, 3.57; N, 12.59%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(4-nitrophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_{10}**)

IR (KBr, cm^{-1}): 3252, 3097 (NH), 3062 (CH aromatic), 2926 (CH_2), 1641 (C=O), 1610 (C=N), 1593 (C=C aromatic), 1506 (amide II), 1491, 1336 (NO_2), 1259, 1028 (C-O-C), 848 (para-substituted benzene), 744 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.94-7.75 (m, 4H, ArH), 7.52 (s, 1H, CONH, D_2O exchangeable), 7.42-6.96 (m, 4H, ArH), 4.97 (s, 2H, OCH_2), 4.32 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ 168.41 (C=O), 166.99 (C=N thiadiazole), 165.29 (C=N benzothiazole), 162.32 (C=N thiadiazole), 161.28 (O-Ar), 139.98, 128.16, 127.86, 122.20, 122.06, 121.45, 121.23, 114.19, 114.06, 66.43, 65.04. ESI-MS: *m/z* 459 ($M^{+\cdot}$). Anal. Calcd. for $C_{18}H_{13}N_5O_4S_3$: C, 47.05; H, 2.83; N, 15.25. Found: C, 47.02; H, 2.87; N, 15.22%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(4-chloro-3-methylphenoxy)methyl]-1,3,4-thiadiazol-2-yl}acetamide (**TDZ_{11}**)

IR (KBr, cm^{-1}): 3284, 3100 (NH), 3010 (CH aromatic), 2956 (CH_3), 2928 (CH_2), 1625 (C=O), 1615 (C=N), 1573 (C=C aromatic), 1525 (amide II), 1244, 1041 (C-O-C), 796 (C-Cl),

746 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.13-7.82 (m, 4H, ArH), 7.74-7.57 (m, 3H, ArH), 7.26 (s, 1H, CONH, D_2O exchangeable), 5.20 (s, 2H, OCH_2), 3.89 (s, 2H, SCH_2), 2.22 (s, 3H, CH_3). MS: *m/z* 467 (M^{+}+4). Anal. Calcd. for $C_{19}H_{15}ClN_4O_2S_3$: C, 49.24; H, 3.23; N, 12.09. Found: C, 49.27; H, 3.20; N, 12.04%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2,4-dichlorophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_{12}**)

IR (KBr, cm^{-1}): 3252, 3105 (NH), 3054 (CH aromatic), 2928 (CH_2), 1637 (C=O), 1605 (C=N), 1595 (C=C aromatic), 1506 (amide II), 1246, 1033 (C-O-C), 799 (C-Cl), 750 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.98-7.81 (m, 4H, ArH), 7.79-7.64 (m, 3H, ArH), 7.29 (s, 1H, CONH, D_2O exchangeable), 5.16 (s, 2H, OCH_2), 3.95 (s, 2H, SCH_2). ESI-MS: *m/z* 484 (M^{+}). Anal. Calcd. for $C_{18}H_{12}Cl_2N_4O_2S_3$: C, 44.62; H, 2.47; N, 11.57. Found: C, 44.59; H, 2.44; N, 11.53%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2,4,6-tribromophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_{13}**)

IR (KBr, cm^{-1}): 3290, 3111 (NH), 3063 (CH aromatic), 2940 (CH_2), 1635 (C=O), 1604 (C=N), 1597 (C=C aromatic), 1518 (amide II), 1244, 1062 (C-O-C), 740 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.06-7.84 (m, 4H, ArH), 7.79-7.72 (m, 2H, ArH), 7.16 (s, 1H, CONH, D_2O exchangeable), 5.13 (s, 2H, OCH_2), 3.89 (s, 2H, SCH_2). ESI-MS: *m/z* 651 (M^{+}). Anal. Calcd. for $C_{18}H_{11}Br_3N_4O_2S_3$: C, 33.17; H, 1.68; N, 8.60. Found: C, 33.14; H, 1.62; N, 8.58%.

3.1.3. Synthesis of some Novel 2-Pyrazoline Incorporated 2-Mercaptobenzothiazoles

The intermediate chalcones (**CHA_{1-19}**) were prepared[183] (Furniss *et al.*, 2005) by the base catalyzed Claisen-Schmidt condensation of the aromatic ketones (**ACP_{1-3}**) with different aromatic aldehydes (**ALD_{1-7}**)

3.1.3.1. Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-disubstituted-4,5-dihydro-1H-pyrazol-1-yl)ethanones (**PYZ_{1-19}**)

General procedure. To a solution of appropriately substituted chalcone (**CHA_{1-19}**) (0.01 mol) in absolute ethanol (75 mL), benzothiazol-2-ylthio acetic acid hydrazide (**MBH_1**) (0.01 mol) and few drops of glacial acetic acid were added. The reaction mixture was refluxed for 14-16 h on a water bath. After completion of reaction the content of flask was reduced under vacuum, cooled and poured onto crushed ice and kept overnight. The separated solid was filtered, washed several times with water, dried and recrystallized from suitable solvents to give

the title compounds **PYZ_{1-19}** (**Scheme 4**). Physical data of compounds **PYZ_{1-19}** are presented in **Table 15**.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**PYZ_1**)

IR (KBr, cm^{-1}): 3034 (CH aromatic), 2931(CH_2), 1689 (C=O), 1640 (C=N), 1600 (C=C aromatic), 769 (C-S-C). ^1H NMR (DMSO-d_6): δ 8.01-7.53 (m, 4H, ArH), 7.51-7.23 (m, 10H, ArH), 6.30 (t, 1H, CH pyrazoline), 4.73 (s, 2H, SCH_2CO), 4.21 (d, 2H, CH_2 pyrazoline). ^{13}C NMR (DMSO-d_6): δ 169.07 (C=O), 166.14 (C=N benzothiazole), 163.46 (C=N pyrazoline), 159.19, 152.53, 136.86, 135.58, 130.91, 129.20, 129.02, 128.72, 128.31, 126.97, 126.28, 124.52, 121.74, 121.10, 65.04, 55.78, 35.62. ESI-MS: *m/z* 430 ($M^{+\cdot}$+1). Anal. Calcd. for $C_{24}H_{19}N_3OS_2$: C, 67.11; H, 4.46; N, 9.78. Found: C, 67.15; H, 4.42; N, 9.75%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[5-(2-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_2**)

IR (KBr, cm^{-1}): 3061 (CH aromatic), 2922 (CH_2), 1681 (C=O), 1618 (C=N), 1585 (C=C aromatic), 719 (C-Cl). ^1H NMR (DMSO-d_6): δ 8.15-7.68 (m, 4H, ArH), 7.57-7.37 (m, 9H, ArH), 7.12 (t, 1H, CH pyrazoline), 4.70 (s, 2H, SCH_2CO), 4.44 (d, 2H CH_2 of pyrazoline. ^{13}C NMR (DMSO-d_6): δ 169.45 (C=O), 166.34 (C=N benzothiazole), 163.75 (C=N pyrazoline), 152.58, 149.10, 136.77, 134.70, 133.43, 133.14, 130.80, 129.67, 129.29, 128.97, 128.39, 127.58, 126.33, 124.42, 121.50, 121.09, 63.28, 55.24, 35.66. EI-MS Calcd. for $C_{24}H_{18}ClN_3OS_2$ (464.0022): *m/z* 465.0039 ($M^{+\cdot}$+H), 437, 269, 239, 207, 179, 166, 144, 134, 122, 113, 102, 76.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_3**)

IR (KBr, cm^{-1}): 3080 (CH aromatic), 2920 (CH_2), 1674 (C=O), 1640 (C=N), 1604 (C=C aromatic), 1531, 1350 (NO_2), 761 (C-S-C). ^1H NMR (DMSO-d_6): δ 8.33-7.72 (m, 5H, ArH), 7.62-7.27 (m, 8H, ArH), 6.48 (t, 1H, CH pyrazoline), 4.75 (s, 2H, SCH_2CO), 4.23 (d, 2H, CH_2 pyrazoline). ^{13}C NMR (DMSO-d_6): δ 169.18 (C=O), 166.38 (C=N benzothiazole), 163.49 (C=N pyrazoline), 152.56, 150.06, 139.73, 139.47, 137.10, 134.66, 129.49, 129.35, 128.98, 128.28, 128.17, 126.26, 124.48, 124.34, 121.70, 121.03, 65.54, 55.25, 35.73. ESI-MS: *m/z* 474 ($M^{+\cdot}$). Anal. Calcd. for $C_{24}H_{18}N_4O_3S_2$: C, 60.74; H, 3.82; N, 11.81. Found: C, 60.78; H, 3.80; N, 11.84%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_4**)

IR (KBr, cm^{-1}): 3061 (CH aromatic), 2968 (CH_3), 2933 (CH_2), 1668 (C=O), 1658 (C=N), 1600 (C=C aromatic), 1255, 1031 (C-O-C), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.02-7.57 (m, 6H, ArH), 7.53-6.94 (m, 7H, ArH), 6.75 (t, 1H, CH pyrazoline), 4.82 (s, 2H, SCH_2CO), 4.47 (d, 2H, pyrazoline), 3.78 (s, 3H, OCH_3). EI-MS Calcd. for $C_{25}H_{21}N_3O_2S_2$ (459.5831): *m/z* 459.5895 ($M^{+\cdot}$), 383, 248, 210, 183, 168, 153, 138, 134, 111, 94, 76, 62.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-phenyl-5-(2-phenylethenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethanone (**PYZ_5**)

IR (KBr, cm^{-1}): 3051(CH aromatic), 2928 (CH_2), 1680 (C=O), 1647 (C=N), 1615 (C=C aliphatic), 1585 (C=C aromatic), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.12-7.86 (m, 4H, ArH), 7.78-7.42 (m, 10H, ArH), 6.96 (m, 2H, CH=CH), 6.71-6.65 (m, 1H, CH pyrazoline), 4.76 (s, 2H, SCH_2CO), 4.34-4.25 (m, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 455 ($M^{+\cdot}$). Anal. Calcd. for $C_{26}H_{21}N_3OS_2$: C, 68.54; H, 4.65; N, 9.22. Found: C, 68.51; H, 4.66; N, 9.26%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[5-(4-dimethylaminophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_6**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2970 (CH_3), 2912 (CH_2), 1666 (C=O), 1642 (C=N), 1589 (C=C aromatic), 756 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.02-7.61 (m, 4H, ArH), 7.59-7.13 (m, 9H, ArH), 6.73 (t, 1H, CH pyrazoline), 4.71 (s, 2H, SCH_2CO), 4.36 (d, 2H, CH_2 pyrazoline), 2.17 (s, 6H, 2xCH_3). ESI-MS: *m/z* 473 ($M^{+\cdot}$). Anal. Calcd. for $C_{26}H_{24}N_4OS_2$: C, 66.07; H, 5.12; N, 11.85. Found: C, 66.10; H, 5.08; N, 11.82%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_7**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2937 (CH_2), 1683 (C=O), 1645 (C=N), 1597 (C=C aromatic), 750 (C-S-C), 717 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.01-7.57 (m, 6H, ArH), 7.48-7.17 (m, 7H, ArH), 6.83 (t, 1H, CH pyrazoline), 4.73 (s, 2H, SCH_2CO), 4.28 (d, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 464 ($M^{+\cdot}$). Anal. Calcd. for $C_{24}H_{18}ClN_3OS_2$: C, 62.12; H, 3.91; N, 9.06. Found: C, 62.09; H, 3.90; N, 9.02%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(4-chlorophenyl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_8**)

IR (KBr, cm^{-1}): 3090 (CH aromatic), 2924 (CH_2), 1681 (C=O), 1618 (C=N), 1597 (C=C aromatic), 748 (C-S-C), 717 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.05-7.74 (m, 4H, ArH), 7.62-7.37 (m, 8H, ArH), 6.72 (t, 1H, CH pyrazoline), 4.69 (s, 2H, SCH_2CO), 4.39 (d, 2H, CH_2, pyrazoline). ESI-MS: *m/z* 499 ($M^{+\cdot}$+1). Anal. Calcd. for $C_{24}H_{17}Cl_2N_3OS_2$: C, 57.83; H, 3.41; N, 8.43. Found: C, 57.86; H, 3.46; N, 8.40%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[5-(3-nitrophenyl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_9**)

IR (KBr, cm^{-1}): 3064 (CH aromatic), 2922, (CH_2), 1668 (C=O), 1622 (C=N), 1602 (C=C aromatic), 1512, 1259 (NO_2), 746 (C-S-C), 719 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.20-7.60 (m, 8H, ArH), 7.52-7.33 (m, 4H, ArH), 7.01 (t, 1H, CH pyrazoline), 4.71 (s, 2H, SCH_2CO), 4.48 (d, 2H, CH_2 pyrazoline). ^{13}C NMR (DMSO-d_6): δ 169.18 (C=O), 166.01 (C=N, benzothiazole), 163.52 (C=N, pyrazoline), 152.52, 148.25, 147.59, 137.57, 137.22, 135.47, 134.68, 134.35, 133.93, 130.66, 130.06, 128.46, 126.30, 124.40, 123.53, 121.89, 64.16, 55.22, 35.57. ESI-MS: *m/z* 509 ($M^{+\cdot}$). Anal. Calcd. for $C_{24}H_{17}ClN_4O_3S_2$: C, 56.63; H, 3.37; N, 11.01. Found: C, 56.54; H, 3.30; N, 10.97%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_{10}**)

IR (KBr, cm^{-1}): 3056 (CH aromatic), 2912 (CH_2), 1681 (C=O), 1622 (C=N), 1602 (C=C aromatic), 1377 (CH_3), 1203, 1033 (C-O-C), 746 (C-S-C), 717 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.00-7.59 (m, 5H, ArH), 7.56-6.95 (m, 7H, ArH), 6.76 (t, 1H, CH pyrazoline), 4.74 (s, 2H, SCH_2CO), 4.45 (d, 2H, CH_2 pyrazoline), 3.78 (s, 3H, OCH_3). EI-MS Calcd. for $C_{25}H_{20}ClN_3O_2S_2$ (494.0282): *m/z* 495.0402 ($M^{+\cdot}$+H), 323, 297, 282, 268, 219, 206, 178, 165, 134, 120, 107, 76.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(4-chlorophenyl)-5-(2-phenylethenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethanone (**PYZ_{11}**)

IR (KBr, cm^{-1}): 3026 (CH aromatic), 2916 (CH_2), 1683 (C=O), 1649 (C=N), 1605 (C=C aliphatic), 1583 (C=C aromatic), 746 (C-S-C), 669 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.27-7.52 (m, 8H, ArH), 7.46-7.12 (m, 5H, ArH), 6.92 (m, 2H, CH=CH), 6.72-6.67 (m, 1H, CH pyrazoline), 4.70 (s, 2H, SCH_2CO), 4.42-4.34 (m, 2H, CH_2 pyrazoline). ^{13}C NMR (DMSO-d_6): δ 169.23 (C=O), 166.31 (C=N, benzothiazole), 163.74 (C=N, pyrazoline), 152.95, 152.57, 137.73, 136.20, 135.70, 134.71, 130.62, 128.88, 128.61, 128.03, 126.81, 126.33, 124.43, 121.78, 121.28, 121.09, 62.42, 55.34, 35.66. ESI-MS: *m/z* 491 ($M^{+\cdot}$+1). Anal. Calcd. for $C_{26}H_{20}ClN_3OS_2$: C, 63.73; H, 4.11; N, 8.57. Found: C, 63.70; H, 4.09; N, 8.54%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(4-chlorophenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_{12}**)

IR (KBr, cm^{-1}): 3049 (CH aromatic), 2928 (CH_2), 1654 (C=O), 1647 (C=N), 1601, 1587 (C=C aromatic), 1458 (CH_2), 1220, 1010 (C-O-C), 752 (C-S-C), 721 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.11-7.76 (m, 4H, ArH), 7.45-6.85 (m, 7H, ArH), 6.68 (t, 1H, CH pyrazoline), 4.78 (s, 2H,

SCH_2CO), 4.49 (d, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 454 ($M^{+\cdot}$). Anal. Calcd. for $C_{22}H_{16}ClN_3O_2S_2$: C, 58.21; H, 3.55; N, 9.26. Found: C, 58.25; H, 3.54; N, 9.29%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[5-(4-dimethylaminophenyl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_{13}**)

IR (KBr, cm^{-1}): 3086 (CH aromatic), 2962 (CH_3), 2926 (CH_2), 1666 (C=O), 1647 (C=N), 1589 (C=C aromatic), 756 (C-S-C), 727 (C-Cl). ^1H NMR (DMSO-d_6): δ 8.02-7.54 (m, 6H, ArH), 7.50-6.94 (m, 6H, ArH), 6.76 (t, 1H, CH pyrazoline), 4.77 (s, 2H, SCH_2CO), 4.42 (d, 2H, CH_2 pyrazoline), 2.16 (s, 6H, 2x CH_3). ESI-MS: *m/z* 507 ($M^{+\cdot}$). Anal. Calcd. for $C_{26}H_{23}ClN_4OS_2$: C, 61.58; H, 4.57; N, 11.05. Found: C, 61.60; H, 4.55; N, 11.01%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(3-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_{14}**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2920 (CH_2), 1683 (C=O), 1618 (C=N), 1595 (C=C aromatic), 1529, 1348 (NO_2), 754 (C-S-C). ^1H NMR (DMSO-d_6): δ 8.21-7.78 (m, 4H, ArH), 7.72-7.37 (m, 9H, ArH), 6.81 (t, 1H, CH pyrazoline), 4.81 (s, 2H, SCH_2CO), 4.47 (d, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 474 ($M^{+\cdot}$). Anal. Calcd. for $C_{24}H_{18}N_4O_3S_2$: C, 60.74; H, 3.82; N, 11.81. Found: C, 60.71; H, 3.80; N, 11.79%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(3-nitrophenyl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_{15}**)

IR (KBr, cm^{-1}): 3057 (CH aromatic), 2924 (CH_2), 1685 (C=O), 1647 (C=N), 1602 (C=C aromatic), 1527, 1348 (NO_2), 704 (C-Cl). ^1H NMR (DMSO-d_6): δ 8.09-7.54 (m, 6H, ArH), 7.49-7.16 (m, 6H, ArH), 6.79 (t, 1H, CH pyrazoline), 4.75 (s, 2H, SCH_2CO), 4.44 (d, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 510 ($M^{+\cdot}$+1). Anal. Calcd. for $C_{24}H_{17}ClN_4O_3S_2$: C, 56.63; H, 3.37; N, 11.01. Found: C, 56.66; H, 3.34; N, 11.05%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(3-nitrophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_{16}**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2956 (CH_3), 2920 (CH_2), 1670 (C=O), 1625 (C=N), 1600 (C=C), 1527, 1348 (NO_2), 1249, 1031 (C-O-C), 756 (C-S-C). ^1H NMR (DMSO-d_6): δ 8.12-7.59 (m, 4H, ArH), 7.46-7.21 (m, 8H, ArH), 6.78 (t, 1H, CH pyrazoline), 4.71 (s, 2H, SCH_2CO), 4.48 (d, 2H, CH_2 pyrazoline), 3.78 (s, 3H, OCH_3). ESI-MS: *m/z* 504 ($M^{+\cdot}$). Anal. Calcd. for $C_{25}H_{20}N_4O_4S_2$: C, 59.51; H, 4.00; N, 11.10. Found: C, 59.55; H, 3.97; N, 11.08%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(3-nitrophenyl)-5-(2-phenylethenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethanone (**PYZ_{17}**)

IR (KBr, cm^{-1}): 3080 (CH aromatic), 2965 (CH_3), 2931 (CH_2), 1666 (C=O), 1612 (C=N), 1608 (C=C aliphatic), 1590 (C=C aromatic), 1525, 1320 (NO_2), 756 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.34-7.43 (m, 8H, ArH), 7.41-7.18 (m, 5H, ArH), 7.09-6.88 (m, 2H, CH=CH), 6.68-6.64 (m, 1H, CH pyrazoline), 4.69 (s, 2H, SCH_2CO), 4.44-4.40 (m, 2H, CH_2 pyrazoline). ^{13}C NMR (DMSO-d_6): δ 169.39 (C=O), 166.20 (C=N benzothiazole), 163.96 (C=N pyrazoline), 152.54, 151.69, 147.86, 138.46 137.99, 136.12, 135.25, 130.04, 128.68, 128.00, 126.90, 126.38, 124.59, 123.78, 121.91, 121.75, 121.09, 120.94, 62.52, 55.74, 35.63. ESI-MS: *m/z* 501 ($M^{+\cdot}$). Anal. Calcd. for $C_{26}H_{20}N_4O_3S_2$: C, 62.38; H, 4.03; N, 11.19. Found: C, 62.35; H, 4.05; N, 11.21%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[5-(furan-2-yl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_{18}**)

IR (KBr, cm^{-1}): 3082 (CH aromatic), 2927 (CH_2), 1680 (C=O), 1618 (C=N), 1602 (C=C aromatic), 1531, 1346 (NO_2), 1195, 1018 (C-O-C), 746 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.09-7.74 (m, 4H, ArH), 7.66-6.89 (m, 7H, ArH), 6.72 (t, 1H, CH pyrazoline), 4.80 (s, 2H, SCH_2CO), 4.49 (d, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 464 ($M^{+\cdot}$). Anal. Calcd. for $C_{22}H_{16}N_4O_4S_2$: C, 56.88; H, 3.47; N, 12.06. Found: C, 56.84; H, 3.43; N, 12.10%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[3-(3-nitrophenyl)-5-(4-dimethylaminophenyl)-4,5-dihydro -1H-pyrazol-1-yl]ethanone (**PYZ_{19}**)

IR (KBr, cm^{-1}): 3052 (CH aromatic), 2962 (CH_3), 2922 (CH_2), 1683 (C=O), 1654 (C=N), 1602 (C=C aromatic), 1521, 1361 (NO_2), 756 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.11-7.59 (m, 4H, ArH), 7.47-7.16 (m, 8H, ArH), 6.76 (t, 1H, CH pyrazoline), 4.76 (s, 2H, SCH_2CO), 4.47 (d, 2H, CH_2 pyrazoline), 2.18 (s, 6H, 2xCH_3). ESI-MS: *m/z* 518 ($M^{+\cdot}$). Anal. Calcd. for $C_{26}H_{23}N_5O_3S_2$: C, 60.33; H, 4.48; N, 13.53. Found: C, 60.30; H, 4.45; N, 13.58%.

3.1.3.2. Synthesis of 2-(1,3-benzothiazol-2-ylsulfonyl)-1-(3,5-disubstituted-4,5-dihydro-1H-pyrazol-1-yl)ethanones (**PYS_{1-9}**)

General procedure. To a suspension of appropriate 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-disubstituted-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (**PYZ_2**, **PYZ_4**, **PYZ_{6-11}** or **PYZ_{14}**) (0.05 mol) in acetic acid (10 mL), a 30% aqueous solution of hydrogen peroxide (0.125 mol, 1.5 mL) and a catalytic amount of sodium tungstate were added successively. The resulting mixture was stirred at room temperature for 12-14 h. After completion of reaction the mixture was poured into 200 mL of 20% aqueous solution of sodium chloride and neutralized with sodium bicarbonate. The separated solid was filtered, washed several times with water, dried and recrystallized from appropriate solvent to give the title compounds **PYS_{1-9}** (**Scheme 4**). Physical data of compounds **PYS_{1-9}** are presented in **Table 16**.

2-(1,3-Benzothiazol-2-ylsulfonyl)-1-[5-(2-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYS_1**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2922 (CH_2), 1683 (C=O), 1653 (C=N), 1599 (C=C aromatic), 1344, 1126 (SO_2), 754 (C-S-C), 669 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.03-7.81(m, 4H, ArH), 7.73-7.69 (m, 9H, ArH), 7.07 (t, 1H, CH pyrazoline), 4.85 (s, 2H, SCH_2CO), 4.51 (d, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 496 ($M^{+\cdot}$). Anal. Calcd. for $C_{24}H_{18}ClN_3O_3S_2$: C, 58.12; H, 3.66; N, 8.47. Found: C, 58.10; H, 3.62; N, 8.50%.

2-(1,3-Benzothiazol-2-ylsulfanyl)-1-[5-(4-methoxyphenyl)]-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**PYS_2**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2968 (CH_3), 2933 (CH_2), 1680 (C=O), 1647 (C=N), 1602 (C=C aromatic), 1340, 1176 (SO_2), 1251, 1028 (C-O-C), 761 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.12-7.59 (m, 4H, ArH), 7.52-6.99 (m, 9H, ArH), 6.78 (t, 1H, CH pyrazoline), 4.78 (s, 2H, SCH_2CO), 4.47 (d, 2H, CH_2 pyrazoline), 3.78 (s, 3H, OCH_3)..ESI-MS: *m/z* 491 ($M^{+\cdot}$). Anal. Calcd. for $C_{25}H_{21}N_3O_4S_2$: C, 61.08; H, 4.31; N, 8.55. Found: C, 61.04; H, 3.36; N, 8.51%.

2-(1,3-Benzothiazol-2-ylsulfonyl)-1-{5-[4-(dimethylamino)phenyl]-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl}ethanone (**PYS_3**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2922 (CH_2), 1679 (C=O), 1635 (C=N), 1595 (C=C aromatic), 1338, 1159 (SO_2), 1018, 1219 (C-O-C), 761 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.21-7.59 (m, 4H, ArH), 7.51-6.91 (m, 9H, ArH), 6.79 (t, 1H, CH pyrazoline), 4.84 (s, 2H, SCH_2CO), 4.50 (d, 2H, CH_2 pyrazoline), 2.37 (s, 6H, 2xCH_3). ESI-MS: *m/z* 505 ($M^{+\cdot}$). Anal. Calcd. for $C_{26}H_{24}N_4O_3S_2$: C, 61.88; H, 4.79; N, 11.10. Found: C, 61.86; H, 4.76; N, 11.08%.

2-(1,3-Benzothiazol-2-ylsulfonyl)-1-[3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYS_4**)

IR (KBr, cm^{-1}): 3061 (CH aromatic), 2930 (CH_2), 1683 (C=O), 1622 (C=N), 1595 (C=C aromatic), 1319, 1199 (SO_2), 717 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.18-7.62 (m, 4H, ArH), 7.58-7.32 (m, 9H, ArH), 7.12 (t, 1H, CH pyrazoline), 4.86 (s, 2H, SCH_2CO), 4.48 (d, 2H, CH_2 pyrazoline). EI-MS Calcd. for $C_{24}H_{18}ClN_3O_3S_2$ (496.0010): *m/z* 496.0201 ($M^{+\cdot}$), 448, 439, 263, 249, 235, 219,178, 159, 150, 135, 125, 101, 75.

2-(1,3-Benzothiazol-2-ylsulfonyl)-1-[3-(4-chlorophenyl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYS_5**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2927 (CH_2), 1681 (C=O), 1618 (C=N), 1597 (C=C), 1313, 1126 (SO_2), 748 (C-S-C), 717, 669 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.23-7.57 (m, 4H, ArH), 7.41-6.98 (m, 8H, ArH), 6.82 (t, 1H, CH pyrazoline), 4.81 (s, 2H, SCH_2CO), 4.43 (d, 2H,

CH_2 pyrazoline). ESI-MS: *m/z* 530 ($M^{+\cdot}$). Anal. Calcd. for $C_{24}H_{17}Cl_2N_3O_3S_2$: C, 54.34; H, 3.23; N, 7.92. Found: C, 54.30; H, 3.26; N, 7.89%.

2-(1,3-Benzothiazol-2-ylsulfonyl)-1-[3-(4-chlorophenyl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYS_6**)

IR (KBr, cm^{-1}): 3091 (CH aromatic), 2918 (CH_2), 1689 (C=O), 1624 (C=N), 1595 (C=C aromatic), 1525, 1352 (NO_2), 1329, 1155 (SO_2), 761 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.17-7.64 (m, 4H, ArH), 7.61-6.98 (m, 8H, ArH), 6.67 (t, 1H, CH pyrazoline), 4.84 (s, 2H, SCH_2CO), 4.52 (d, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 541 ($M^{+\cdot}$). Anal. Calcd. for $C_{24}H_{17}ClN_4O_5S_2$: C, 53.28; H, 3.17; N, 10.36. Found: C, 53.25; H, 3.20; N, 10.38%.

2-(1,3-Benzothiazol-2-ylsulfonyl)-1-[3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYS_7**)

IR (KBr, cm^{-1}): 3064 (CH aromatic), 2955 (CH_3), 2933 (CH_2), 1683 (C=O), 1625 (C=N), 1595 (C=C aromatic), 1305, 1172 (SO_2), 1259, 1010 (C-O-C), 756 (C-S-C), 719 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.12-7.67 (m, 4H, ArH), 7.56-6.97 (m, 8H, ArH), 6.75 (t, 1H, CH pyrazoline), 4.83 (s, 2H, SCH_2CO), 4.48 (d, 2H, CH_2 pyrazoline), 3.81 (s, 3H, OCH_3). ESI-MS: *m/z* 526 ($M^{+\cdot}$). Anal. Calcd. for $C_{25}H_{20}ClN_3O_4S_2$: C, 57.08; H, 3.83; N, 7.99. Found: C, 58.12; H, 3.87; N, 8.13%.

2-(1,3-Benzothiazol-2-ylsulfonyl)-1-[3-(4-chlorophenyl)-5-(2-phenylethenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYS_8**)

IR (KBr, cm^{-1}): 3026 (CH aromatic), 2926 (CH_2), 1683 (C=O), 1649 (C=N), 1622 (C=C aliphatic), 1591 (C=C aromatic), 1323, 1176 (SO_2), 761 (C-S-C), 669 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 7.99-7.70 (m, 4H, ArH), 7.63-7.12 (m, 9H, ArH), 6.79 (m, 2H, CH=CH), 6.70-6.63 (m, 1H, CH pyrazoline ring), 4.80 (s, 2H, SCH_2CO), 4.43-4.34 (m, 2H, CH_2 pyrazoline ring). ESI-MS: *m/z* 524 ($M^{+\cdot}$+2). Anal. Calcd. for $C_{26}H_{20}ClN_3O_3S_2$: C, 59.82; H, 3.86; N, 8.05. Found: C, 59.79; H, 3.89; N, 8.02%.

2-(1,3-Benzothiazol-2-ylsulfonyl)-1-[3-(3-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYS_9**)

IR (KBr, cm^{-1}): 3059 (CH aromatic), 2922 (CH_2), 1683 (C=O), 1616 (C=N), 1590 (C=C aromatic), 1531, 1348 (NO_2), 1317, 1159 (SO_2), 761 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.14-7.69 (m, 4H, ArH), 7.62-7.21 (m, 9H, ArH), 6.65 (t, 1H, CH pyrazoline), 4.88 (s, 2H, SCH_2CO), 4.47 (d, 2H, CH_2 pyrazoline). ESI-MS: *m/z* 506 ($M^{+\cdot}$). Anal. Calcd. for $C_{24}H_{18}N_4O_5S_2$: C, 56.91; H, 3.58; N, 11.06. Found: C, 56.89; H, 3.55; N, 11.02%.

3.2. BIOLOGICAL EVALUATION

Animals were procured from J. S. S. College of Pharmacy, Ooty Animal House and were maintained in colony cages at 23±2 ºC, relative humidity of 45-50%, maintained under 12 h light and dark cycle and fed with the standard rat pellet diet (Hindustan Liver Ltd., Mumbai). Prior approval of the Local Animal Ethical Committee was obtained to carry out the experimental work on animals (Annextures I-III).

3.2.1. Biological Evaluation of some Novel 1,3,4-Oxadiazole and Acetohydrazide Incorporated 2-Mercaptobenzothiazoles

3.2.1.1. Antimicrobial Activity

Antibacterial activity of newly synthesized compounds **AOX_{1-15}**, **ODZ_{1-15}** and **ACH_{1-5}** were assayed by the cup plate method[177] (Barry ALJ, 1999) on Muller Hinton agar (Hi-media) plates against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 10400), *Escherichia coli* (NCTC 10418) and *Pseudomonas aeruginosa* (NCTC 10662). Solutions of the test compounds and ciprofloxacin were prepared in dimethylsulfoxide (DMSO) at concentration of 100 and 10 µg mL^{-1}, respectively. The selected strains of bacteria were inoculated into 10 mL of sterile Muller Hinton nutrient broth, and incubated at 37 ºC for 24 h. The final inoculum size was 10^6 CFU mL^{-1} (Colony Forming Unit mL^{-1}). Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. Using a punch, 8 mm diameter wells were made on these seeded agar plates and 100 µL solutions of each test compound in dimethylsulfoxide (DMSO) were added into each labeled well with the help of micropipette. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and incubated in an upright position at 37 ºC for 24 h. The diameter of inhibition zones was measured in millimetre and the results are summarized in **Table 1**.

Test compounds **AOX_{1-15}**, **ODZ_{1-15}** and **ACH_{1-5}** were also evaluated for their antifungal potential on Sabouraud dextrose agar (Hi-media) plates against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 1344). Solutions of the test compounds (100 µg mL^{-1}) and ketoconazole (20 µg mL^{-1}) were prepared in dimethylsulfoxide (DMSO). The selected strains of fungi were inoculated into 10 mL of sterile Sabouraud dextrose broth, and incubated at 26 ºC for 48-72 h. The final inoculum size was 10^6 CFU mL^{-1} (Colony Forming Unit mL^{-1}). Using a sterile cotton swab, the Sabouraud dextrose broth cultures were swabbed on the surface of sterile

Sabouraud dextrose agar plates. Using a punch, 8 mm diameter wells were made on these seeded agar plates and 100 µL solutions of each test compound in dimethylsulfoxide (DMSO) were added into each labeled well with the help of micropipette. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and incubated in an upright position at 26 ºC for 48-72 h. The incubation chamber was kept sufficiently humid. The diameter of inhibition zones was measured in millimetre and the results are summarized in **Table 1**.

3.2.1.2. Pharmacological Activity

The synthesized compounds **AOX_{4-6}**, **ODZ_{2-13}** and **ACH_{1-3}** were evaluated for their acute toxicity, analgesic and anti-inflammatory activities. Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Student's *t*-test to assess the statistical significance.

3.2.1.2.1. Acute Toxicity

Acute oral toxicity was performed following the Organization of Economic Cooperation and Development (OECD-423) guidelines (acute toxic class method). Swiss albino mice (n=3) of either sex (44-55 g) selected by random sampling were used for the study. The animals were fasted for 3-4 h with water *ad libitum*, after which the test compounds were administered orally as suspension (1% CMC solution) at the doses of 50, 100, 250, 500 and 1000 mg kg^{-1} and the mice were observed for three days.

3.2.1.2.2. Analgesic Activity

The analgesic activity of the synthesized compounds was evaluated by the tail immersion method[178] (Di Stasi *et al*., 1988) using albino mice of either sex (38-46 g). The animals were divided randomly into nineteen groups of six animals each. The animals were fasted for 24 h before the experiment with free access to water. First group was taken as control and received appropriate volumes of carboxymethyl cellulose (CMC) solution (1% w/v in water) orally. Last group received reference drug paracetamol (50 mg kg^{-1}) and rest of the group was treated orally with test drugs (100 mg kg^{-1}) suspended in CMC solution (1% w/v in water). The animals were held in position by a suitable restrainer with the tail extending out. The lower 5 cm of tail was gently immersed into thermostatistically controlled water at 55±0.5 °C. The time in seconds for the tail withdrawal was taken as the reaction time with a cut-off time of immersion, set at 10 second for both control as well as treated groups of animals. The reaction time was recorded at

0.5, 1, 2 and 3 h after treatment. The percent analgesic activity was calculated by the following formula,

$$(T_2\text{-}T_1/10\text{-}T_1)x100$$

where T_1 is the reaction time (second) before treatment and T_2 is the reaction time (second) after treatment. The analgesic activity results are summarized in **Table 2**.

3.2.1.2.3. Anti-inflammatory Activity

The acute anti-inflammatory activity results of the synthesized compounds were determined following the carrageenan induced paw oedema method[179] (Winter *et al.*, 1962) using Wistar albino rats of either sex (155-160 g). The animals were divided randomly into nineteen groups of six animals each. The animals were fasted for 24 h before the experiment with free access to water. First group was taken as control and received appropriate volumes of carboxymethyl cellulose (CMC) solution (1% w/v in water) orally. Last groups received reference drug diclofenac (20 mg kg^{-1}) and rest of the groups was treated orally with test drugs (100 mg kg^{-1}) suspended in CMC solution (1.0% w/v in water). Thirty minutes after administration of the test compounds, 0.1 mL carrageenan solution (1.0% w/v in sterile saline) was injected into the sub-plantar tissue of right hind paw of each rat. The volume of paw was measured at different time intervals of 0.5, 1, 2 and 3 h after carrageenan injection by means of plethysmometer (UGO Basile 7140, India). The percentage protection against inflammation was calculated by the following formula,

$$(Vc–Vt)/Vc\ x100$$

where Vc is the oedema volume in control group and Vt is the oedema volume in groups treated with the test compounds. The anti-inflammatory activity results are summarized in **Table 3**.

3.2.1.2.4. Ulcerogenic effects

The test compounds **ODZ_4**, **ODZ_6** and **ODZ_{10}** were evaluated for their acute ulcerogenic potential according to the method reported by Cioli *et al.*[180] (1979) using Wistar albino rats of either sex (155-160 g). The animals were divided randomly into five groups of six animals each. The test compounds (200 mg kg^{-1}) and diclofenac sodium (20 mg kg^{-1}) were administered orally as suspension (1% w/v CMC solution). Control group received appropriate volumes of CMC solution orally. Food but not water was removed 24 h before administration of the test compounds. After compound treatment, the rats were fed with normal diet for 17 h and then sacrificed. Their stomachs were removed, cut out along the greater curvature and washed with distilled water and then gently cleaned by dipping in normal saline. The mucosal damage was

examined by means of a magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring systems: 0.5 redness; 1.0 spot ulcers; 1.5 hemorrhagic streaks; 2.0 ulcers > 3 but ≤ 5; 3.0 ulcers > 5. The mean score of each treated group minus the mean score of control group was regarded as the severity index of gastric mucosal damage. Results are summarized in **Table 4**.

3.2.2. Biological Evaluation of some Novel 1,3,4-Oxadiazole, 1,3,4-1,2,4-Triazole and Thiadiazole Incorporated 2-Mercaptobenzothiazoles

3.2.2.1. Antimicrobial Activity

Newly synthesized compounds **OXZ_{1-13}**, **TRZ_{1-3}**, **ATZ_1**, **ATZ_2** and **TDZ_{1-10}** were assayed by the cup plate method[177] (Barry ALJ, 1999) as described in *Section 3.2.1.1*. Antibacterial activity was evaluated on Muller Hinton agar (Hi-media) plates (37 °C, 24 h) against *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29213), *Shigella dysenteriae* and *Pseudomonas aeruginosa* (NCTC 10662). Test compounds were also evaluated for their antifungal potential on Sabouraud dextrose agar (Hi-media) plates (26 °C, 48-72 h) against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 1344). Solutions of the test compounds (100 µg mL^{-1}), ciprofloxacin (10 µg mL^{-1}) and ketoconazole (20 µg mL^{-1}) were prepared in dimethylsulfoxide (DMSO) and the results (**Table 5**) were recorded as the average diameter of inhibition zones (three independent determinations) of bacterial or fungal growth around the disks in mm.

3.2.2.2. Pharmacological Activity

The synthesized compounds **OXZ_2**, **OXZ_4**, **OXZ_{8-11}**, **TRZ_1**, **TRZ_3**, **TDZ_1**, **TDZ_2**, **TDZ_4** and **TDZ_{8-10}** were evaluated for their acute toxicity, analgesic and anti-inflammatory activities. Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Student's *t*-test to assess the statistical significance.

3.2.2.2.1. Acute Toxicity

Acute oral toxicity was performed following the Organization of Economic Cooperation and Development (OECD-423) guidelines (acute toxic class method). Swiss albino mice (n=3) of either sex (36-47 g) selected by random sampling were used for the study. The animals were fasted for 3-4 h with water *ad libitum*, after which the test compounds were administered orally

as suspension (1% CMC solution) at the doses of 50, 100, 250, 500 and 1000 mg kg^{-1} and the mice were observed for three days.

3.2.2.2.2. Analgesic Activity

The analgesic activity of the synthesized compounds was evaluated by the tail immersion method[178] (Di Stasi *et al*., 1988) using albino mice of either sex (32-40 g). The animals were divided randomly into fifteen groups of six animals each. The animals were fasted for 24 h before the experiment with free access to water. The test compounds (100 mg kg^{-1}) and paracetamol (50 mg kg^{-1}) were administered orally as suspension (1.0% w/v CMC solution). The control rats received appropriate volumes of CMC solution orally. The analgesic activity of the synthesized compounds was evaluated by following the same procedure as described in *Section 3.2.1.2.2.* and results are summarized in **Table 6**.

3.2.2.2.3. Anti-inflammatory Activity

The acute anti-inflammatory activity results of the synthesized compounds were determined following the carrageenan induced paw oedema method[179] (Winter *et al*., 1962) in Wistar albino rats of either sex (155-160 g). The animals were divided randomly into fifteen groups of six animals each. The animals were fasted for 24 h before the experiment with free access to water. The test compounds (100 mg kg^{-1}) and diclofenac sodium (20 mg kg^{-1}) were administered orally as suspension (1.0% w/v CMC solution). The control rats received appropriate volumes of CMC solution orally. The acute anti-inflammatory activity of the synthesized compounds was determined following the same procedure as described in *Section 3.2.1.2.3.* and results are summarized in **Table 7**.

3.2.2.2.4. Ulcerogenic effects

The test compounds **OXZ_4**, **OXZ_9**, **TDZ_4** and **TDZ_9** were evaluated for their acute ulcerogenic potential according to the method reported by Cioli *et al.*[180] (1979) in Wistar albino rats of either sex (155-160 g). The animals were divided randomly into six groups of six animals each. The test compounds (200 mg kg^{-1}) and diclofenac sodium (20 mg kg^{-1}) were administered orally as suspension (1% w/v CMC solution). Control group received appropriate volumes of CMC solution orally. Food but not water was removed 24 h before administration of the test compounds. The test compounds were evaluated for their acute ulcerogenic potential following the same procedure as described in *Section 3.2.1.2.4.* and results are summarized in **Table 8**.

3.2.3. Biological Evaluation of some Novel 2-Pyrazoline Incorporated 2-Mercaptobenzothiazoles

3.2.3.1. Antimicrobial Activity

Newly synthesized compounds **PYZ_{1-19}** and **PYS_{1-9}** were assayed by the cup plate method[177] (Barry ALJ, 1999) as described in *Section 3.2.1.1.* Antibacterial activity was evaluated on Muller Hinton agar (Hi-media) plates (37 °C, 24 h) against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 10400), *Escherichia coli* (NCTC 10418) and *Pseudomonas aeruginosa* (NCTC 10662). Test compounds were also evaluated for their antifungal potential on Sabouraud dextrose agar (Hi-media) plates (26 °C, 48-72 h) against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 1344). Solutions of the test compounds (100 µg mL^{-1}), ciprofloxacin (10 µg mL^{-1}) and ketoconazole (20 µg mL^{-1}) were prepared in dimethylsulfoxide (DMSO) and the results (**Table 9**) were recorded as the average diameter of inhibition zones (three independent determinations) of bacterial or fungal growth around the disks in mm.

3.2.3.2. Pharmacological activity

The synthesized compounds **PYZ_{4-6}**, **PYZ_{8-10}**, **PYZ_{13}**, **PYZ_{14}**, **PYZ_{17}** and **PYS_{1-3}** were evaluated for their acute toxicity, analgesic and anti-inflammatory activities. Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Student's *t*-test to assess the statistical significance.

3.2.3.2.1. Acute Toxicity

Acute oral toxicity was performed following the Organization of Economic Cooperation and Development (OECD-423) guidelines (acute toxic class method). Swiss albino mice (n=3) of either sex (41-48 g) selected by random sampling were used for the study. The animals were fasted for 3-4 h with water *ad libitum*, after which the test compounds were administered orally as suspension (1% CMC solution) at the doses of 50, 100, 250, 500 and 1000 mg kg^{-1} and the mice were observed for three days.

3.2.3.2.2. Analgesic Activity

The analgesic activity of the synthesized compounds was evaluated by the tail immersion method[178] (Di Stasi *et al.*, 1988) using albino mice of either sex (35-45 g). The animals were

divided randomly into thirteen groups of six animals each. The animals were fasted for 24 h before the experiment with free access to water. The test compounds (100 mg kg^{-1}) and paracetamol (50 mg kg^{-1}) were administered orally as suspension (1.0% w/v CMC solution). The control rats received appropriate volumes of CMC solution orally. The analgesic activity of the synthesized compounds was evaluated by following the same procedure as described in *Section 3.2.1.2.2.* and results are summarized in **Table 10**.

3.2.3.2.3. Anti-inflammatory Activity

The acute anti-inflammatory activity results of the synthesized compounds were determined following the carrageenan induced paw oedema method[179] (Winter *et al.*, 1962) in Wistar albino rats of either sex (155-160 g). The animals were divided randomly into thirteen groups of six animals each. The animals were fasted for 24 h before the experiment with free access to water. The test compounds (100 mg kg^{-1}) and diclofenac sodium (20 mg kg^{-1}) were administered orally as suspension (1.0% w/v CMC solution). The control rats received appropriate volumes of CMC solution orally. The acute anti-inflammatory activity of the synthesized compounds was determined following the same procedure as described in *Section 3.2.1.2.3.* and results are summarized in **Table 11**.

3.2.3.2.4. Ulcerogenic effects

The test compounds **PYZ_9**, **PYZ_{10}** and **PYZ_{14}** were evaluated for their acute ulcerogenic potential according to the method reported by Cioli *et al.*[180] (1979) in Wistar albino rats of either sex (162-173 g). The animals were divided randomly into five groups of six animals each. The test compounds (200 mg kg^{-1}) and diclofenac sodium (20 mg kg^{-1}) were administered orally as suspension (1% w/v CMC solution). Control group received appropriate volumes of CMC solution orally. Food but not water was removed 24 h before administration of the test compounds. The test compounds were evaluated for their acute ulcerogenic potential following the same procedure as described in *Section 3.2.1.2.4.* and results are summarized in **Table 12**.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Synthesis and Biological Evaluation of some Novel 1,3,4-Oxadiazole and Acetohydrazide Incorporated 2-Mercaptobenzo-thiazoles

4.1.1. Chemistry

The synthesis of the title compounds **ODZ_{1-15}** and **ACH_{1-5}** is outlined in **Scheme 1**. The key intermediate ethyl (benzothiazol-2-ylthio)acetate (**MBE_1**) was prepared according to the literature procedure[117] (Karali *et al.*, 2004). The reactions of phenols (**PHE_{1-15}**) with ethyl chloroacetate in dry acetone in the presence of anhydrous K_2CO_3 resulted in the formation of corresponding ethyl aryloxyacetates (**EST_{1-15}**). These esters (**EST_{1-15}**) were conveniently converted to the 2-aryloxyacetohydrazides[168] (Holla *et al.*, 1992) (**HYD_{1-15}**) by refluxing it with hydrazine hydrate (99%) in absolute ethanol. Hydrazides (**HYD_{1-15}**) on reactions with cyanogen bromide in absolute ethanol gave the corresponding 5-(aryloxy)methyl-1,3,4-oxadiazol-2-amines (**AOX_{1-15}**). Further reactions of compounds **AOX_{1-15}** with ethyl-2-(benzothiazol-2-ylthio) acetate (**MBE_1**) and $MgCl_2$ (0.5 equiv.) in THF[176] (Guo *et al.*, 2001) furnished the title compounds 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*-[5-(aryloxymethyl)-1,3,4-oxadiazol-2-yl]acetamides (**ODZ_{1-15}**). In a similar manner reactions of compounds **HYD_1**, **HYD_2**, **HYD_4**, **HYD_5** and **HYD_{10}** with ethyl-2-(benzothiazol-2-ylthio) acetate (**MBE_1**) and $MgCl_2$ (0.5 equiv.) in dichloromethane furnished the title compounds 2-(1,3-benzothiazol-2-ylsulfanyl)-n'-[(aryloxy) acetyl] acetohydrazides (**ACH_{1-5}**).

The infrared (IR) spectra of compounds **AOX_{1-15}** showed C-O-C vibrations of the oxadiazole ring[160,163] (Karthikeyan *et al.*, 2008; Padmavathi *et al.*, 2009) in the region 1282-1246 cm^{-1}. The C=N stretching observed at 1664-1642 cm^{-1} is due to the ring closure. The formation of oxadiazole ring in **AOX_2** was supported by its ^1H NMR spectrum (**Figure 7a-c**), which

showed a multiplet signal for four aromatic protons at δ 7.38-7.04 ppm and singlet signals at δ 7.15 and 5.15 ppm due to the NH_2 and OCH_2 fragments, respectively. Its mass spectrum showed a molecular ion peak at *m/z* 226 which is in conformity with the molecular formula $C_9H_8ClN_3O_2$.

The IR spectra of 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*-[5-(aryloxymethyl)-1,3,4-oxadiazol -2-yl]acetamides (**ODZ$_{1\text{-}15}$**) showed characteristic absorption bands in the regions 3470-3105 cm^{-1} (NH), 1695-1651 cm^{-1} (C=O) and 1298-1251 cm^{-1} (C-O-C oxadiazole). In the ^{1}H NMR spectrum[160,161] (Karthikeyan *et al*., 2008; Islam *et al.,* 2008) of **ODZ$_7$** (**Figure 8a,b**), two singlet signals were observed at δ 5.50 and 4.58 ppm which were ascribed to the OCH_2 and SCH_2 protons, respectively. Also, two multiplet signals observed in the region at δ 8.12-7.52 and 7.50-7.22 ppm were assigned to the four and seven aromatic protons and a broad singlet signal at δ 7.77 ppm was assigned to the NH fragment of acetamido group present at second position of the oxadiazole ring. Further, LC mass spectrum showed ($M^{+\cdot}$+2) peak at *m/z* 451 which is in conformity with its molecular formula $C_{22}H_{16}N_4O_3S_2$.

The IR spectra of compounds **ACH$_{1\text{-}5}$** showed two carbonyl stretching bands in the regions 1685-1664 and 1647-1625 cm^{-1} and absorption bands in the region 3313-3100 cm^{-1}, characteristic of a NHNH group, was observed. The ^{1}H NMR spectrum[174,175] (Ahmad *et al*., 2009; Verma *et al*., 1984) of compound **ACH$_1$** showed two multiplet signals at δ 7.95-7.75 and 7.64-7.25 ppm for four aromatic protons each and singlet signals at δ 8.89, 5.43 and 4.53 ppm due to the NHNH, OCH_2 and SCH_2 fragments, respectively. The electrospray ionization (ESI) mass spectrum of compound **ACH$_1$** (**Figure 15**) displayed molecular ion ($M^{+\cdot}$) peak at *m/z* 408 which is in conformity with its molecular formula $C_{17}H_{14}ClN_3O_3S_2$. The specrum is dominated by *m/z* 102 ion (base peak) resulting from the fragmentation of $[C_8H_5NS_2]^{+\cdot}$ ion (*m/z* 181) which could be expected as the major fragmentation pathway of **ACH$_1$**. Moreover, the spectrum exibited an intense peak at *m/z* 42 which is due to the loss of $[NHCO]^{+\cdot}$ fragment. A proposed mechanism of fragmentation and the *m/z* of the fragments is given in **Scheme 6**.

The ^{13}C NMR spectra were recorded for a few members of the title compounds **AOX$_9$**, **ODZ$_4$**, **ODZ$_7$**, **ODZ$_{10}$** and **ACH$_5$**. The ^{13}C NMR spectrum (in DMSO-d_6) of **AOX$_9$** (**Figure 10**) showed two signals at δ 165.64 and 158.04 ppm due to the azomethine carbons of the oxadiazole ring. The six signals at δ 150.55, 139.57, 134.24, 125.03, 121.28 and 115.32 ppm were assigned to the phenyl ring carbons and a signal at δ 66.60 ppm was observed due to the OCH_2 fragment. The ^{13}C NMR spectrum (in DMSO-d_6) of 1,3,4-oxadiazole analog **ODZ$_4$** (**Figure 11**) displayed two signals at δ 165.59 and 163.58 ppm was assigned due to the azomethine carbon of the

oxadiazole ring[160] (Karthikeyan *et al.*, 2008). The carbonyl carbon (amide) in the same compound exhibited a signal at δ 168.08-ppm while the azomethine carbon of the benzothiazole ring[116] (Kumar *et al.*, 2005) was observed at δ 166.29 ppm. The OCH_2 and SCH_2 fragments carbon signals were observed at δ 65.59 and 60.87 ppm, respectively. The twelve signals at δ 156.92, 152.35, 134.75, 132.82, 130.13, 129.29, 128.18, 126.32, 122.85, 121.79, 121.09 and 120.72 ppm were assigned to the phenyl ring carbons. In the ^{13}C NMR spectrum of **ACH_5** (in DMSO-d_6, **Figure 13**), two signals assigned to the carbonyl carbons were observed at δ 168.29 and 167.91 ppm and a signal assigned to the azomethine carbon of the benzothiazole ring was observed at δ 166.99 ppm. The OCH_2 and SCH_2 fragments carbon signals were observed at δ 68.89 and 60.80 ppm and ten signals at δ 165.29, 152.32, 151.28, 134.85, 126.31, 124.50, 121.79, 121.07, 118.41, 117.83 ppm were assigned to the phenyl ring carbons.

4.1.2. Biological Study

4.1.2.1. Antimicrobial activity

Antimicrobial screening by the cup plate method[177] (Barry ALJ, 1999) displayed variable inhibitory effects of the tested compounds against the growth of the Gram-positive and Gram-negative bacteria, while they were inactive against the two tested strains of fungi (**Table 3**). In comparison to the 1,3,4-oxadiazol-2-amines (inhibition zone 17-21 mm) and acetohydrazides (inhibition zone 13-23 mm), 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethy)-1,3,4-oxadiazol-2-yl]acetamides exhibited higher inhibitory activity against the tested bacterial strains. In this regard compounds **ODZ_4**, **ODZ_6**, **ODZ_{10}**, **ODZ_{11}** and **ODZ_{13}** showed significant inhibitory activity (inhibition zone 25-32 mm) against all the tested bacterial strains. Furthermore, the maximum inhibitory activity was observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (inhibition zone 31 and 32 mm, respectively) in compound **ODZ_{10}** having 4-nitrophenyl group at fifth position of the oxadiazole ring. It was observed that either changing the position of NO_2 group in the phenyl ring from fourth (compound **ODZ_{10}**) to second (compound **ODZ_5**) or replacing it with a electron releasing CH_3 group (compound **ODZ_9**) shifted the spectrum of activity against Gram-positive bacteria *Staphylococcus aureus* and *Escherichia coli* (inhibition zone 27-31 mm). It is also evident from results that a electron withdrawing nitro group in the phenyl ring at fifth position of the oxadiazole ring is optimum for the broad spectrum of antimicrobial activity. In the present study rest of the compounds showed moderate to weak inhibitory activity against all the tested bacterial strains.

4.1.2.2. Pharmacological Activity

4.1.2.2.1. Acute Toxicity

No behavioral changes in animals were observed during the experiment and at the end hematological parameters were estimated and there was no observable change. In the present study, mortality was not observed even at 1000 mg kg^{-1} indicating that the tested compounds are nontoxic to animals.

4.1.2.2.2. Analgesic activity

The analgesic activity of the synthesized compounds **AOX_{4-6}**, **ODZ_{2-13}** and **ACH_{1-3}** was evaluated by the tail immersion technique[178] (Di Stasi *et al*., 1988) using albino mice of either sex (n=6). In general compounds belonging to the 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethy)-1,3,4-oxadiazol-2-yl]acetamide series exhibited better analgesic activity in comparison to the 1,3,4-oxadiazol-2-amines and acetohydrazides (**Table 4**). The tested compounds **ODZ_{10}** and **ODZ_{13}** exhibited fast analgesic activity (36.1 and 37.2%, respectively) as evident from observation at 30 min following oral administration (100 mg kg^{-1}) while at 1 h the same compounds exhibited moderate activity (43.4 and 45.5% analgesia, respectively) compared to the reference drug (44.2% analgesia at 50 mg kg^{-1}). At 2 h compounds bearing 3-methylphenyl (**ODZ_4**), 4-nitrophenyl (**ODZ_{10}**) or 2-acetamido-4-chlorophenyl (**ODZ_{13}**) groups at fifth position of the 1,3,4-oxadiazol ring exhibited potent analgesic activity (66.1-85.1%) compared to the reference drug (37.7%). The highest activity (85.1% analgesia) was observed at 2 h in derivative **ODZ_{13}**. It is important to note that at 3 h analgesic activity was maintained in four compounds namely; **ODZ_4**, **ODZ_6**, **ODZ_{10}** and **ODZ_{13}** (70.3-76.5%), whereas rest of the compounds showed a sharp decline in activity. Compounds from both 1,3,4-oxadiazol-2-amine (15.9-36.1% analgesia) and acetohydrazide (10.4-32.6% analgesia) series showed weak to moderate analgesic activities noticeably less than the reference drug (**Figure 18**).

4.1.2.2.3. Anti-inflammatory activity

The anti-inflammatory activity results determined using the carrageenan induced paw oedema method[179] (Winter *et al*., 1962) in rats are summarized in **Table 5**. It is clear that at 30 min after carrageenan injection, two compounds, namely **ODZ_4** and **ODZ_{13}** showed rapid onset of action (50.2 and 50.3% inhibition, respectively at a dose of 100 mg kg^{-1}). At 1 h the tested compounds bearing 3-methylphenyl (**ODZ_4**), 1-naphthyl (**ODZ_6**) and 4-nitrophenyl (**ODZ_{10}**) and 2-acetamido-4-chlorophenyl (**ODZ_{13}**) groups at fifth position of the 1,3,4-oxadiazole ring

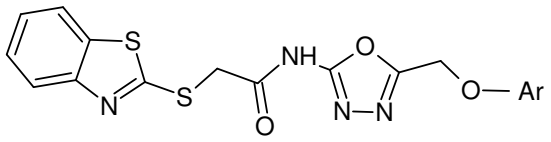
exhibited moderate anti-inflammatory activitiy (42.3-53.9% inhibition). Whereas at 2 and 3 h compounds **ODZ_4**, **ODZ_6** and **ODZ_{10}** were effective in inhibiting the paw oedema (58.2-68.2%), when compared with the reference drug (69.3 and 66.2%, respectively at a dose of 20 mg kg^{-1}). In this regard the maximum activity (68.2%) was observed at 2 h in derivative **ODZ_{10}**. Rest of the investigated compounds showed variable degree of anti-inflammatory activity ranging between weak to moderate (9.1-48.7%). In general, a marked decrease in activity was observed except for compounds **ODZ_4, ODZ_6** and **ODZ_{10}** at 3 h following carrageenan injection**.** It was also observed that substitution of 2,4,6-tri-Br (**ODZ_3**), 4-Cl-2-CH_3 (**ODZ_8**) or 2,4-di-Cl (**ODZ_{11}**) groups in the phenyl ring at fifth position of the oxadiazole ring markedly decreased the anti-inflammatory activity. Compounds from both 1,3,4-oxadiazol-2-amine (10.3-36.6%) and ayloxyacetohydrazide (11.4-39.6%) series showed weak to moderate anti-inflammatory activity noticeably less than the reference drug (**Figure 19**).

4.1.2.2.4. Ulcerogenic effects

Compounds **ODZ_4**, **ODZ_6** and **ODZ_{10}** that exhibited higher analgesic and anti-inflammatory profiles in the pre-mentioned animal models and promising antibacterial activities were further evaluated for their ulcerogenic potential in rats according to the method reported by Cioli *et al.*[180] (1979). The results indicated low ulcerogenic activity of the tested compounds ranging from 2.4±0.6 to 4.4±0.8 (**Table 6**) whereas the standard drug diclofenac sodium showed high severity index of 5.1±0.8. The maximum reduction in ulcerogenic activity (2.4±0.6) was observed in compound **ODZ_{10}**. The other tested compounds also exhibited better GI safety profile as compared to the standard drug diclofenac sodium (**Figure 20**).

In summary, we have synthesized novel 2-mercaptobenzothiazole derivatives containing 1,3,4-oxadiazole (**ODZ_{1-15}**) and aryloxyacetohydrazide (**ACH_{1-5}**) moieties with the objective of developing dual anti-inflammatory-antimicrobial agents with minimal ulcerogenic activity. Among these, oxadiazole derivative **ODZ_{10}** showed the most prominent and consistent activity with a significant reduction of gastrointestinal toxicity. Therefore compound **ODZ_{10}** would represent a fruitful matrix for the development of a new class of dual acting antimicrobial and analgesic-anti-inflammatory agents.

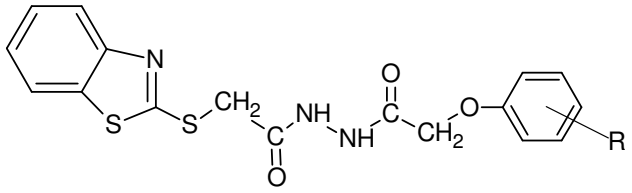
Table 1. Physical data of the synthesized 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethy)-1,3,4-oxadiazol-2-yl]acetamides (**ODZ_{1-15}**)



Compound	Ar	Solv. Cryst.	M.p.(°C)	[a]Yield (%)
ODZ_1	2-Cl.C_6H_4	methanol	150	66
ODZ_2	4-Cl.C_6H_4	ethanol	161	57
ODZ_3	2,4,6-tri-Br.C_6H_2	ethanol	181	73
ODZ_4	3-CH_3.C_6H_4	methanol	148	67
ODZ_5	4-CH_3.C_6H_4	methanol	134	66
ODZ_6	1-naphthyl	methanol	156	68
ODZ_7	2-naphthyl	acetone	141	64
ODZ_8	4-Cl-2-CH_3.C_6H_3	ethanol	157	71
ODZ_9	2-NO_2.C_6H_4	ethanol	198	70
ODZ_{10}	4-NO_2.C_6H_4	ethanol	180	73
ODZ_{11}	2,4-di-Cl.C_6H_3	ethanol	110	64
ODZ_{12}	2,6-di-Cl.C_6H_3	methanol	>300	58
ODZ_{13}	2-CH_3CONH-4-Cl.C_6H_3	methanol	186	63
ODZ_{14}	2-CH_3CONH-4-CH_3.C_6H_3	methanol	176	68
ODZ_{15}	4-CH_3CONH-C_6H_4	ethanol	191	57

[a]Isolated yield

Table 2. Physical data of the synthesized 2-(1,3-benzothiazol-2-ylsulfanyl)-n'-[(aryloxy)acetyl] acetohydrazides (**ACH_{1-5}**)



Compound	Ar	[a,]M.p.(°C)	[b]Yield (%)
ACH_1	2-Cl	104	66
ACH_2	4-Cl	148	65
ACH_3	3-CH_3	155	72
ACH_4	4-CH_3	158	63
ACH_5	4-NO_2	168	59

[a]Solvent crystallization: ethanol
[b]Isolated yield

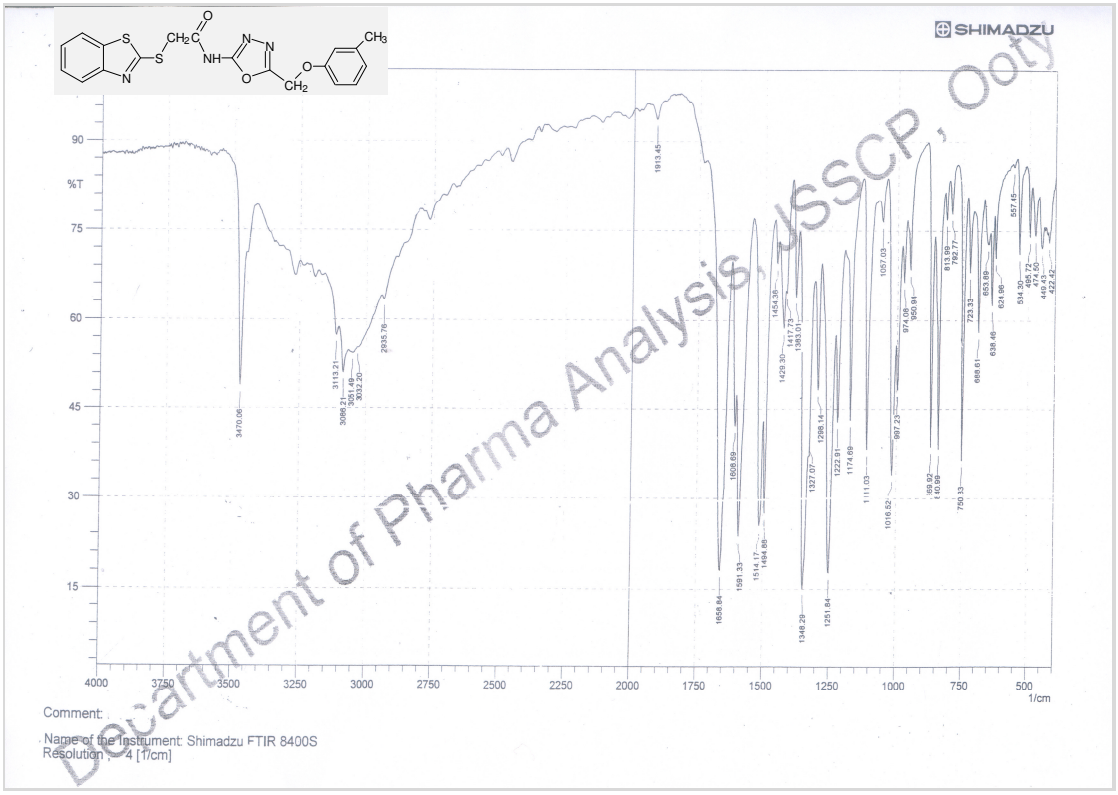


Figure 4. Infrared spectrum of the synthesized compound **ODZ$_4$**

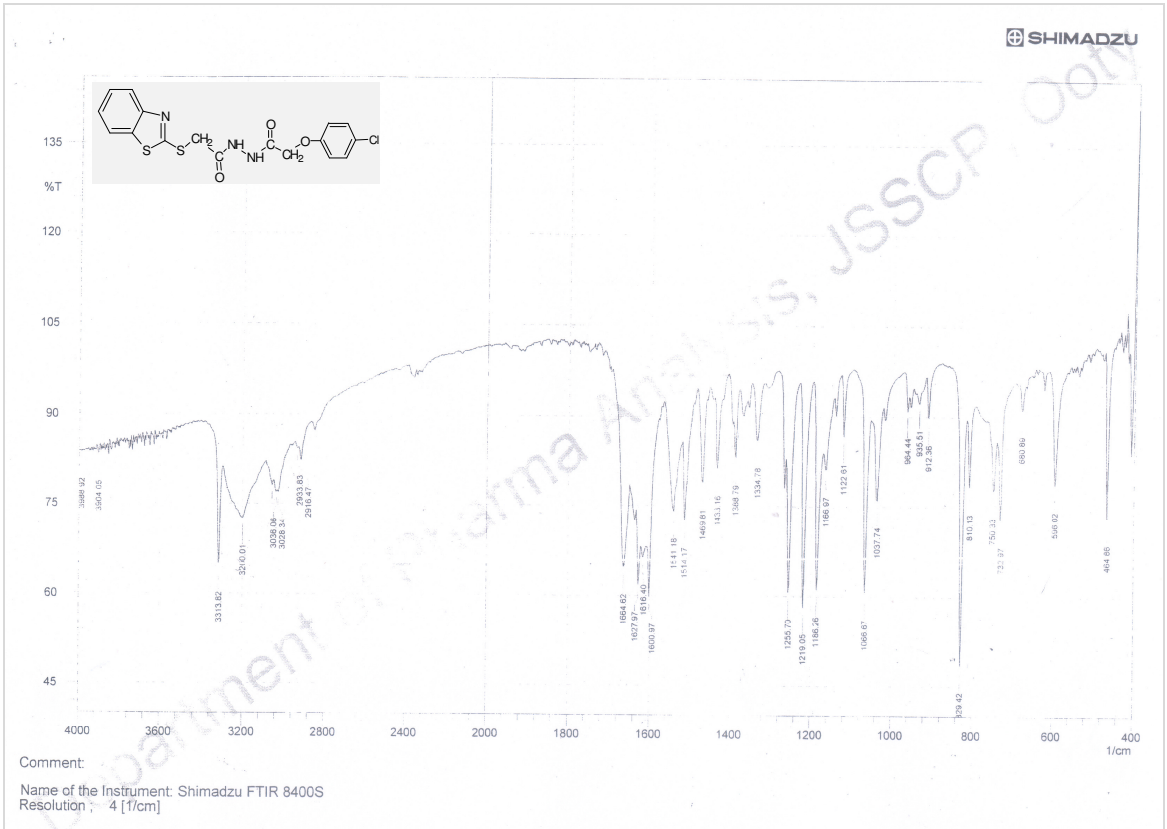


Figure 5. Infrared spectrum of the synthesized compound **ACH$_2$**

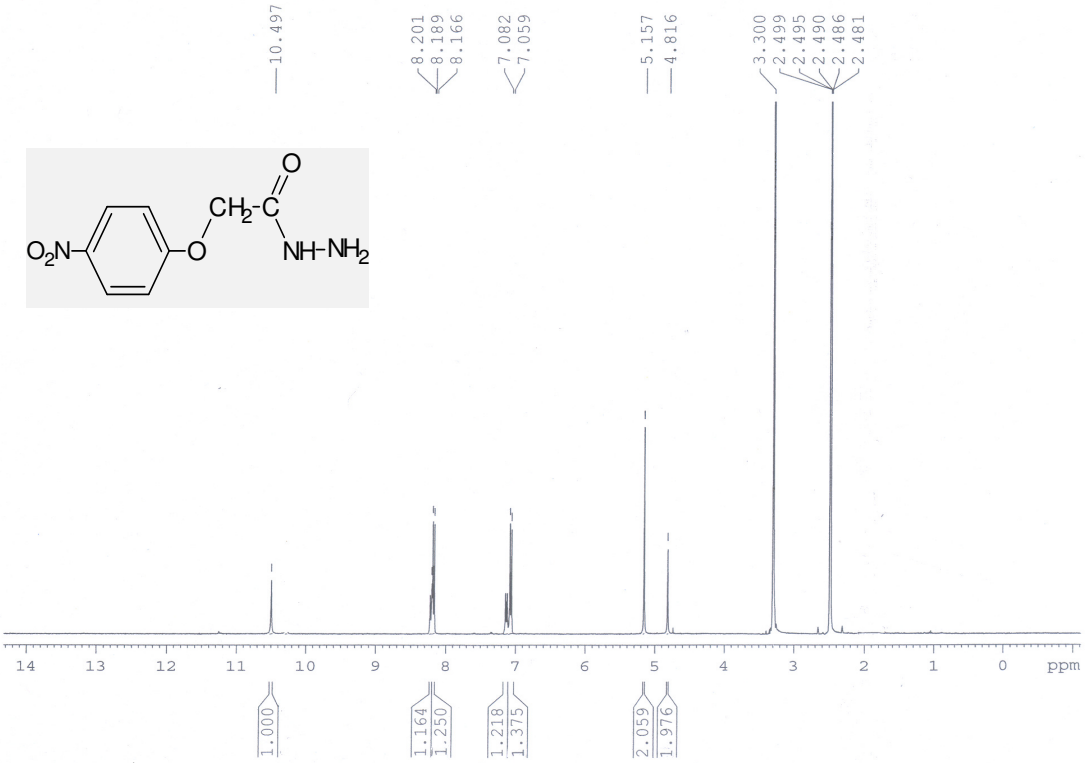


Figure 6. [1]H NMR spectrum of the synthesized compound **HYD_{10}**

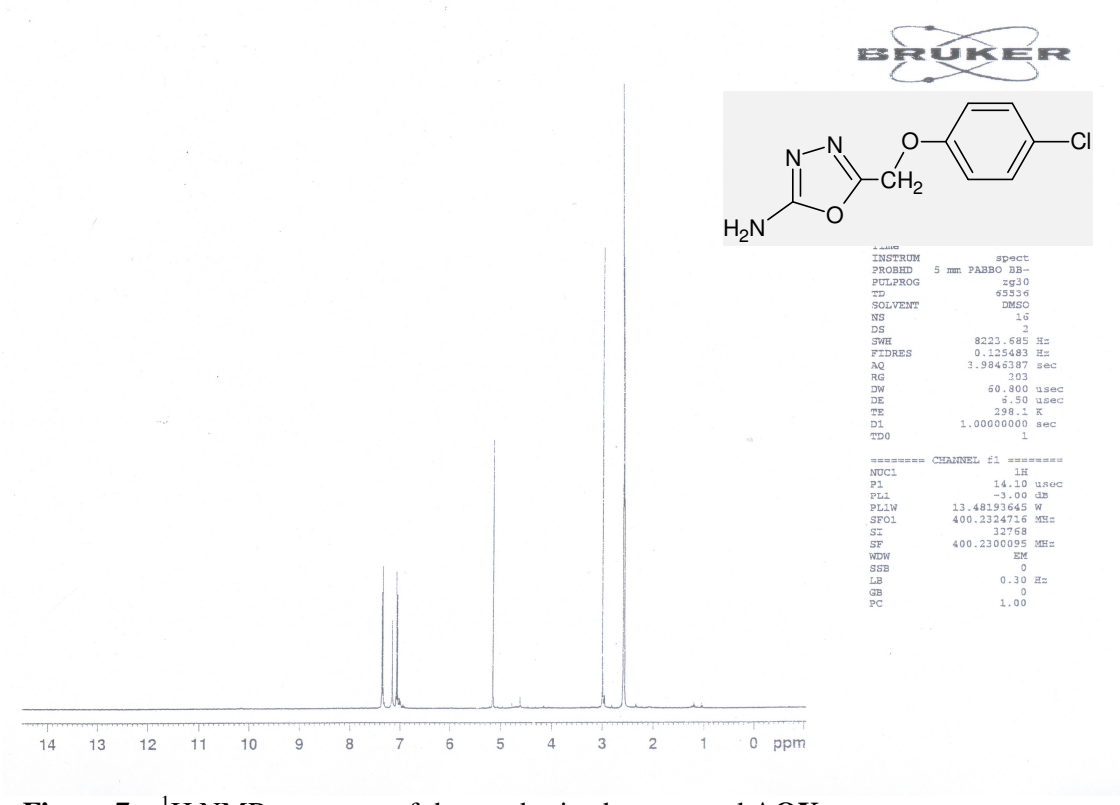


Figure 7a. [1]H NMR spectrum of the synthesized compound **AOX_2**

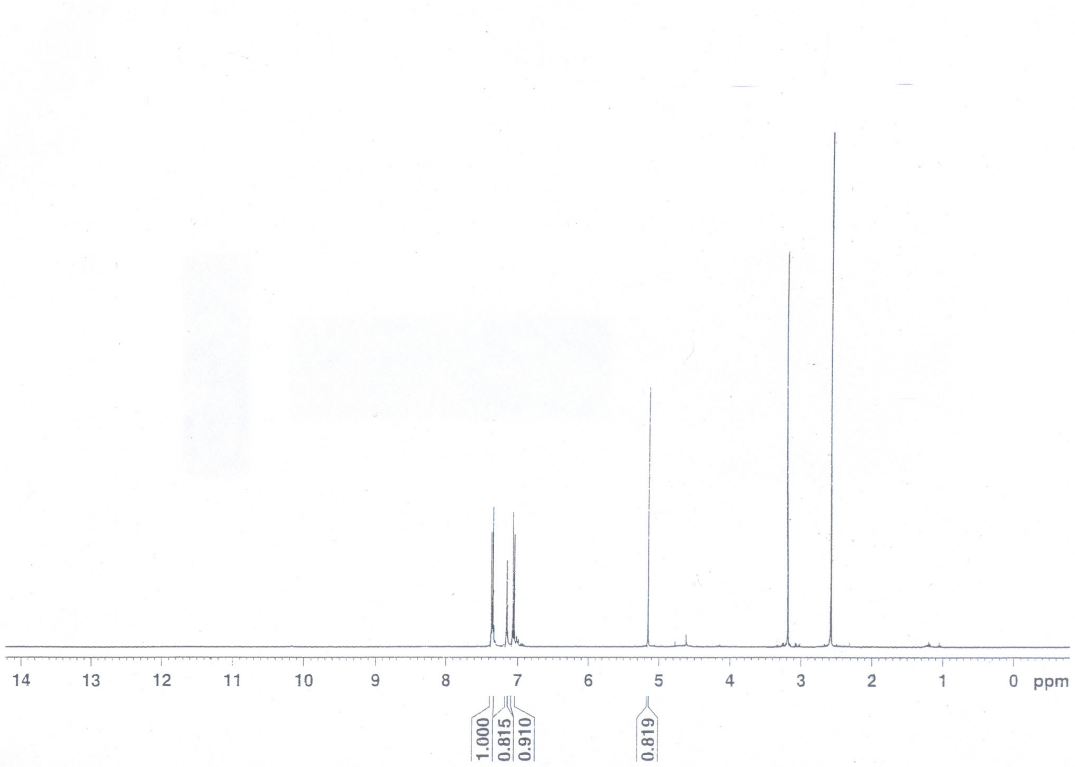


Figure 7b. ^{1}H NMR spectrum of the synthesized compound **AOX_2**

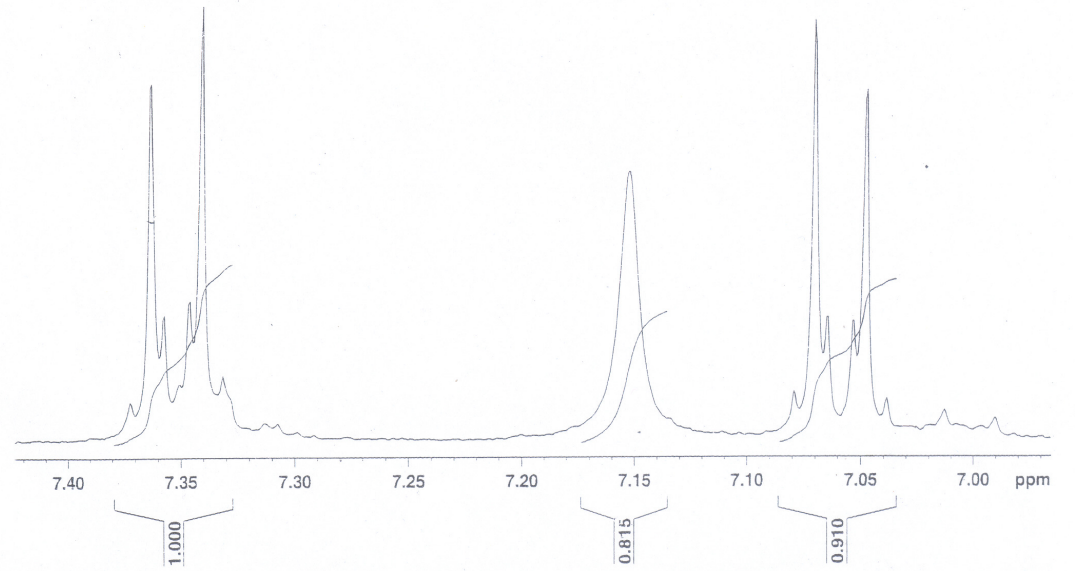


Figure 7c. Expansion of the ^{1}H NMR spectrum of the synthesized compound **AOX_2**

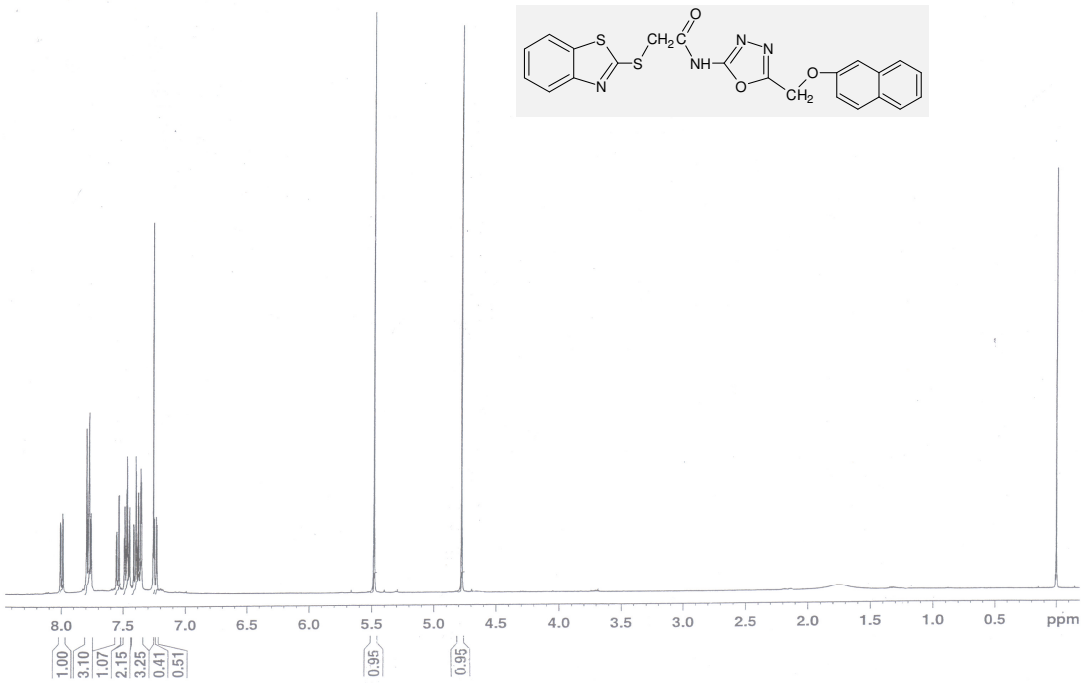


Figure 8a. ^{1}H NMR spectrum of the synthesized compound **ODZ$_7$**

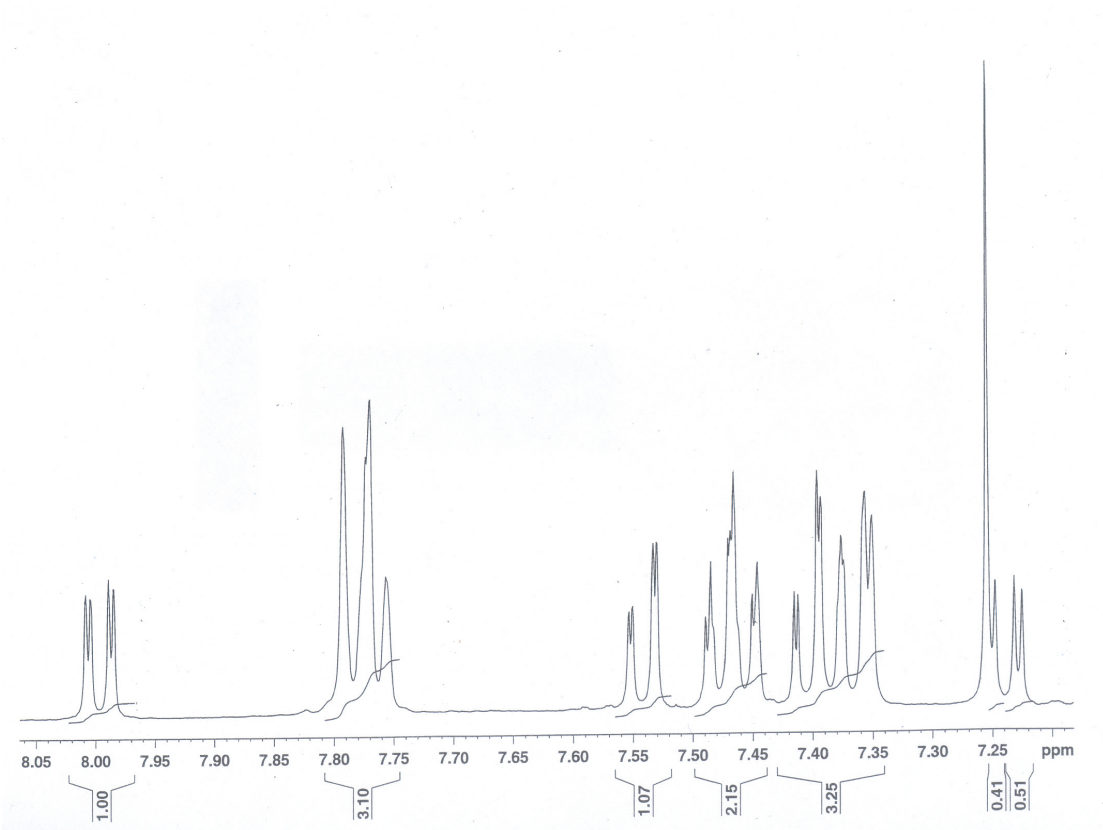


Figure 8b. Expansion of the ^{1}H NMR spectrum of the synthesized compound **ODZ$_7$**

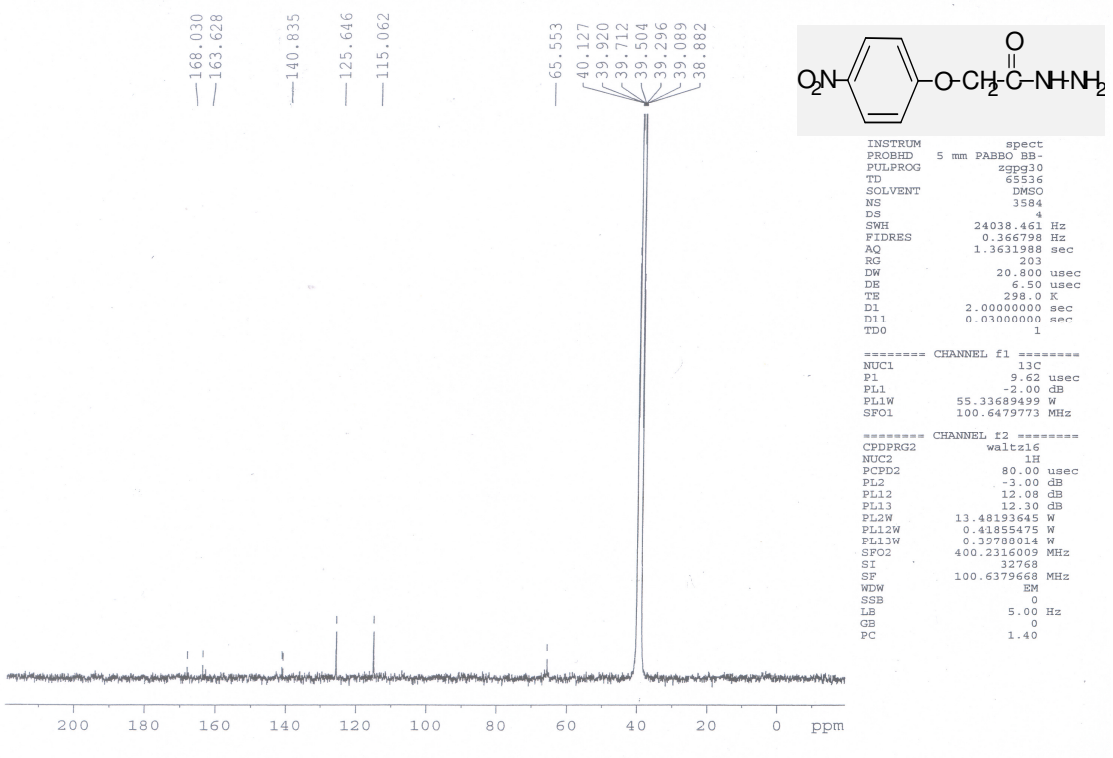


Figure 9. ^{13}C NMR spectrum of the synthesized compound **HYD_{10}**

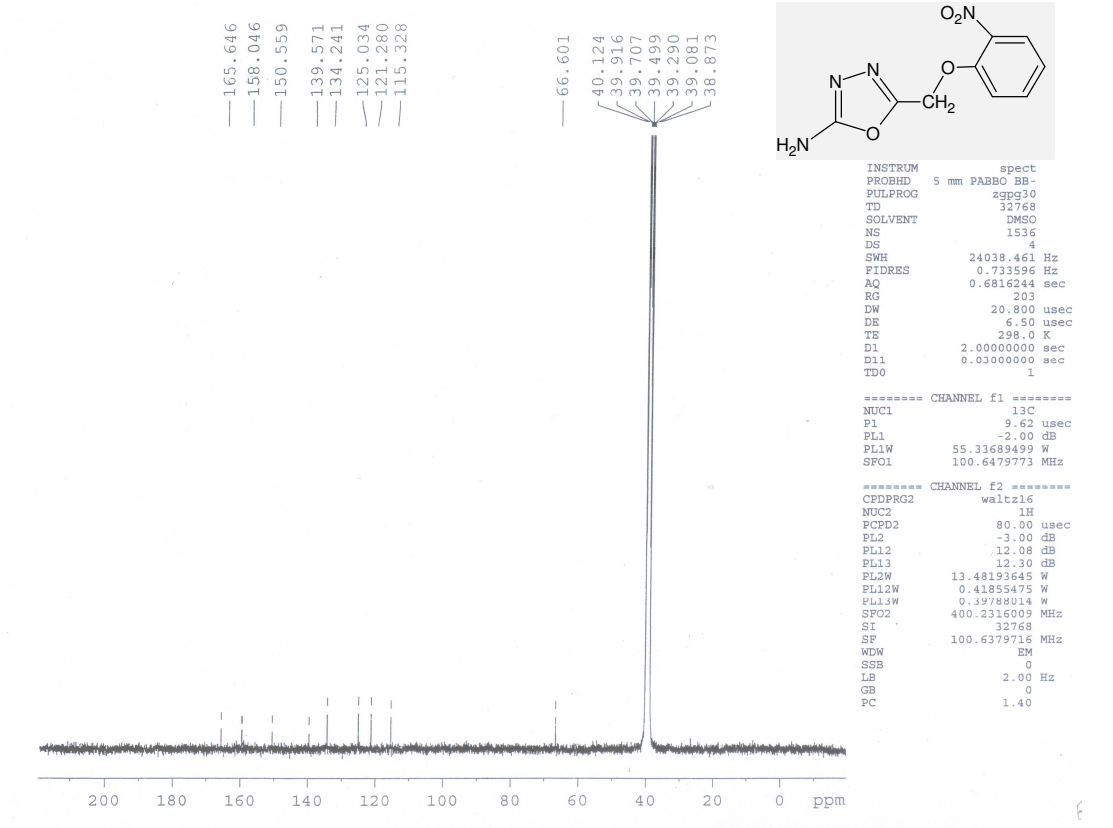


Figure10. ^{13}C NMR spectrum of the synthesized compound **AOX_9**

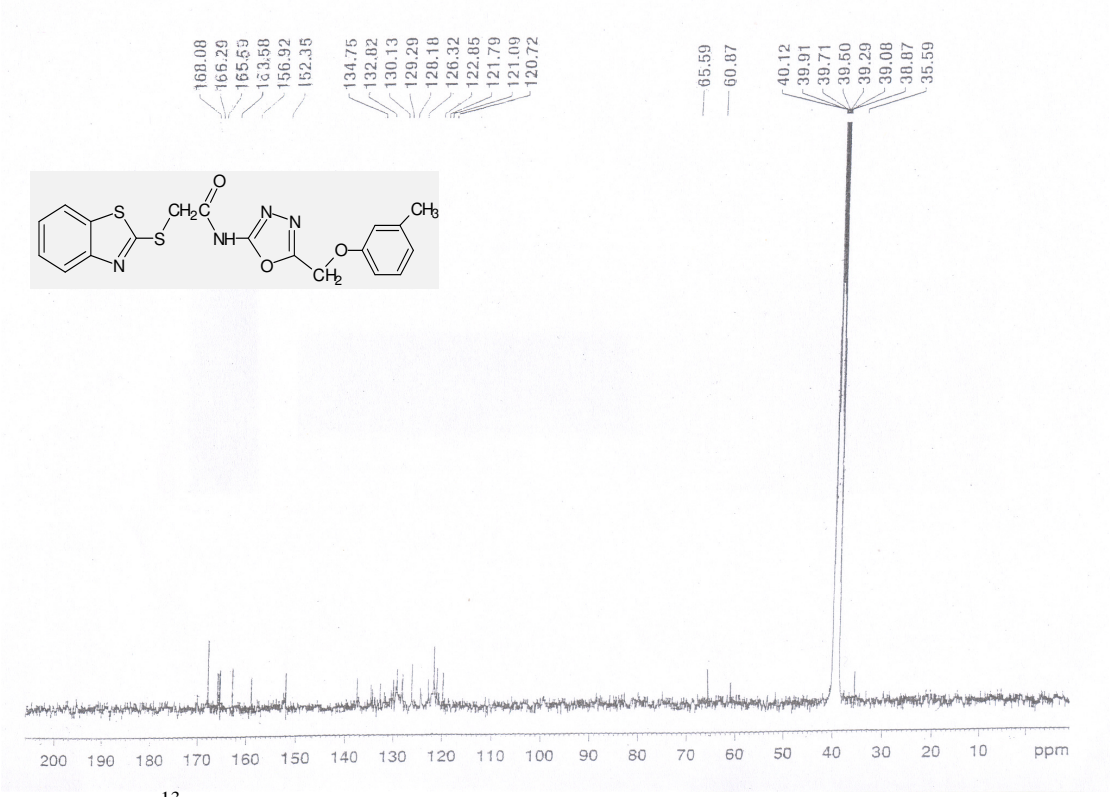


Figure 11. ^{13}C NMR spectrum of the synthesized compound **ODZ$_4$**

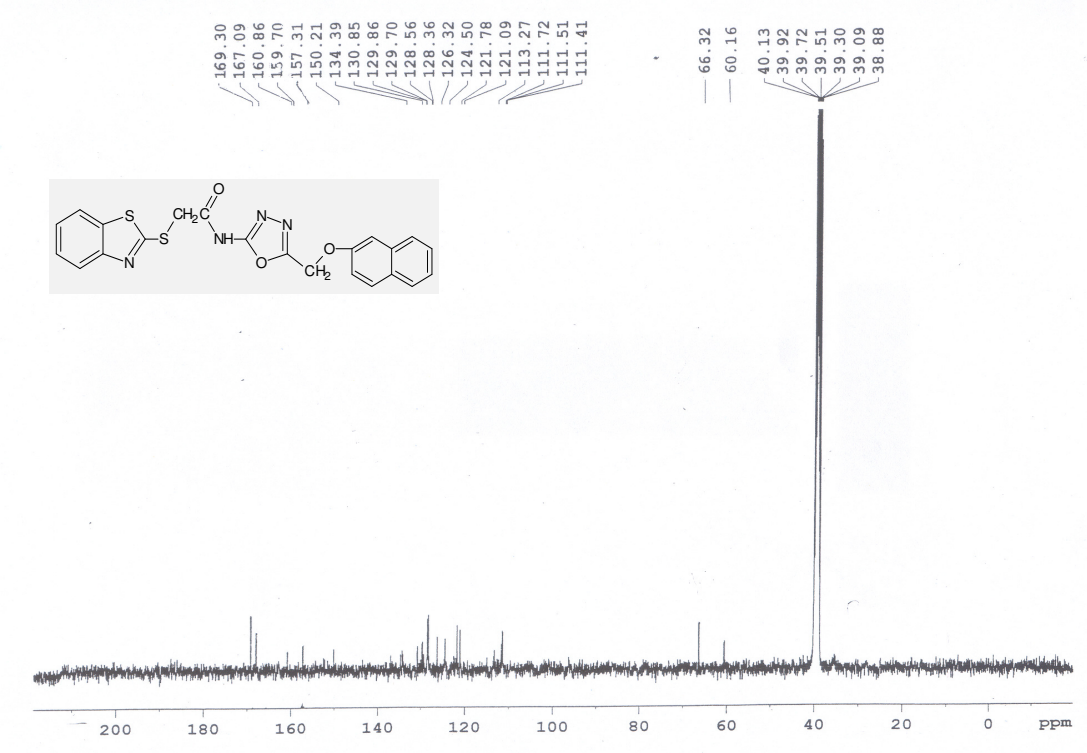


Figure 12. ^{13}C NMR spectrum of the synthesized compound **ODZ$_7$**

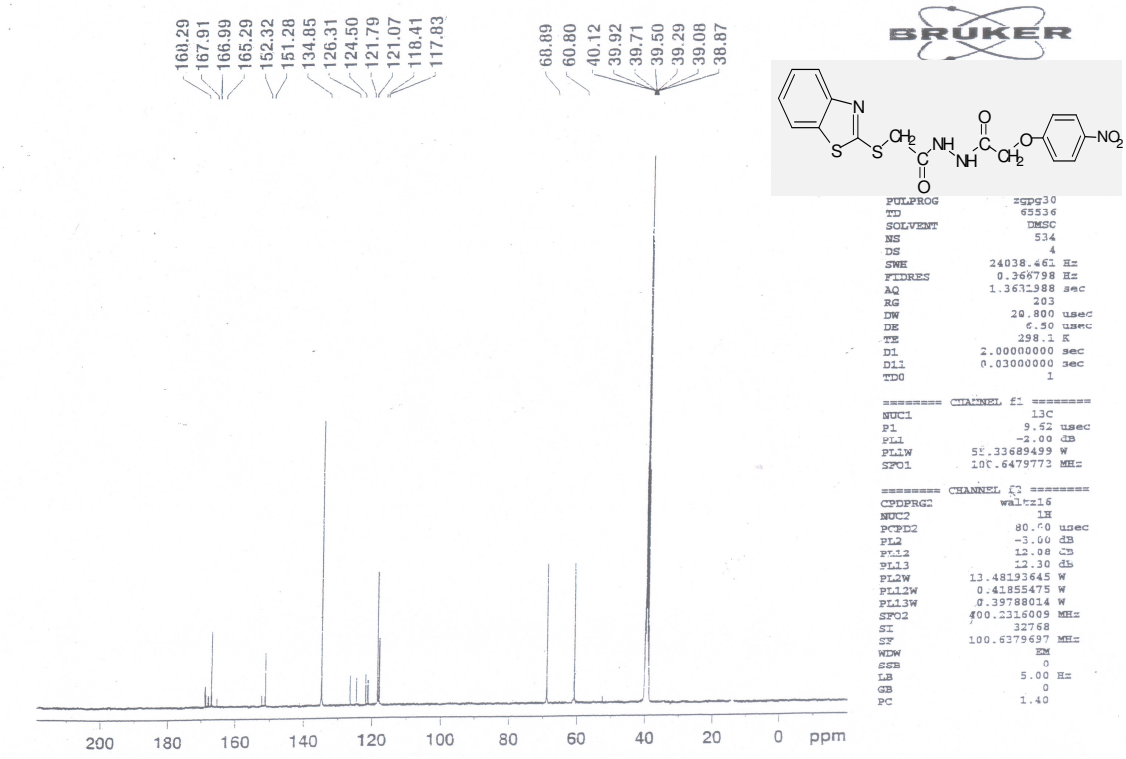


Figure 13. ^{13}C NMR spectrum of the synthesized compound **ACH_5**

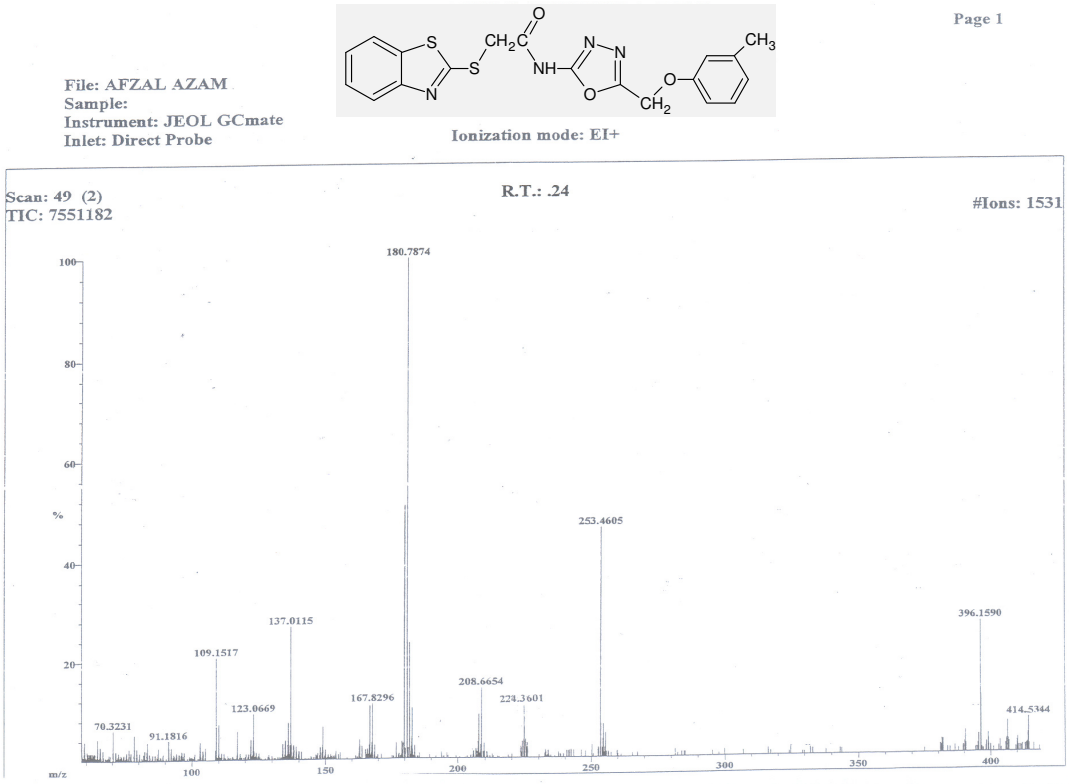


Figure 14. Electron impact mass spectrum of the synthesized compound **ODZ_4**

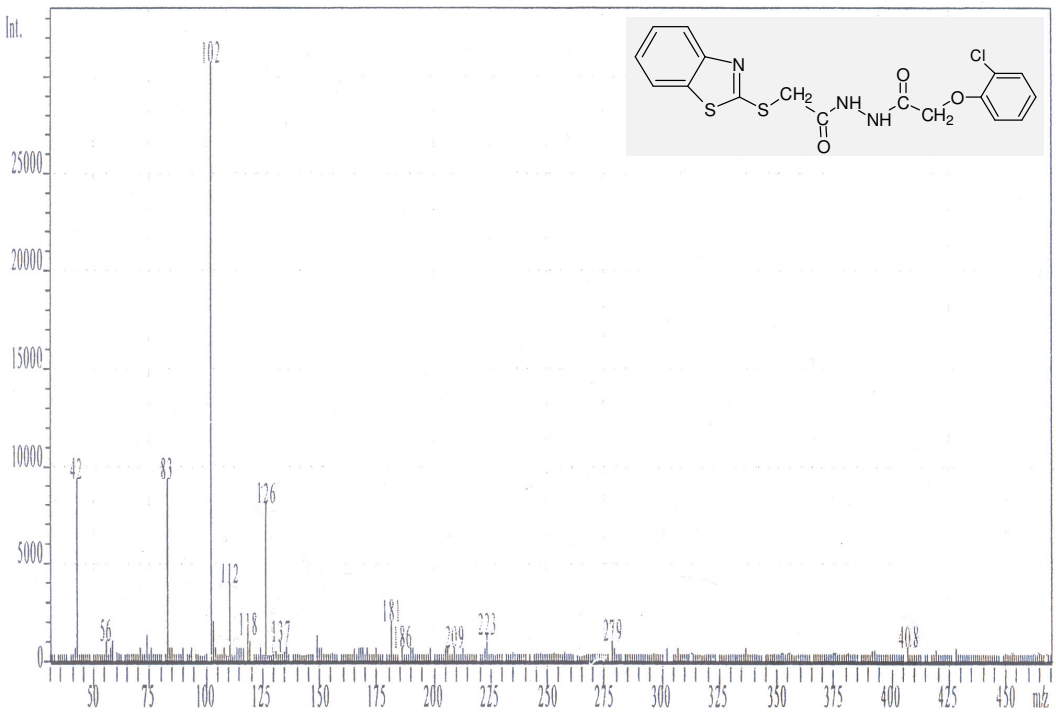
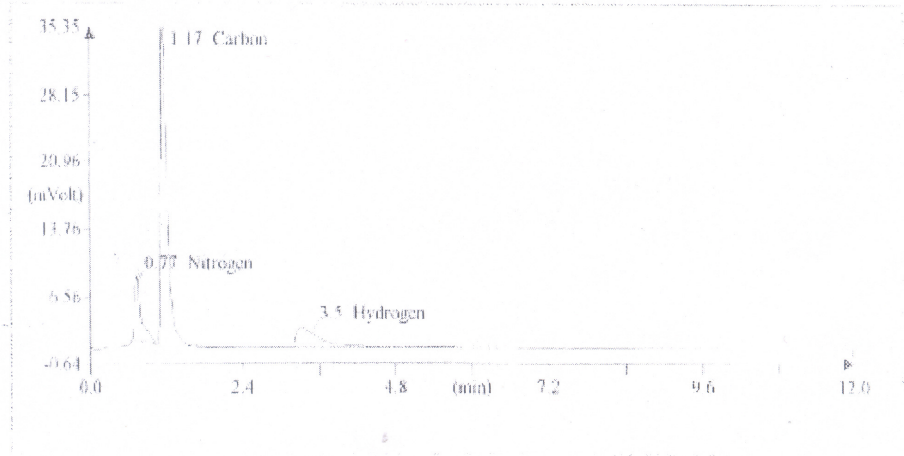


Figure 15. Electron spray ionization mass spectrum of the synthesized compound **ACH_1**

FLASH EA 1112 SERIES CHNS REPORT
THERMO FINNIGAN

Company name:
Method filename: C:\Progam Files\Thermo Finnigan\Eager 300 for A1112\data\First Run\N C H S syster
Sample ID: M-10 (24) (# 472)
Analysis type: Unknown
Chromotogram filename: Azam-08122009-1.dat
Sample weight in mg: 1.412

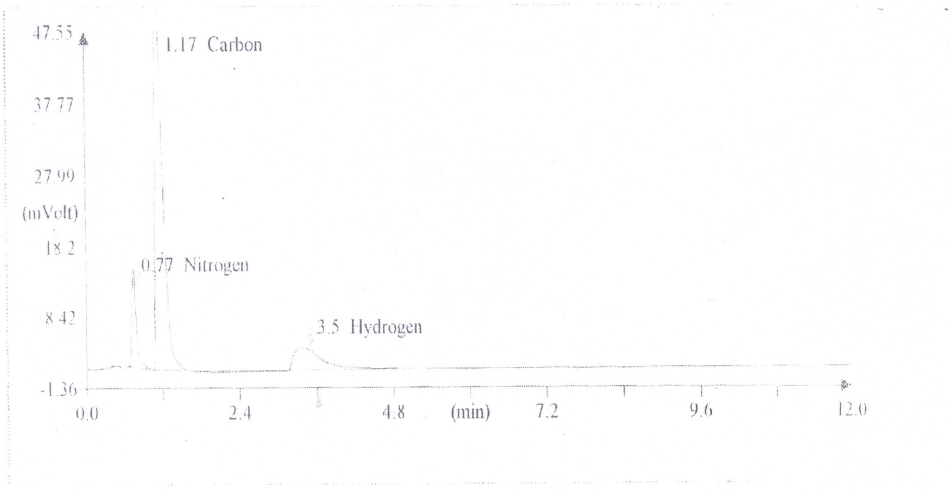


Peak Number (#)	Component Name	Elements %	Area (.1* uV* sec)
1	Nitogen	13.570	542751
2	Carbon	55.303	3536998
3	Hydrogen	3.914	577547
		72.787	4657296

Figure 16. CHN analysis spectrum of the synthesized compound **ODZ$_4$**

FLASH EA 1112 SERIES CHNS REPORT
THERMO FINNIGAN

Company name:
Method filename: C:\Progam Files\Thermo Finnigan\Eager 300 for A1112\data\First Run\N C H S syster
Sample ID: M-10 (24) (# 473)
Analysis type: Unknown
Chromotogram filename: Azam-08122009-1.dat
Sample weight in mg: 1.245



Peak Number (#)	Component Name	Elements %	Area (.1* uV* sec)
1	Nitrogen	13.420	871329
2	Carbon	48.783	3058500
3	Hydrogen	3.345	723344
		65.548	4653173

Figure 17. CHN analysis spectrum of the synthesized compound **ACH_5**

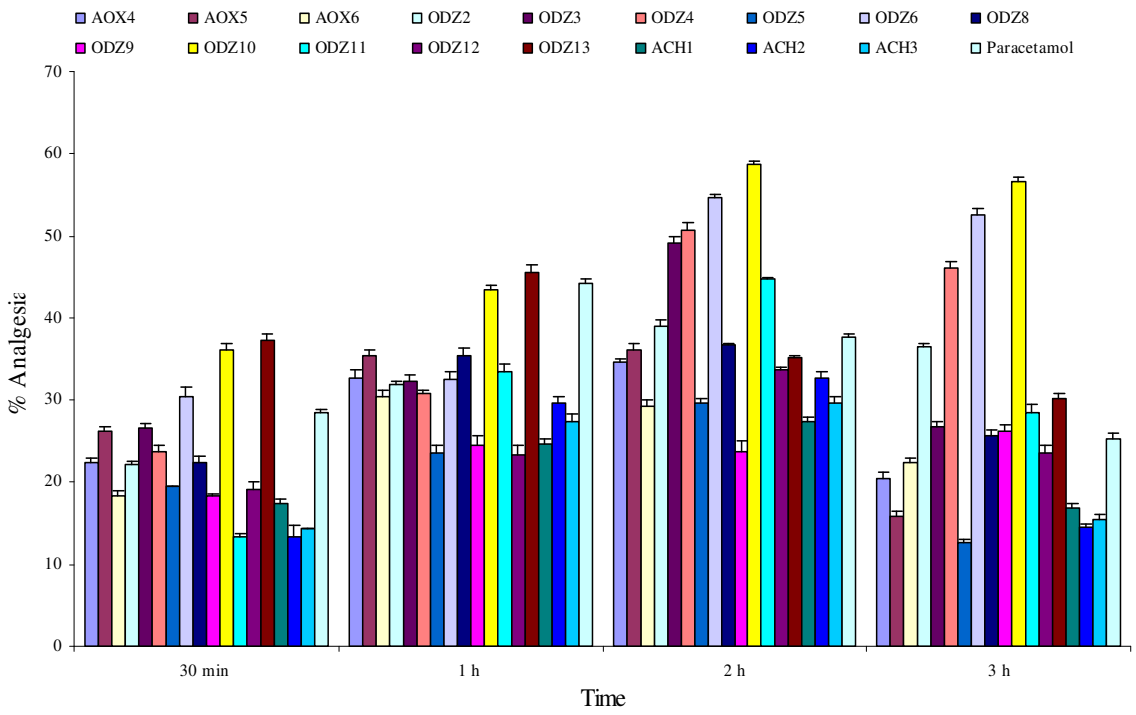


Figure 18. Analgesic activity of some selected 1,3,4-oxadiazole and acetohydrazide incorporated 2-mercaptobenzothiazoles in mice by the tail immersion method

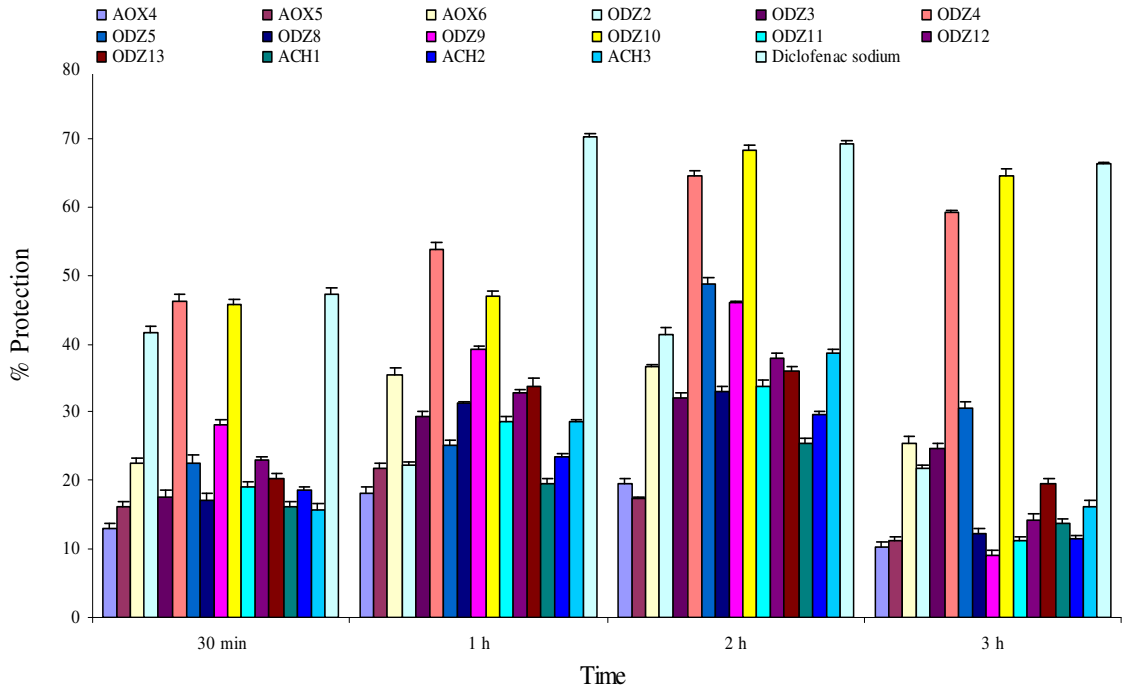
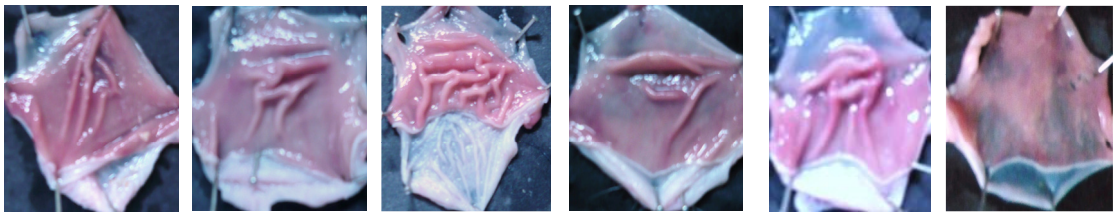
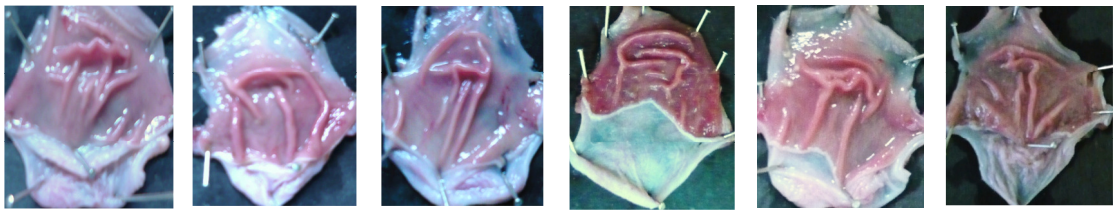


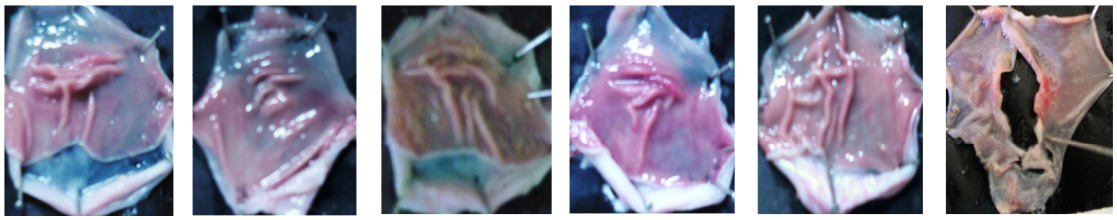
Figure 19. Anti-inflammatory activity of some selected 1,3,4-oxadiazole and acetohydrazide incorporated 2-mercaptobenzothiazoles by the carrageenan induced rat paw oedema method



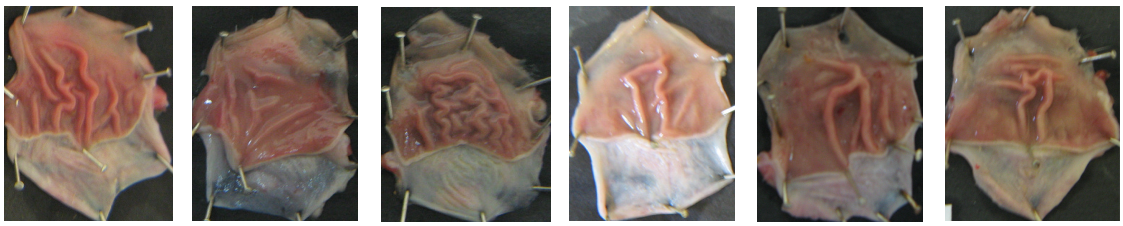
Control carboxymethyl cellulose



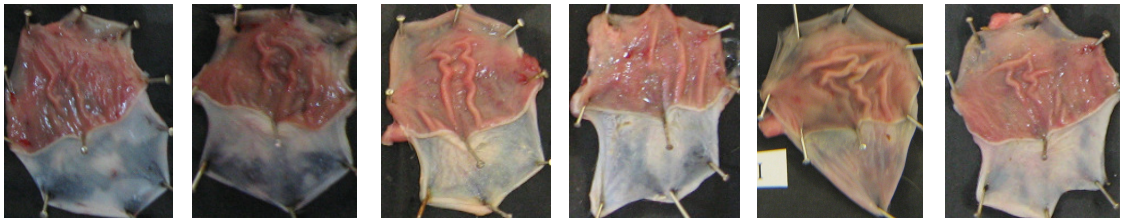
Compound **ODZ$_4$**



Compound **ODZ$_6$**

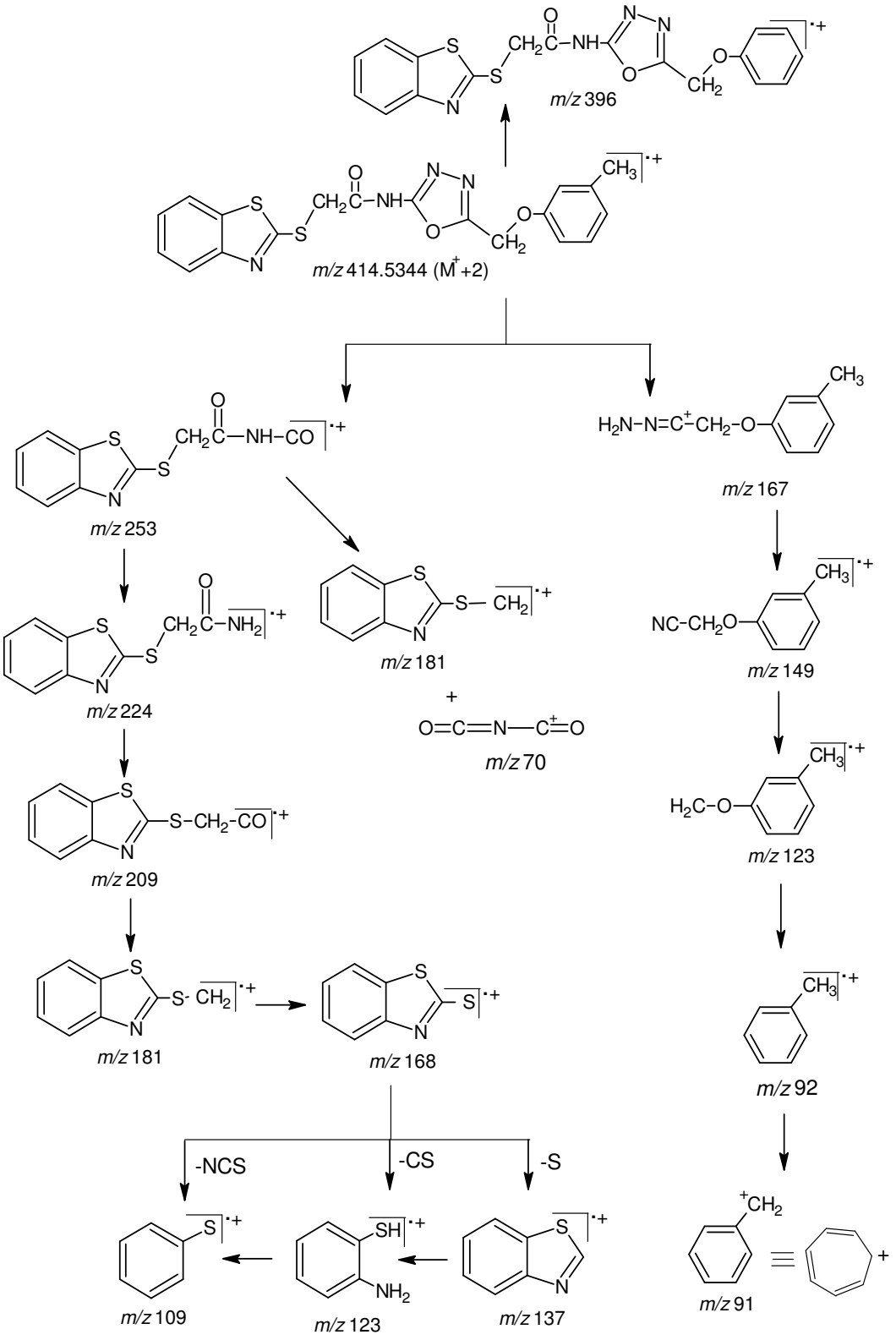


Compound **ODZ$_{10}$**

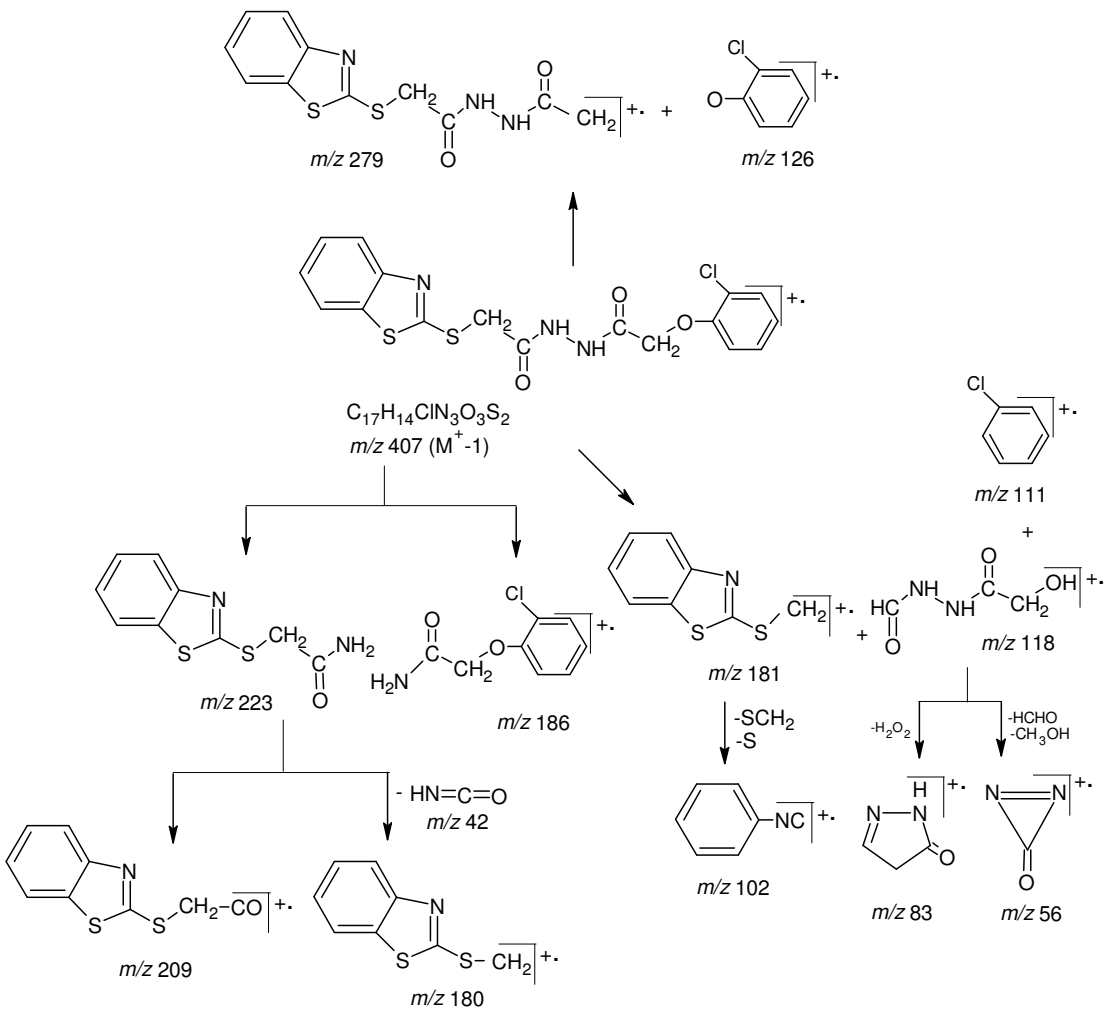


Diclofenac sodium

Figure 20. Ulcerogenic potential of some 1,3,4-oxadiazole incorporated 2-mercaptobenzothiazoles by the Cioli *et al.* method



Scheme 5. Possible mass fragmentation pattern of the synthesized compound **ODZ$_4$**



Scheme 6. Possible mass fragmentation pattern of the synthesized compound**ACH$_1$**

Table 3. Antimicrobial screening of 1,3,4-oxadiazole and acetohydrazide incorporated 2-mercaptobenzothiazoles by the cup plate method.

Compd.	Zone of inhibition (mm)[a,b]					
	S.a.	*B.s.*	*E.c.*	*P.a.*	*C.a.*	*A.n.*
AOX_1	14	15	13	15	-	-
AOX_2	13	16	15	14	-	-
AOX_3	16	13	15	14	-	-
AOX_4	18	19	19	18	-	-
AOX_5	17	20	21	19	-	-
AOX_6	21	18	19	20	-	-
AOX_7	14	16	13	16	-	-
AOX_8	14	16	13	15	-	-
AOX_9	13	14	16	14	-	-
AOX_{10}	15	13	16	12	-	-
AOX_{11}	13	15	13	13	-	-
AOX_{12}	13	15	13	14	-	-
AOX_{13}	13	14	13	17	-	-
AOX_{14}	14	13	14	13	-	-
AOX_{15}	13	14	14	16	-	-
ODZ_1	24	25	24	26	-	-
ODZ_2	26	24	27	25	-	-
ODZ_3	24	26	25	24	-	-
ODZ_4	29	30	30	29	-	-
ODZ_5	31	29	12	14	-	-
ODZ_6	30	25	27	28	-	-
ODZ_7	18	17	20	19	-	-
ODZ_8	18	17	19	18	-	-
ODZ_9	30	27	19	17	-	-
ODZ_{10}	31	30	29	32	-	-
ODZ_{11}	26	29	28	27	-	-
ODZ_{12}	18	19	20	17	-	-
ODZ_{13}	30	29	30	28	-	-
ODZ_{14}	14	16	13	15	-	-
ODZ_{15}	13	15	13	16	-	-
ACH_1	14	13	17	20	-	-
ACH_2	-	14	18	22	-	-
ACH_3	-	14	18	19	-	-
ACH_4	14	13	15	18	-	-
ACH_5	13	14	16	23	-	-
DMSO	-	-	-	-	-	-
Ciprofloxacin	30	32	33	31	-	-
Ketoconazole	-	-	-	-	32	28

Test compounds, ciprofloxacin and ketoconazole were tested at 100, 10 and 20 mg kg^{-1} concentrations, respectively. [a]Average of three readings. [b]**-** indicates no activity. *S.a.: Staphylococcus aureus; B.s.: Bacillus subtilis; E. c.: Escherichia coli; P.a.: Pseudomonas aeruginosa*; *C. a.: Candida albicans*; *A. n.: Aspergillus niger*.

Table 4. Analgesic activity of some selected 1,3,4-oxadiazole incorporated 2-mercaptobenzothiazoles in mice by the tail flick method.

Compd	Percent analgesic activity			
	30 min %Analgesia± SEM	1 h %Analgesia± SEM	2 h %Analgesia± SEM	3 h %Analgesia± SEM
AOX_4	22.4±0.5[c]	32.6±1.0[b]	34.6±0.3[a]	20.5±0.6[a]
AOX_5	26.1±0.7[b]	35.4±0.8[b]	36.1±0.8[c]	15.9±0.6[b]
AOX_6	18.2±0.6[b]	30.3±0.9[c]	29.3±0.7[b]	22.3±0.7[b]
ODZ_2	22.2±0.3[b]	31.8±0.4[c]	39.0±0.8[b]	36.6±0.3[b]
ODZ_3	26.5±0.7[c]	32.3±0.7[c]	49.1±0.8[c]	26.8±0.6[b]
ODZ_4	23.7±0.8[a]	30.7±0.5[a]	66.1±0.9[b]	76.1±0.8[b]
ODZ_5	19.4±0.8[b]	23.4±1.0[b]	29.6±0.6[b]	12.5±0.4[c]
ODZ_6	30.4±1.1[b]	32.4±1.0[b]	54.6±0.3[a]	72.5±0.9[b]
ODZ_8	22.4±0.7[b]	35.4±1.0[b]	36.6±0.3[a]	25.5±0.9[b]
ODZ_9	18.4±0.2[c]	24.4±1.2[c]	44.7±1.3[c]	26.1±0.7[a]
ODZ_{10}	36.1±0.7[b]	43.4±0.6[b]	70.7±0.5[a]	76.5±0.7[b]
ODZ_{11}	13.4±0.3[a]	33.4±1.0[b]	44.6±0.3[a]	28.5±0.9[b]
ODZ_{12}	19.2±0.8[b]	23.4±1.0[b]	33.6±0.3[a]	23.5±0.9[b]
ODZ_{13}	37.2±0.9[b]	45.5±0.9[b]	85.1±0.3[a]	70.3±0.6[a]
ACH_1	17.4±0.6[c]	24.6±0.7[b]	27.4±0.4[a]	16.9±0.5[c]
ACH_2	13.4±1.2[b]	29.6±0.7[c]	32.6±0.8[b]	14.5±0.4[c]
ACH_3	10.4±0 .6[b]	27.4±1.0[c]	29.6±0.7[b]	15.4±0.7[b]
Paracetamol	28.4±0.4[b]	44.2±0.6[a]	37.7±0.4[b]	25.5±0.8[b]

Test compounds and paracetamol were tested at 100 and 50 mg kg^{-1} body weight, respectively. Results are expressed in mean ± SEM (n=6); Significance levels [a]$P<0.05$, [b]$P<0.01$ and [c]$P<0.001$ compared with the respective control.

Table 5. Anti-inflammatory activity of some selected 1,3,4-oxadiazole incorporated 2-mercaptobenzothiazoles by the carrageenan induced rat paw oedema method.

Compd.	Percent protection			
	30 min % Pretection (Mean % protection± SEM)	1 h %Protection (Mean % protection± SEM)	2 h %Protection (Mean % protection±SEM)	3 h %Protection (Mean % protection± SEM)
AOX_4	12.9±0.6[c]	18.1±1.0[a]	19.5±0.7[b]	10.3±0.7[c]
AOX_5	16.1±0.7[b]	21.7±0.8[b]	17.3±0.3[b]	11.2±0.4[c]
AOX_6	22.4±0.7[b]	35.4±1.0[b]	36.6±0.3[a]	25.5±0.9[b]
ODZ_2	41.6±1.0[b]	22.2±0.6[b]	41.2±1.0[b]	21.6±0.6[b]
ODZ_3	17.7 ±0.9[b]	29.2±0.8[b]	31.9±0.8[b]	24.5±0.8[b]
ODZ_4	50.2±0.9[b]	53.9±0.8[b]	64.6±0.7[b]	59.1±0.3[a]
ODZ_5	22.5±1.2[c]	25.2±0.7[b]	48.7±0.9[b]	30.5±1.1[c]
ODZ_6	19.1±1.0[b]	42.3±0.3[b]	63.0±0.6[b]	58.2±0.8[c]
ODZ_8	17.1±0.9[c]	31.3±0.3[b]	33.0±0.7[b]	12.2±0.7[c]
ODZ_9	28.0±0.8[b]	39.0±0.6[b]	45.9±0.4[b]	9.1±0.6[b]
ODZ_{10}	45.7±0.7[a]	47.0±0.7[b]	68.2±0.8[b]	64.6±0.9[b]
ODZ_{11}	19.0±0.8[b]	28.7±0.6[b]	33.8±0.8[c]	11.2±0.4[c]
ODZ_{12}	23.0±0.5[b]	32.7±0.6[b]	37.8±0.8[c]	14.2±0.9[b]
ODZ_{13}	50.3±0.7[b]	51.8±1.1[b]	66.0±0.7[a]	19.4±0.8[c]
ACH_1	16.2±0.6[b]	19.5±0.7[c]	25.3±0.8[c]	13.7±0.6[b]
ACH_2	18.7±0.5[a]	23.4±0.6[b]	39.6±0.6[c]	11.4±0.7[c]
ACH_3	15.7±0.9[c]	28.6±0.3[c]	38.7±0.5[b]	16.2±0.9[b]
Diclofenac sodium	47.2±1.1[c]	70.1±0.6[b]	69.3±0.3[b]	66.2±0.4[b]

Test compounds and diclofenac sodium were tested at 100 and 20 mg kg^{-1} body weight, respectively; results are expressed in mean±SEM (n=6); Significance levels [a]P<0.05, [b]P<0.01 and [c]P<0.001 compared with the respective control.

Table 6. Ulcerogenic effects of some selected 1,3,4-oxadiazole incorporated 2-mercaptobenzothiazoles by the Cioli *et al.'s* method.

Compd.	Control 1% CMC	**ODZ_4**	**ODZ_6**	**ODZ_{10}**	Diclofenac sodium
Severity Index	00.0±0.1[a]	4.4±0.8[b]	3.6±0.5[b]	2.4±0.6[a]	5.1±0.8[c]

Test compounds and diclofenac sodium were tested at 200 and 20 mg kg^{-1}body weight, respectively. Results are expressed in mean ± SEM (n=6); Significance levels [a]P<0.05, [b]P<0.01 and [c]P<0.001 compared with the respective control.

4.2. Synthesis and Biological Evaluation of some Novel 1,3,4-Oxadiazole, 1,2,4-Triazole and 1,3,4-Thiadiazole Incorporated 2-Mercaptobenzothiazoles

4.2.1. Chemistry

The synthesis of the title compounds (**OXZ_{1-13}**), (**TRZ_{1-7}**) and (**TDZ_{1-13}**) is described in **Scheme 2** and **3**. Ethyl (benzothiazol-2-ylthio)acetate (**MBT_1**) was prepared according to the reported method[117] (Karali *et al.*, 2004), and condensed with hydrazine hydrate (99%) in absolute ethanol to obtain benzothiazol-2-ylthio acetic acid hydrazide[118] (Desai *et al.*, 2006) (**MBH_1**). The reactions of phenols (**PHL_{1-13}**) with chloroacetic acid in basic medium[181] (Furniss *et al.*, 2005) resulted in the formation of corresponding aryloxyacetic acid derivatives (**AOA_{1-13}**). Cyclo-condensation[160] (Karthikeyan *et al.*, 2008) of these acids with hydrazide (**MBH_1**) in the presence of phosphorous oxychloride gave the desired 1,3,4-oxadiazol derivatives (**OXZ_{1-13}**). Further condensation[163] (Padmavathi *et al.,* 2009) of the 1,3,4-oxadiazol derivatives **OXZ_{2-5}**, **OXZ_9**, **OXZ_{10}** and **OXZ_{12}** with hydrazine hydrate (99%) in n-butanol furnished the corresponding 1,2,4-triazol-4-amine derivatives (**TRZ_{1-7}**). On the other hand, cyclization[182] (Jadhav *et al.*, 2008) of **AOA_{1-13}** with thiosemicarbazide in the presence of phosphorous oxychloride gave the corresponding 1,3,4-thiadiazol-2-amine derivatives (**ATZ_{1-13}**). The reactions of compounds **ATZ_{1-13}** with ethyl (benzothiazol-2-ylthio)acetate (**MBE_1**) in refluxing absolute ethanol afforded the corresponding 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl]acetamides (**TDZ_{1-13}**). The structures of the newly synthesized compounds were confirmed by the analytical and spectral data.

The infrared (IR) spectra of compounds **OXZ_{1-13}** showed C-O-C vibrations of the oxadiazole ring[160,163] (Karthikeyan *et al.*, 2008; Padmavathi *et al.*, 2009) in the region 1311-1249 cm^{-1}. The C=N stretching observed at 1660-1599 cm^{-1} is due to the ring closure. The proton magnetic resonance (^1H NMR) spectrum[160,161] (Karthikeyan *et al.*, 2008; Islam *et al.*, 2008) of **OXZ_1** (in $CDCl_3$) showed multiplet signals at δ 7.99-7.68 and 7.54-6.98 ppm for four and five aromatic protons and singlet signals at δ 5.62 and 3.50 ppm due to the OCH_2 and SCH_2 fragments. In the ^{13}C NMR spectrum, the C=N carbon of the benzothiazole ring resonated at δ 166.33 ppm. The C-2 and C-5 carbons of the oxadiazole moiety gave singlets at δ 164.84 and 157.75 ppm, respectively. The chemical shift values of the other aromatic carbons were observed in the expected region. The electron impact (EI) mass spectrum of **OXZ_1** (**Figure 32**) showed [$M^{+\cdot}$+1] peak at *m/z* 356 which is in agreement with its molecular formula

$C_{17}H_{13}N_3O_2S_2$. The spectrum is dominated by *m/z* 148 ion (base peak) due to the fragment $[C_8H_7NO_2]^{+\cdot}$ which could be expected as the major fragmentation pathway of **OXZ$_1$**.The spectrum also exhibited medium intensity peaks at *m/z* 223, 206 and 107, which are consistent with the cleavage of $[C_9H_6N_2OS_2]^{+\cdot}$, $[C_9H_6N_2S_2]^{+\cdot}$ and $[C_7H_7O]^{+\cdot}$, respectively. A proposed mechanism of fragmentation and the *m/z* of the fragments is given in **Scheme 7**.

In the IR spectra of compounds **TRZ$_{1\text{-}7}$** absorption bands in the region 3200-3149 cm^{-1}, characteristic of a NH_2 group, were observed. The formation of triazole ring in **TRZ$_2$** was supported by its ^{1}H NMR spectrum[163,169] (Padmavathi *et al*., 2009; Mullican *et al*., 1993), which showed singlet signals at δ 5.46, 4.89 and 4.67 ppm (**Figure 25a-c**), respectively due to the NH_2, OCH_2 and SCH_2 fragments of 1,2,4-triazol-4-amine. Two multiplet signals observed at δ 7.46-7.20 and 7.09-6.91 ppm were assigned to four aromatic each. In the ^{13}C NMR spectrum[163] (Padmavathi *et al*., 2009) of **TRZ$_2$** (in DMSO-d_6, **Figure 30**), the azomethine carbon of benzothiazole ring resonated at δ 166.99 ppm. The two carbons of the triazole ring appeared at δ 154.16 ppm for the carbon at position 3 and at δ 165.29 ppm for the carbon at ring position 5. The singlet signals at δ 66.97 and 64.80 ppm were assigned to the OCH_2 and SCH_2 fragments. The phenyl carbons appeared as ten singlets at δ 152.88, 134.02, 127.51, 126.54, 125.98, 125.57, 124.85, 121.23, 120.83 and 106.27 ppm. The electon spray ionization (ESI) mass spectrum of compound **TRZ$_2$** showed $[M^{+\cdot}+H_2O]$ peak at *m/z* 422 which is in agreement with its molecular formula $C_{17}H_{14}ClN_5OS_2$. The electron impact (EI) mass spectrum of **TRZ$_3$** (**Figure 33**) exhibited a $[M^{+\cdot}+1]$ peak at *m/z* 384, a base beak at *m/z* 206 which is due to $[C_9H_6N_2S_2]^{+\cdot}$, a medium intensity peak at *m/z* 107 which is due to $[C_7H_7O]^{+\cdot}$ .The spectrum also revealed medium intensity peaks at *m/z* 165, 148 and 133.

In the IR spectra of compounds **ATZ$_{1\text{-}13}$**, the disappearance of C=O stretching bands of the aryloxyacetic acids and detection of strong C=N stretching bands in the region 1653–1620 cm^{-1} are evidence for the ring closure of thiadiazol ring. The ^{1}H NMR spectrum of **ATZ$_1$** showed a multiplet signal for five aromatic protons at δ 7.37-6.98 ppm and singlet signals at δ 7.24 and 5.23 ppm due to the NH_2 and OCH_2 fragments, respectively.

In the IR spectrum of compound **TDZ$_1$** characteristic absorption bands at 3261, 3100 (NH), 1654 (C=O), and 1605 (C=N) cm^{-1} were observed. Its ^{1}H NMR spectrum[166,167] (Kumar *et al*., 2008; Schenone *et al*., 2006) showed multiplet signals at δ 7.33-7.27 and 7.04-6.94 ppm for four and five aromatic protons and singlet signals at δ 5.26 and 4.12 ppm due to the OCH_2 and SCH_2 fragments, respectively. The singlet signal at δ 7.25 ppm assigned to the NH (amide) fragment, disappeared on D_2O exchange (**Figure 27a-c** and **28a-c**). In the ^{13}C NMR

spectrum[116,164] (Kumar *et al*., 2005; Kadi *et al*., 2010), the carbonyl carbon exhibited a singlet at δ 169.07 ppm and the C=N carbon of the benzothiazole ring resonated at δ 166.14 ppm. The C-2 and C-5 carbons of the thiadiazole moiety gave singlets at δ 166.82 and 162.61 ppm, respectively. The remaining carbon signals were observed at the expected chemical shift values. The electrospray ionization (ESI) mass spectrum of compound **TDZ_1** displayed the molecular ion peak at *m/z* 414.

4.2.2. Biological Study

4.2.2.1. Antimicrobial Activity

The antimicrobial screening by the cup plate method[177] (Barry ALJ, 1999) indicated significant inhibitory activity (inhibition zone **>** 19 mm) of the tested compounds **OXZ_8**, **TRZ_1**, **ATZ_1**, **TDZ_4**, **TDZ_8**, **TDZ_9** and **TDZ_{10}** against the Gram-negative bacteria *Pseudomonas aeruginosa* whereas compounds **OXZ_2**, **OXZ_4**, **OXZ_5**, **OXZ_9**, **OXZ_{10}**, **OXZ_{11}**, **TRZ_3**, **TDZ_1** and **TDZ_2** were found to be moderately active (inhibition zone 16-19 mm) against the same microorganism (**Table 11**). Furthermore, the maximum inhibitory activity (inhibition zone 26 mm) was observed in derivative **TDZ_9** having 2-methoxyphenyloxymethyl group at fifth position of the thiadiazole ring. The tested compounds exhibited no activity against Gram-positive microorganism except compounds **TRZ_3** and **TDZ_{10}** which displayed weak inhibitory activity against *Staphylococcus epidermidis* (inhibition zones 17 and 16 mm, respectively). In the present investigation the tested compounds did not posses antifungal activity. It was observed that compound bearing ortho-OCH_3 or para-NO_2 in the phenyl ring of the phenyloxymethyl group at fifth position of the thiadiazole nucleus exhibited significant inhibitory activity against *Pseudomonas aeruginosa*. While a notable decrease or loss in activity was seen when these substituents were replaced with 2-Cl, 4-Cl, 3-CH_3 or 4-CH_3.

4.2.2.2. Pharmacological Activity

4.2.2.2.1. Acute Toxicity

No behavioral changes in animals were observed during the experiment and at the end hematological parameters were estimated and there was no observable change. In the present study, mortality was not observed even at 1000 mg kg^{-1} indicating that the tested compounds are nontoxic to animals.

4.2.2.2.2. Analgesic activity

The analgesic activity of the synthesized compunds was evaluated by the tail immersion method[178] (Di Stasi *et al*., 1988) using mice. The analgesic activity results are summarized in **Table 12**. The tested compounds **OXZ_8**, **OXZ_{11}**, **TDZ_1**, **TDZ_4**, **TDZ_8** and **TDZ_9** exhibited fast analgesic activity (40.0-69.2%) as evident from observation at 30 min following oral administration. At 1 and 2 h compounds **OXZ_4**, **OXZ_8**, **OXZ_{11}**, **TDZ_2**, **TDZ_8** and **TDZ_9** exhibited potent analgesic activity (40.5-76.8%) compared to the standard drug paracetamol (43.8 and 36.4%, respectively at a dose of 50 mg kg^{-1}). In general, a sharp decline in the activity was observed at 3 h following oral administration (**Figure 38**). It was observed that substitution with naphthalen-2-yloxymethyl (**OXZ_8**) group at fifth position of the oxadiazole nucleus or phenyloxymethyl (**TDZ_1**), naphthalen-2-yloxymethyl (**TDZ_8**) or ortho-methoxyphenyloxy-methyl (**TDZ_9**) groups at fifth position of the thiadiazole nucleus enhances the activity. Moreover, it was also observed that substitution of ortho-Cl (**OXZ_2**, **TRZ_1** and **TDZ_2**) or para-NO_2 (**OXZ_{10}** and **TDZ_{10}**) in the phenyl ring of the phenyloxymethyl group at fifth position of the oxadiazole, triazole or thiadiazole nucleus resulted in a marked decrease in analgesic activity.

4.2.2.2.3. Anti-inflammatory activity

The anti-inflammatory activity results determined using the carrageenan induced paw oedema method[179] (Winter *et al*., 1962) in rats are summarized in **Table 13**. The tested compounds **OXZ_2**, **OXZ_{10}**, **OXZ_{11}**, **TDZ_4**, **TDZ_9** and **TDZ_{10}** showed rapid onset of action (50.6-67.9%) as evident from observation at 30 min after carrageenan injection. At 1 h compounds **OXZ_2**, **OXZ_4**, **OXZ_9**, **TRZ_3**, **TDZ_2**, **TDZ_4**, **TDZ_8**, **TDZ_9** and **TDZ_{10}** were effective in inhibiting the paw edema (72.8-81.6%), when compared with the reference drug (72.1% at a dose of 20 mg kg^{-1}). The highest activity was found in derivative **TDZ_4** having 2-methylphenyloxymethyl group at fifth position of the thiadiazole ring (**Figure 39**). At 2 h compounds **TDZ_8**, **TDZ_9** and **TDZ_{10}** showed significant anti-inflammatory activity ranging from 76.0% to 78.7% inhibition. In general, a marked decrease in activity was observed at 3 h following carrageenan injection. It was observed that the anti-inflammatory activity is dependent on both the nature of substituents and the basic skeleton of the molecules.

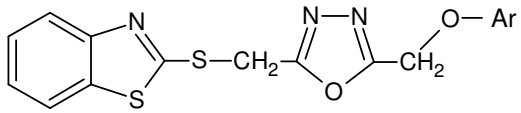
4.2.2.2.4. Ulcerogenic effects

Compounds **OXZ_4**, **OXZ_9**, **TDZ_4** and **TDZ_9** were tested for their ulcerogenic potential according to the method reported by Cioli *et al.*[180] (1979). The tested compounds showed low severity index (2.2±0.3to 3.2±0.5) compared to the standard drug diclofenac sodium (4.4±0.6). The maximum reduction in the ulcerogenic activity was found in the thiadiazole derivatives

TDZ_4 and **TDZ_9** (2.2±0.3 and 2.3±0.3, respectively) (**Table 14**). The other tested compounds also exhibited better GI safety profile as compared to the standard drug diclofenac sodium (**Figure 40**).

In summary, various 2-mercaptobenzothiazole derivatives were prepared with the objective of developing dual anti-inflammatory-antimicrobial agents with minimum ulcerogenic activity. Among these, a thiadiazole derivative 2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(2-methoxyphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**TDZ_9**) showed the most prominent and consistent activity with a significant reduction of gastrointestinal toxicity. Therefore compound **TDZ_9** would represent a fruitful matrix for the development of a new class of dual acting antimicrobial and analgesic-anti-inflammatory agents.

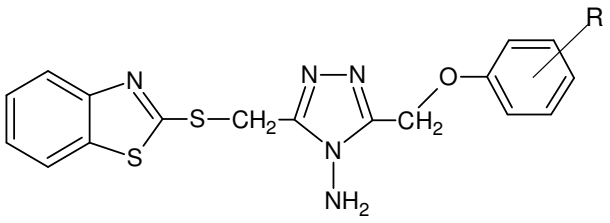
Table 7. Physical data of the synthesized 2-{(benzo[d]thiazol-2-ylthio) methyl}-5-(aryloxymethyl)-1,3,4-oxadiazoles (**OXZ_{1-13}**)



Compound	Ar	Solv. Cryst.	M.p.(°C)	[a]Yield (%)
OXZ_1	C_6H_5	ethanol	160	44
OXZ_2	2-Cl.C_6H_4	ethanol	184	56
OXZ_3	4-Cl.C_6H_4	ethanol	155	67
OXZ_4	2-CH_3.C_6H_4	ethanol	175	54
OXZ_5	3-CH_3.C_6H_4	ethanol	170	54
OXZ_6	4-CH_3.C_6H_4	DMF:water (1:1)	167	43
OXZ_7	1-naphthyl	ethanol	135	63
OXZ_8	2-naphthyl	DMF:ethanol (1:1)	160	55
OXZ_9	2-CH_3O.C_6H_4	DMF:ethanol (1:1)	160	72
OXZ_{10}	4-NO_2.C_6H_4	DMF:acetone (1:1)	182	66
OXZ_{11}	4-Cl-3-CH_3.C_6H_3	methanol	119	61
OXZ_{12}	2,4-di-Cl.C_6H_3	methanol	137	65
OXZ_{13}	2,4,6-tri-Br.C_6H_2	methanol	181	60

[a]Isolated yield

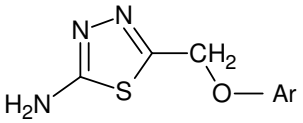
Table 8. Physical data of the synthesized 3-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-5-(aryloxymethyl)-4H-1,2,4-triazol-4-amines (**TRZ_{1-7}**)



Compound	Ar	Solv. Cryst.	M.p. (°C)	[a]Yield (%)
TRZ_1	2-Cl	ethanol	205	69
TRZ_2	4-Cl	ethanol	219	65
TRZ_3	2-CH_3	chloroform	>300	46
TRZ_4	3-CH_3	chloroform	218	57
TRZ_5	2-OCH_3	ethanol	185	88
TRZ_6	4-NO_2	ethanol	>300	62
TRZ_7	2,4-di-Cl	ethanol	226	72

[a]Isolated yield

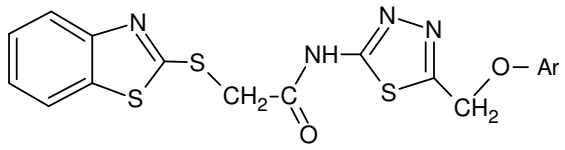
Tables 9. Physical data of the synthesized 5-(aryloxymethyl)-1,3,4-thiadiazol-2-amines (**ATZ_{1-13}**)



Compound	Ar	Solv. Cryst.	M.p. (°C)	[a]Yield (%)
ATZ_1	C_6H_5	ethanol	185	66
ATZ_2	2-Cl.C_6H_4	THF	219	51
ATZ_3	4-Cl.C_6H_4	THF	204-205	58
ATZ_4	2-CH_3.C_6H_4	THF	208	65
ATZ_5	3-CH_3.C_6H_4	THF	181	59
ATZ_6	4-CH_3.C_6H_4	THF	208	53
ATZ_7	1-naphthyl	THF	236-238	72
ATZ_8	2-naphthyl	THF	183	61
ATZ_9	2-CH_3O.C_6H_4	THF	183	61
ATZ_{10}	4-NO_2.C_6H_4	THF	198	56
ATZ_{11}	4-Cl-3-CH_3.C_6H_3	THF	206	54
ATZ_{12}	2,4-di-Cl.C_6H_3	THF	219-220	47
ATZ_{13}	2,4,6-tri-Br.C_6H_2	THF	162	62

[a]Isolated yield

Tables 10. Physical data of the synthesized 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl]acetamides (**TDZ_{1-13}**)



Compound	Ar	Solv. Cryst.	M.p.(°C)	[a]Yield (%)
TDZ_1	C_6H_5	methanol	204	44
TDZ_2	2-Cl.C_6H_4	methanol	232	65
TDZ_3	4-Cl.C_6H_4	methanol	213	71
TDZ_4	2-CH_3.C_6H_4	acetone:THF (1:1)	209	56
TDZ_5	3-CH_3.C_6H_4	methanol	182	59
TDZ_6	4-CH_3.C_6H_4	methanol	225	47
TDZ_7	1-naphthyl	methanol	245	62
TDZ_8	2-naphthyl	THF	193	54
TDZ_9	2-CH_3O.C_6H_4	THF	196	68
TDZ_{10}	4-NO_2.C_6H_4	THF	214	51
TDZ_{11}	4-Cl-3-CH_3.C_6H_3	THF	210	55
TDZ_{12}	2,4-di-Cl.C_6H_3	methanol	129	45
TDZ_{13}	2,4,6-tri-Br.C_6H_2	THF	150	72

[a]Isolated yield

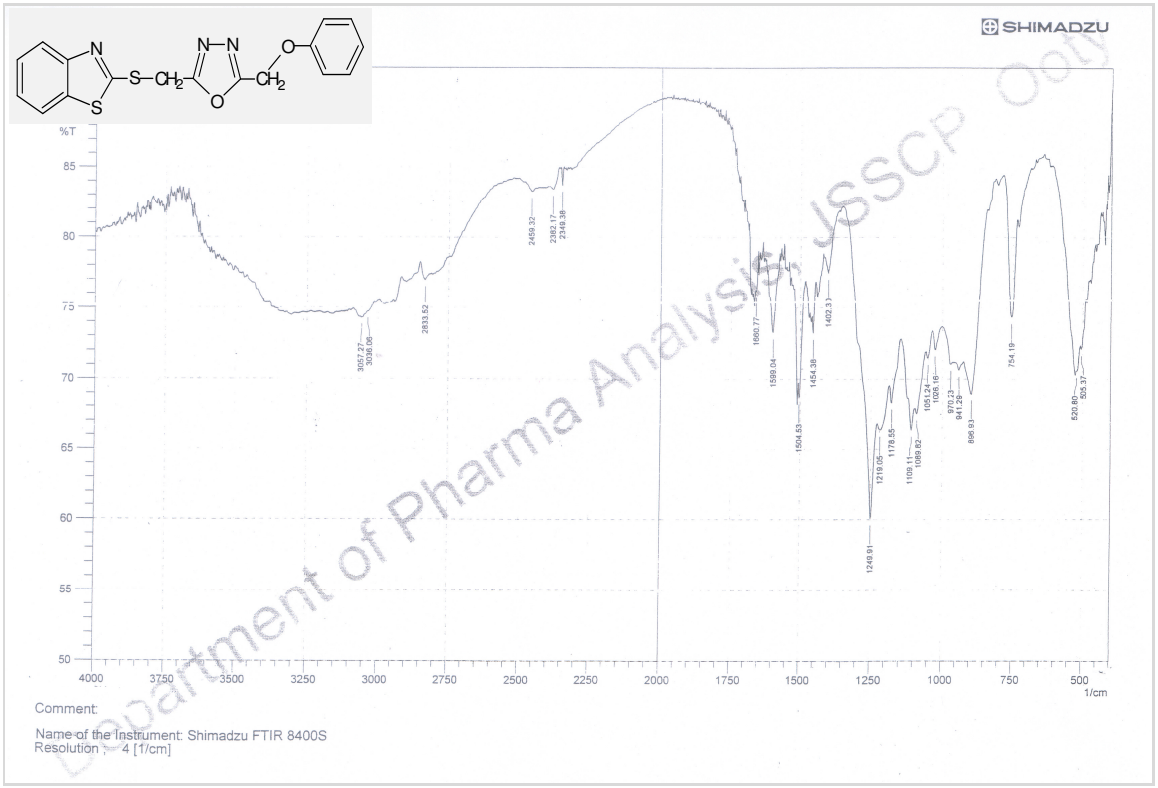


Figure 21. Infrared spectrum of the synthesized compound **OXZ_1**

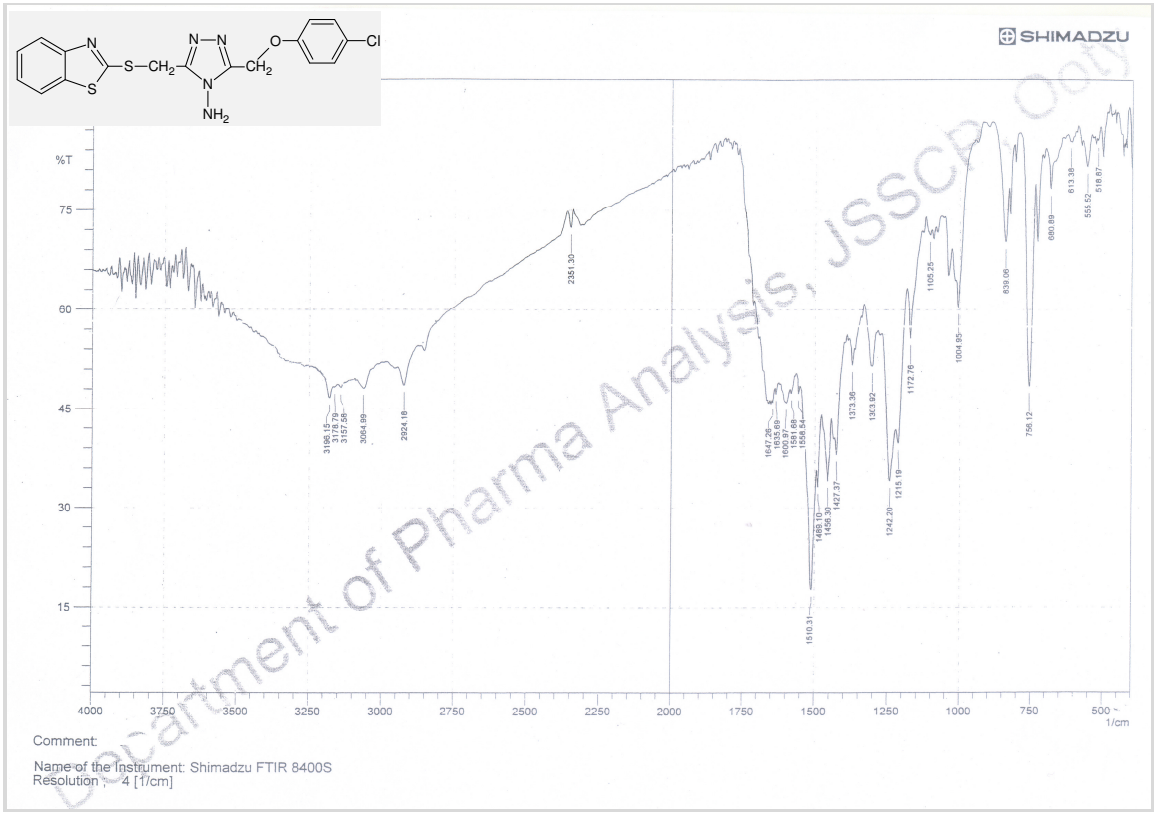


Figure 22. Infrared spectrum of the synthesized compound **TRZ_2**

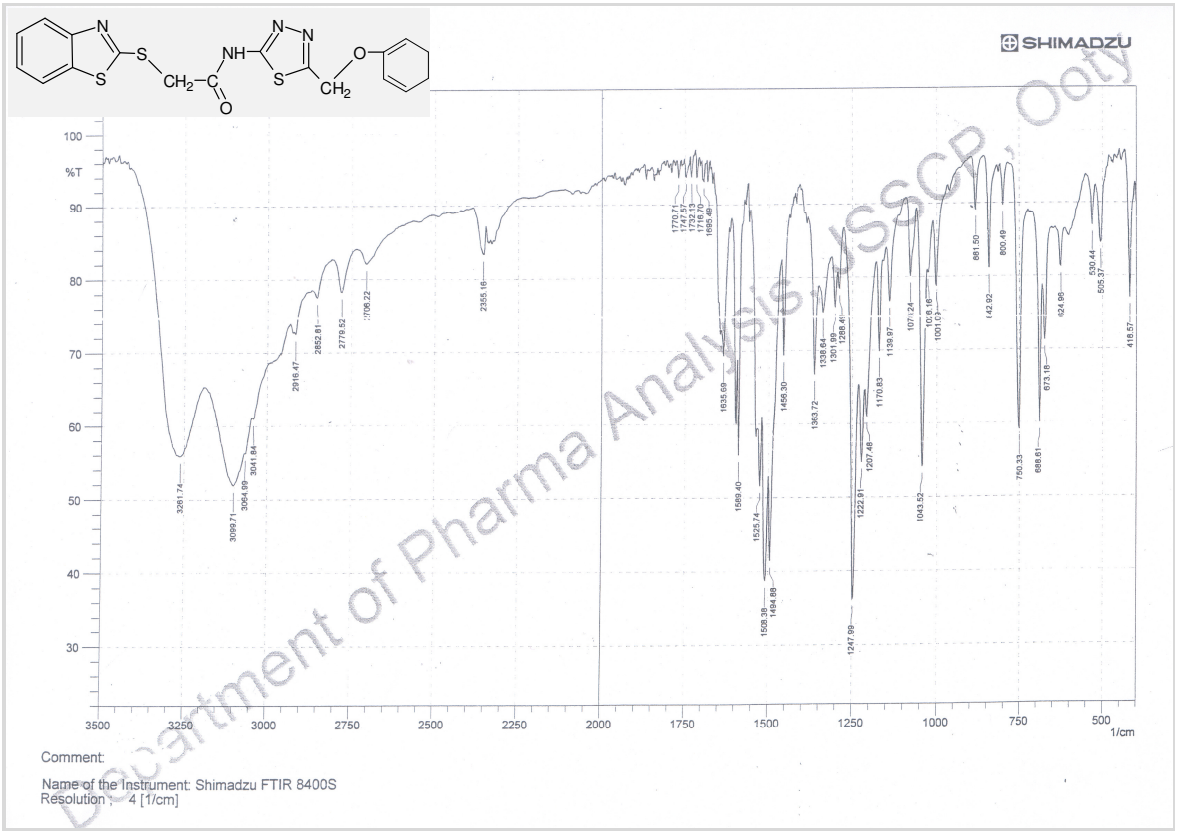


Figure 23. Infrared spectrum of the synthesized compound **TDZ_1**

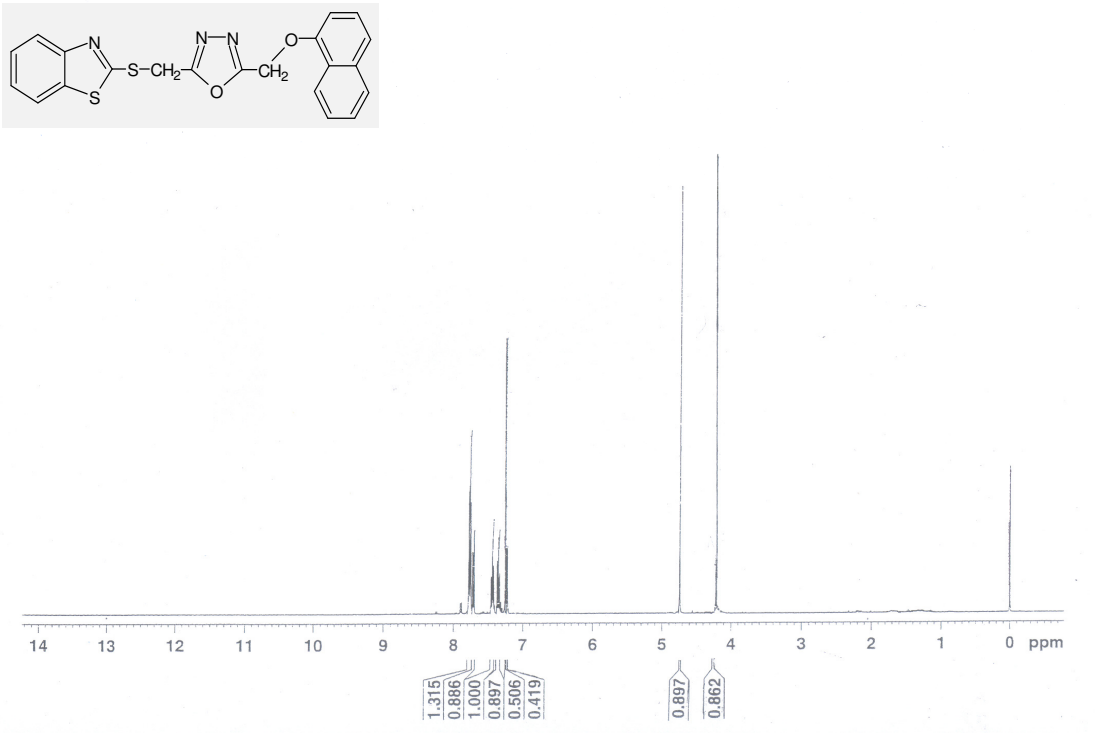


Figure 24a. ^{1}H NMR spectrum of the synthesized compound **OXZ_7**

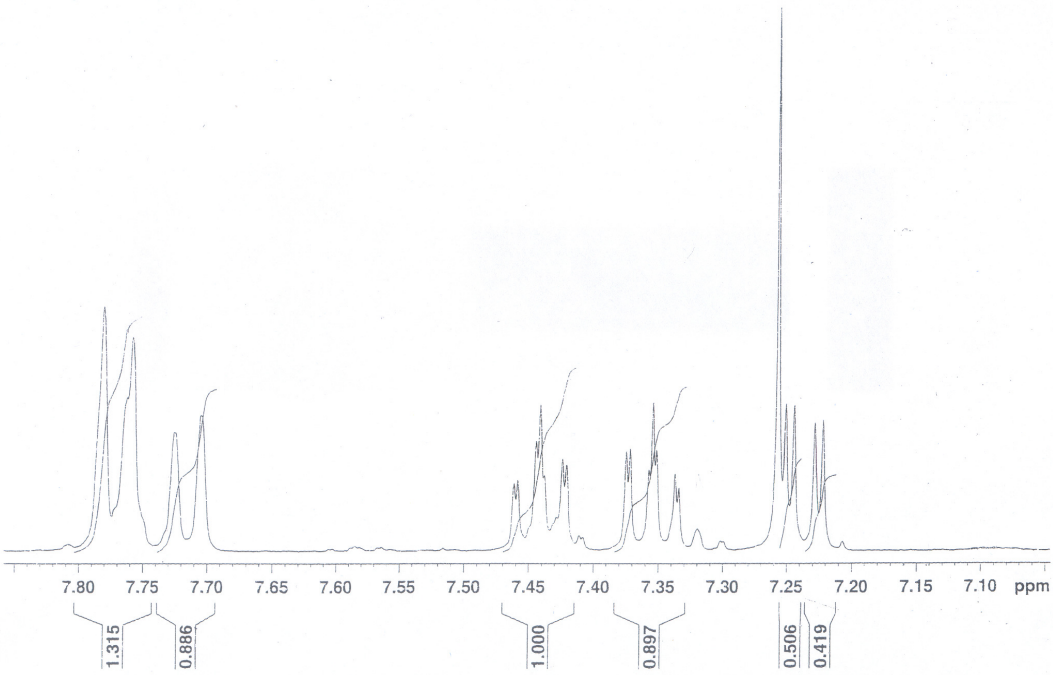


Figure 24b. Expansion of the [1]H NMR spectrum of the synthesized compound **OXZ_7**

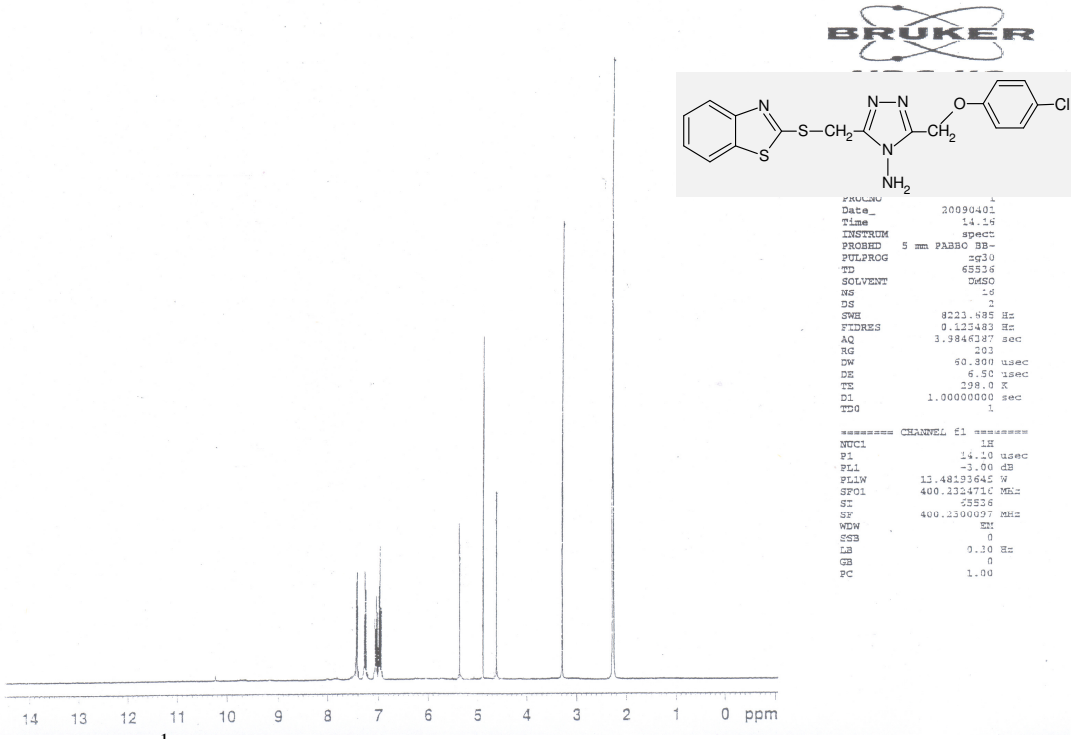


Figure 25a. [1]H NMR spectrum of the synthesized compound **TRZ_2**

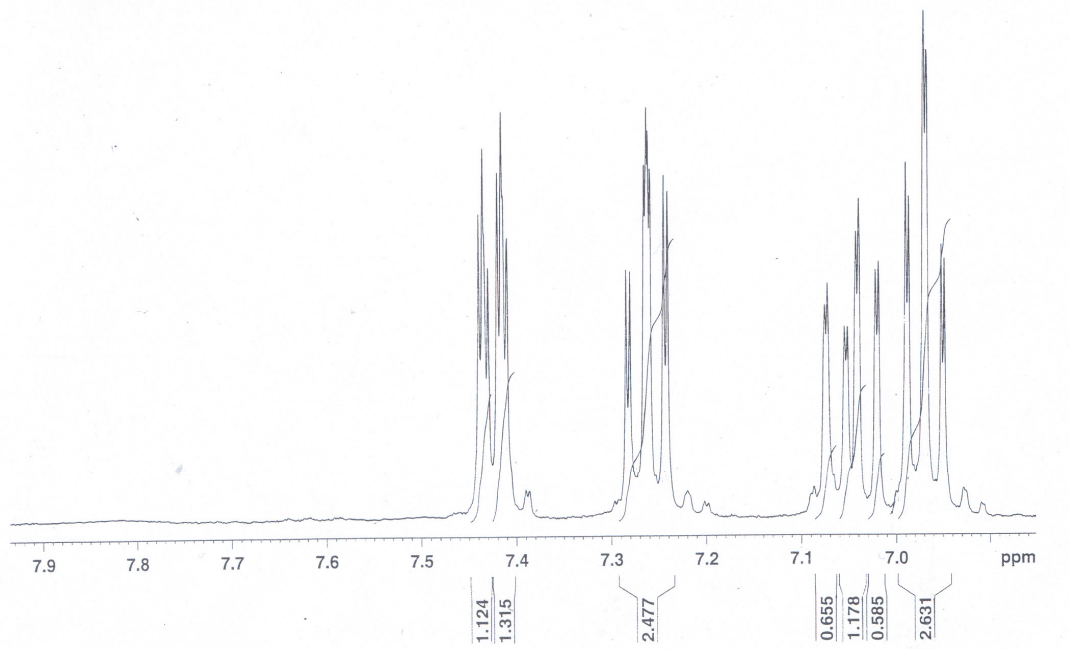


Figure 25b. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TRZ_2**

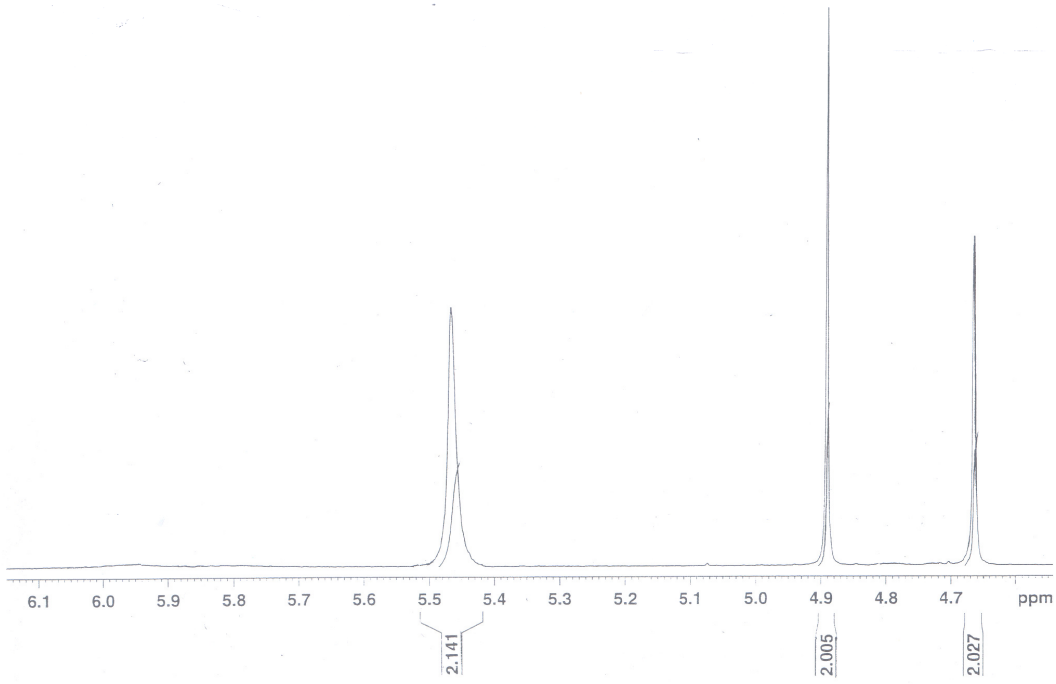


Figure 25c. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TRZ_2**

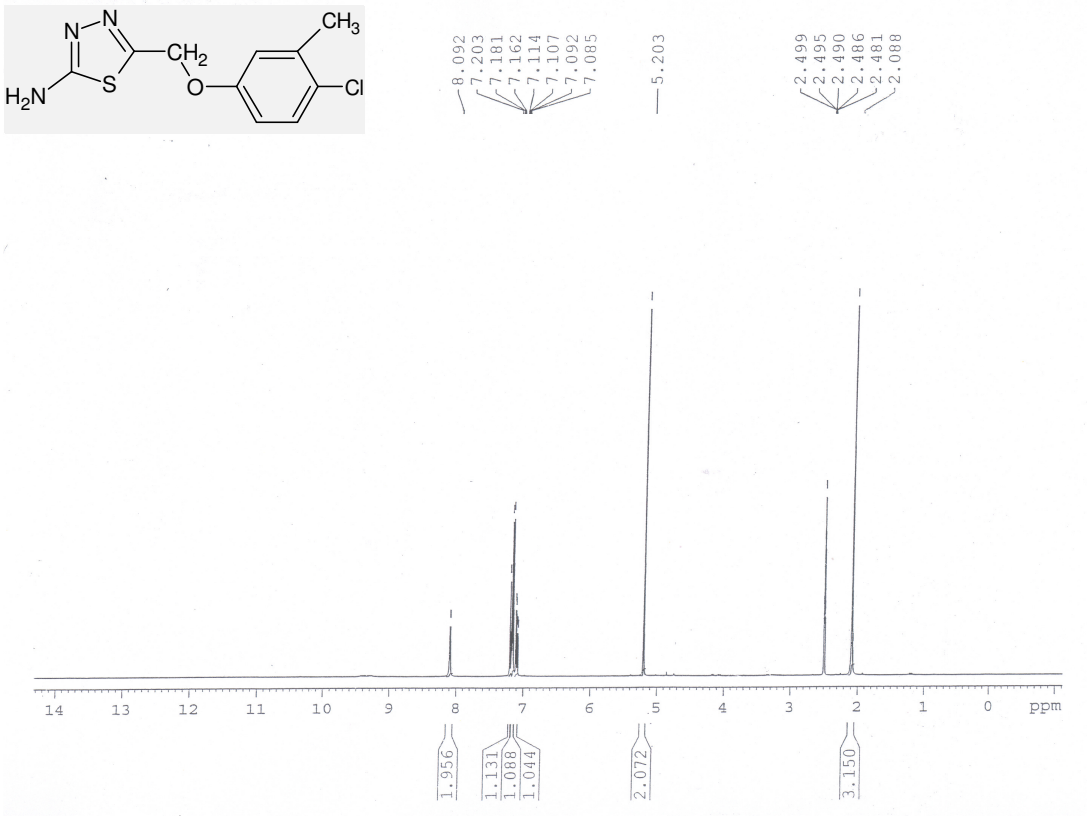


Figure 26. [1]H NMR spectrum of the synthesized compound **ATZ_{11}**

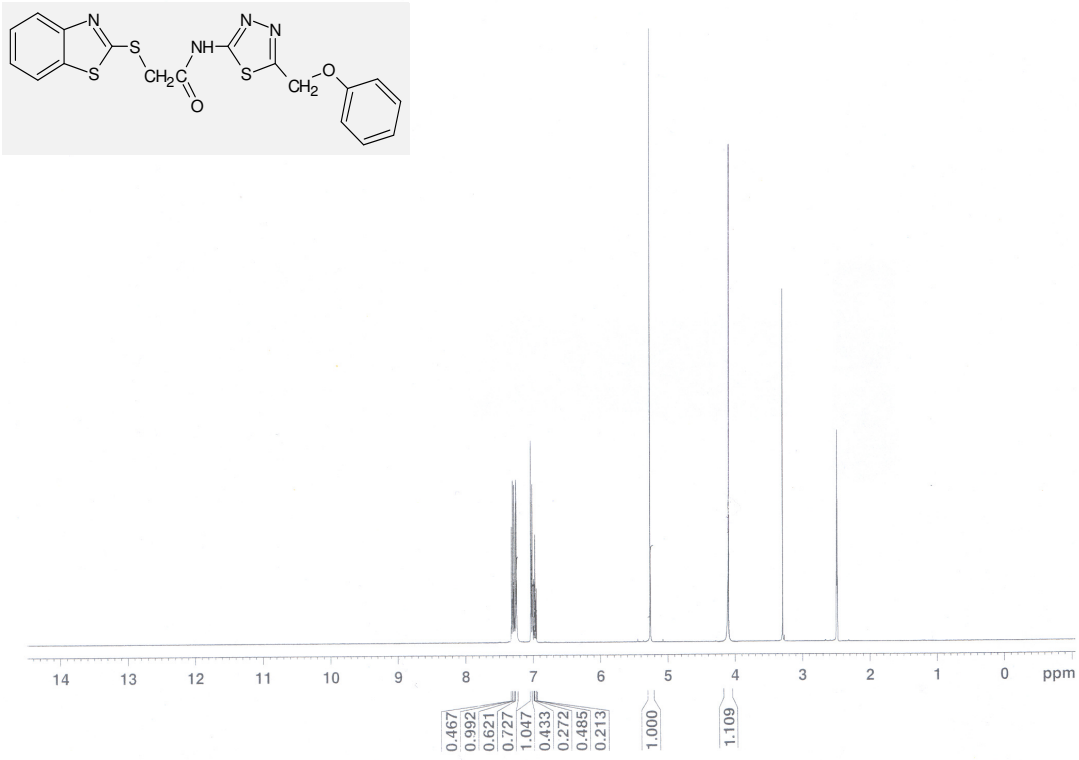


Figure 27a. [1]H NMR spectrum of the synthesized compound **TDZ_1**

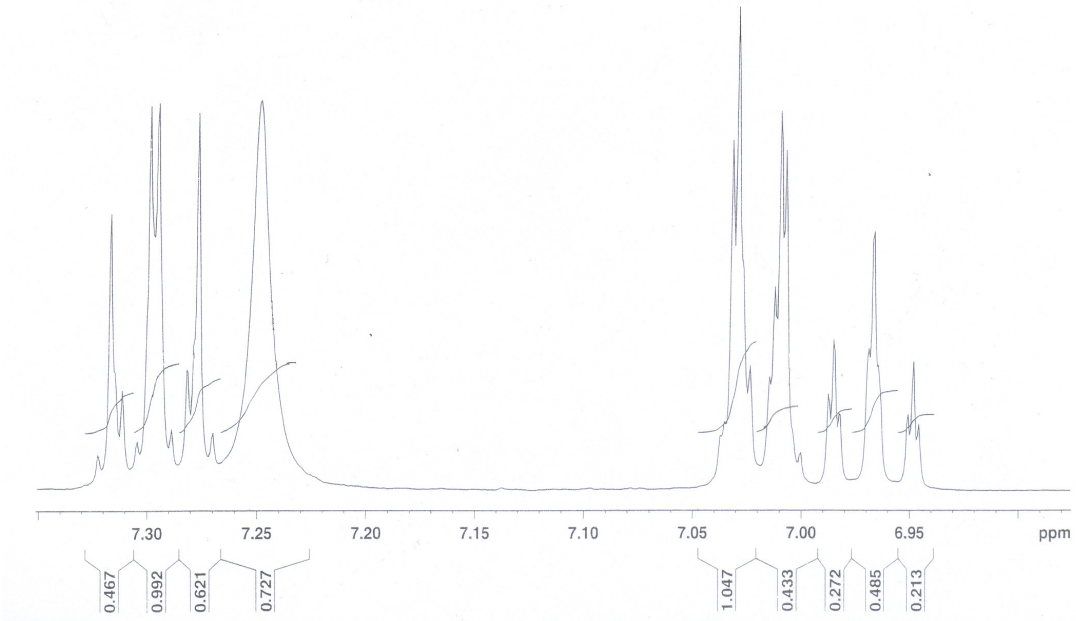


Figure 27b. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TDZ_1**

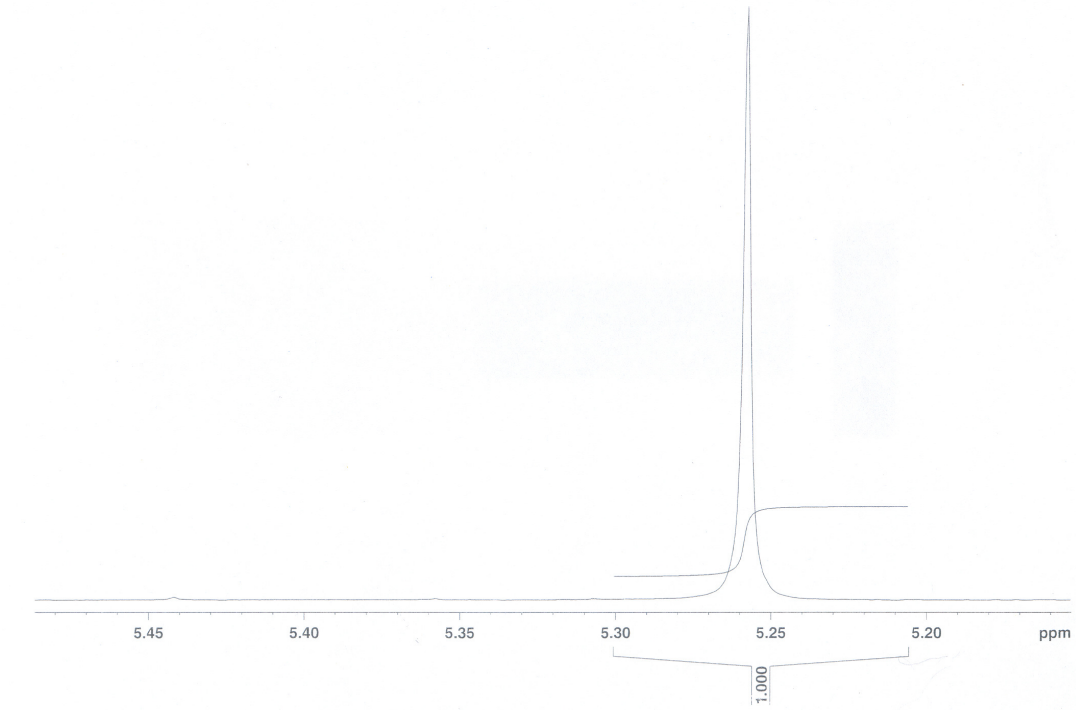


Figure 27c. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TDZ_1**

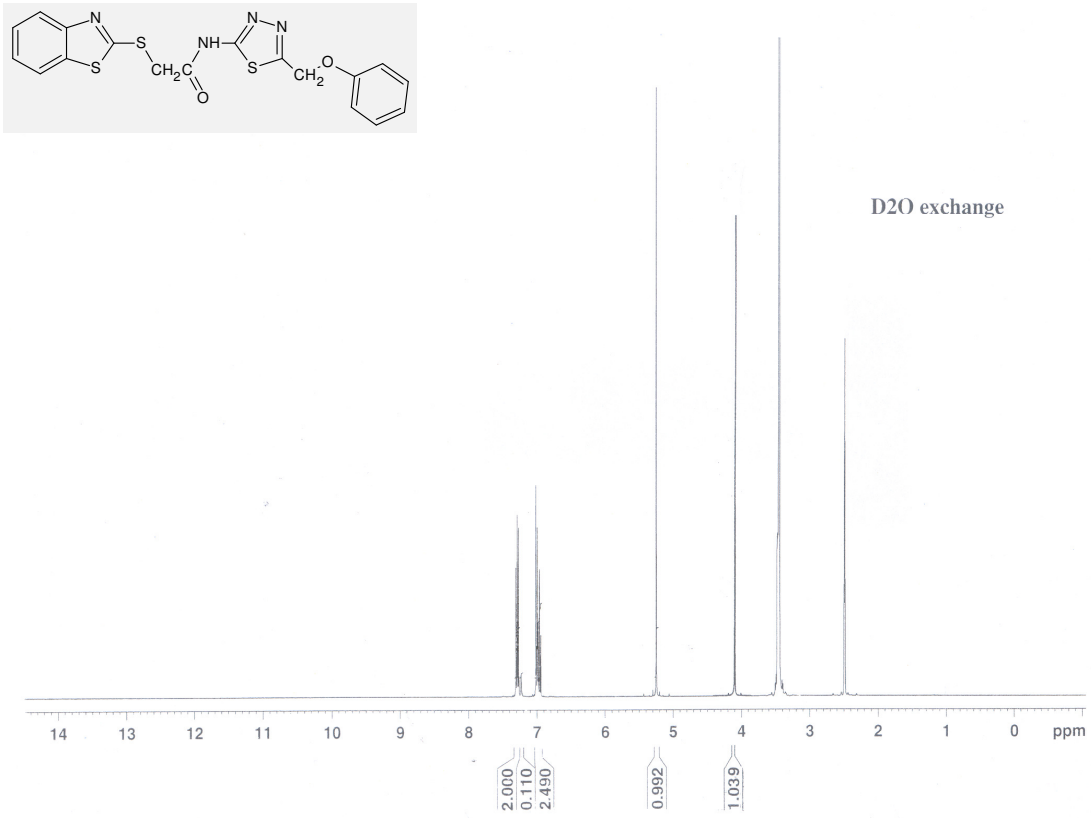


Figure 28a. ^{1}H NMR spectrum of the synthesized compound **TDZ$_1$** (D_2O exchange)

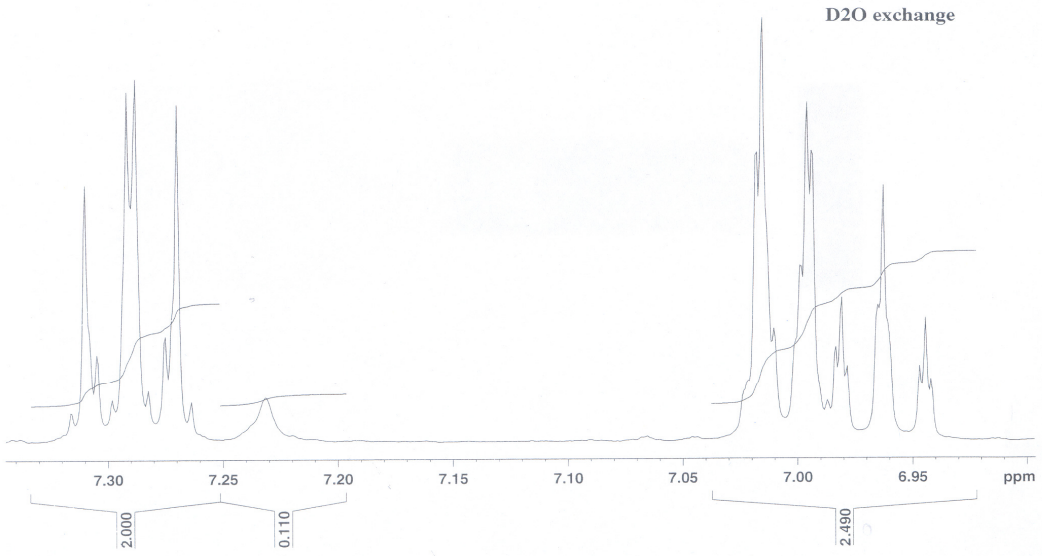


Figure 28b. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TDZ$_1$** (D_2O exchange)

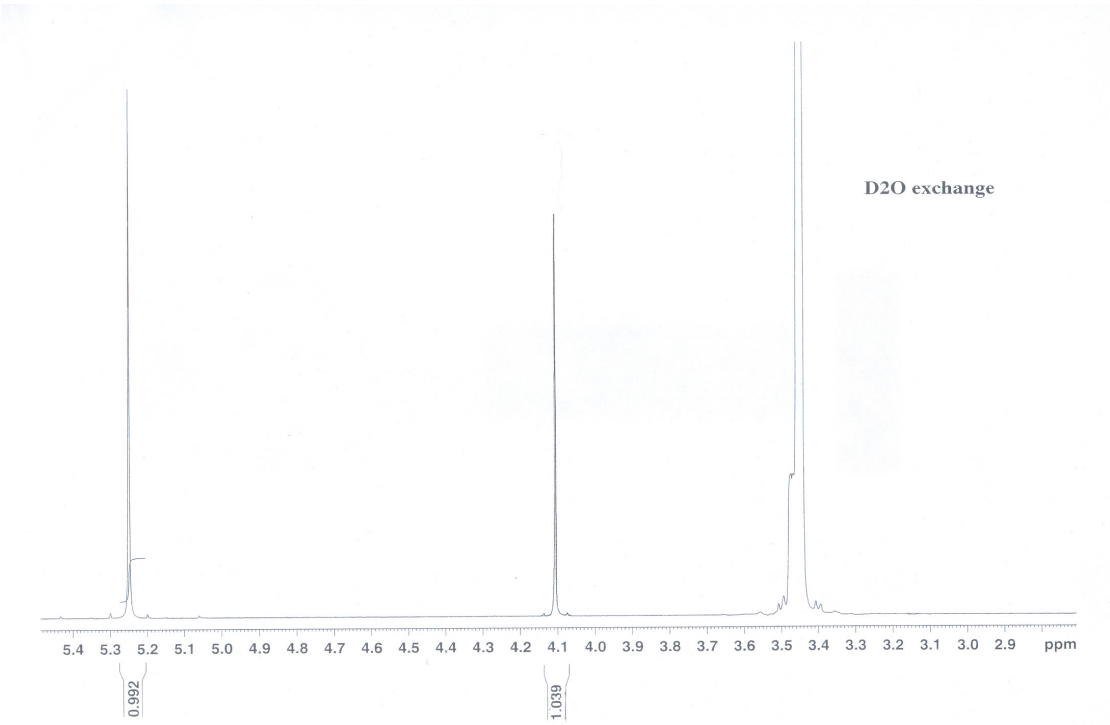


Figure 28c. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TDZ_1** (D_2O exchange)

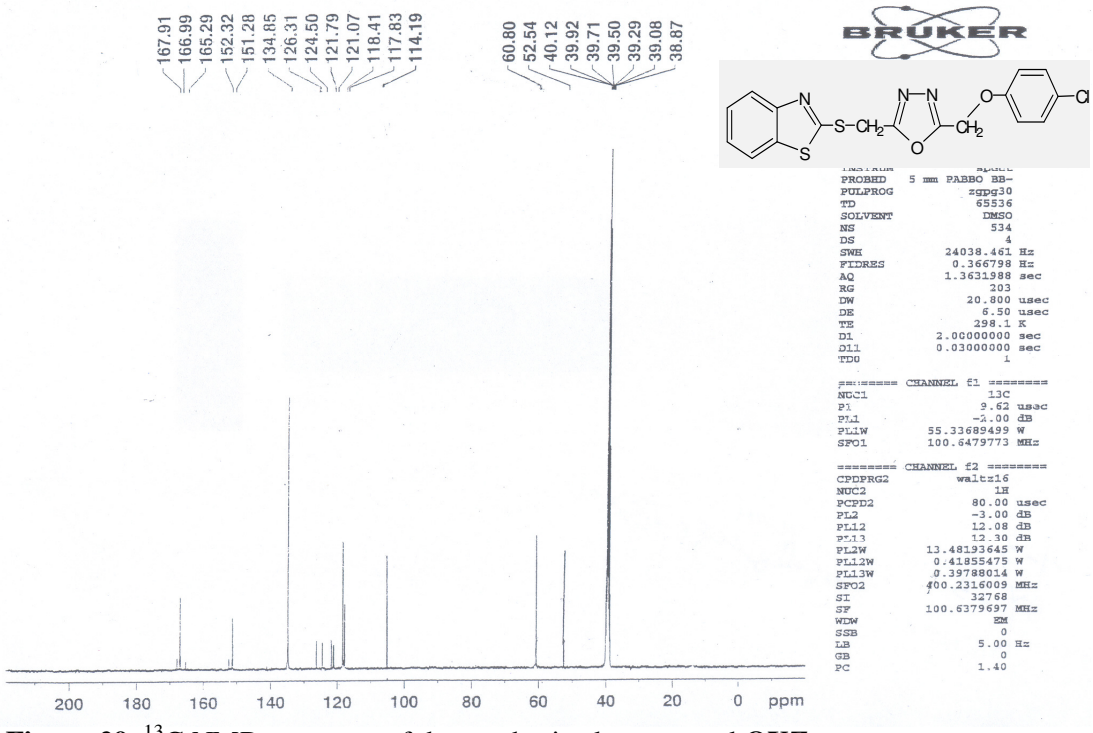


Figure 29. ^{13}C NMR spectrum of the synthesized compound **OXZ_3**

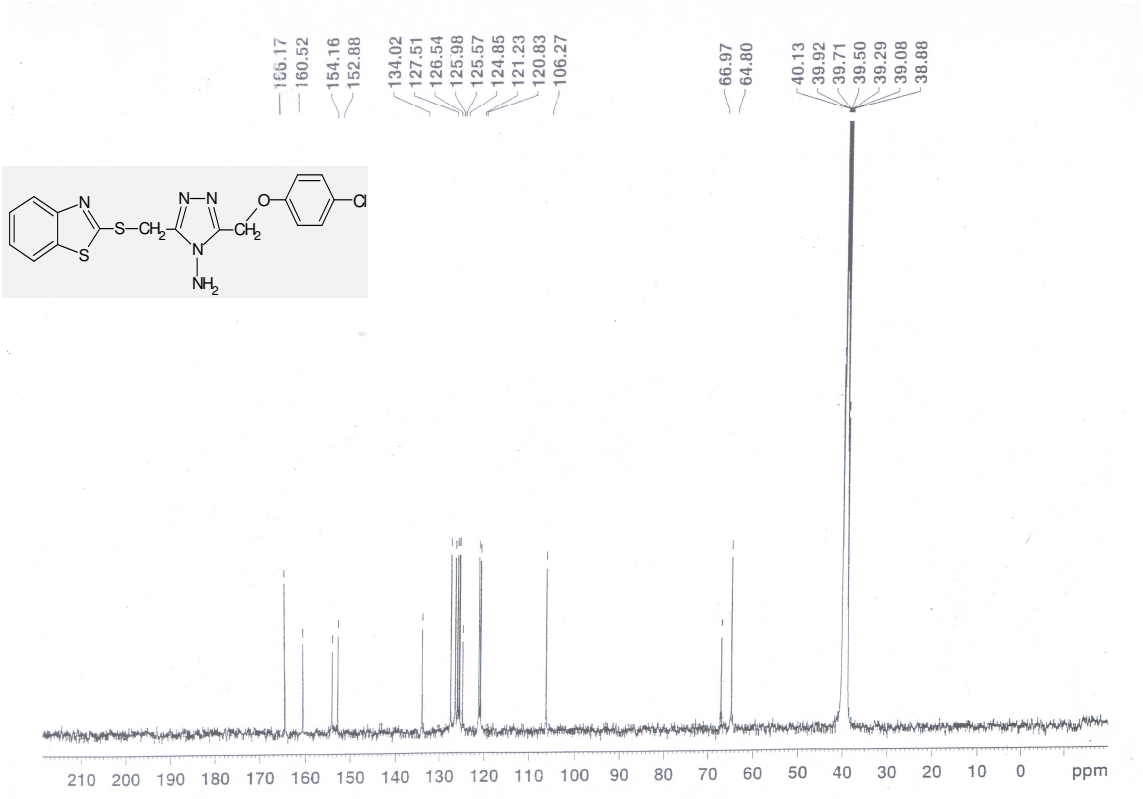


Figure 30. ^{13}C NMR spectrum of the synthesized compound **TRZ$_2$**

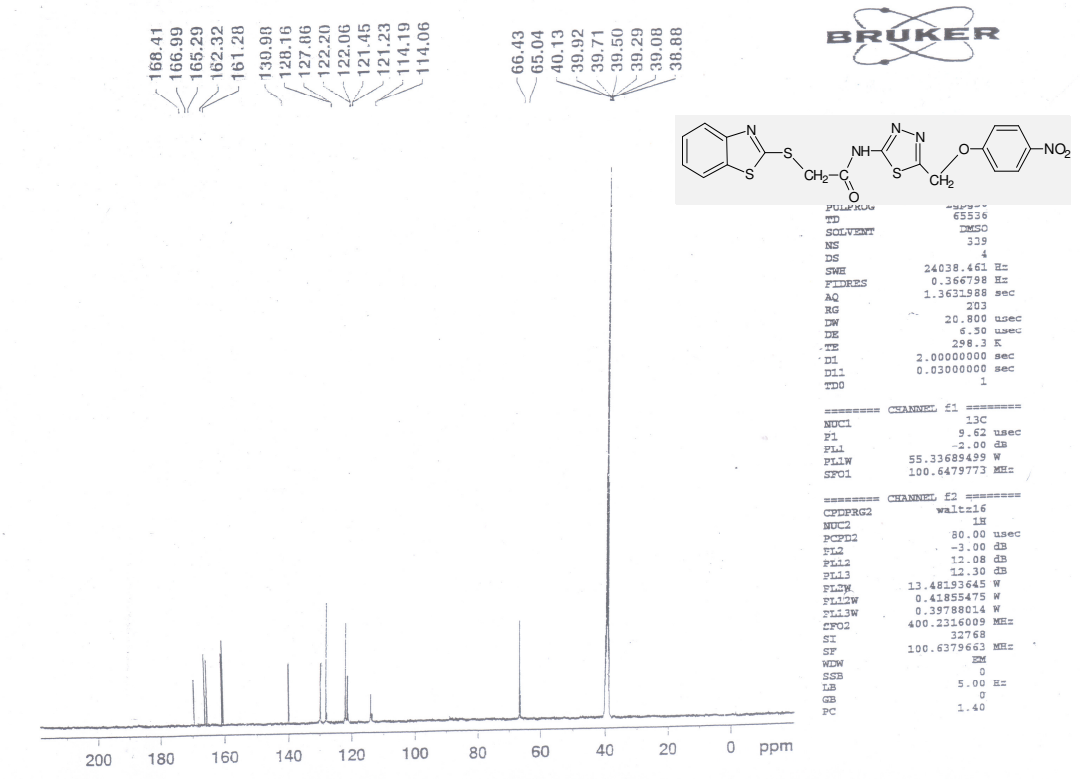


Figure 31. ^{13}C NMR spectrum of the synthesized compound **TDZ$_{10}$**

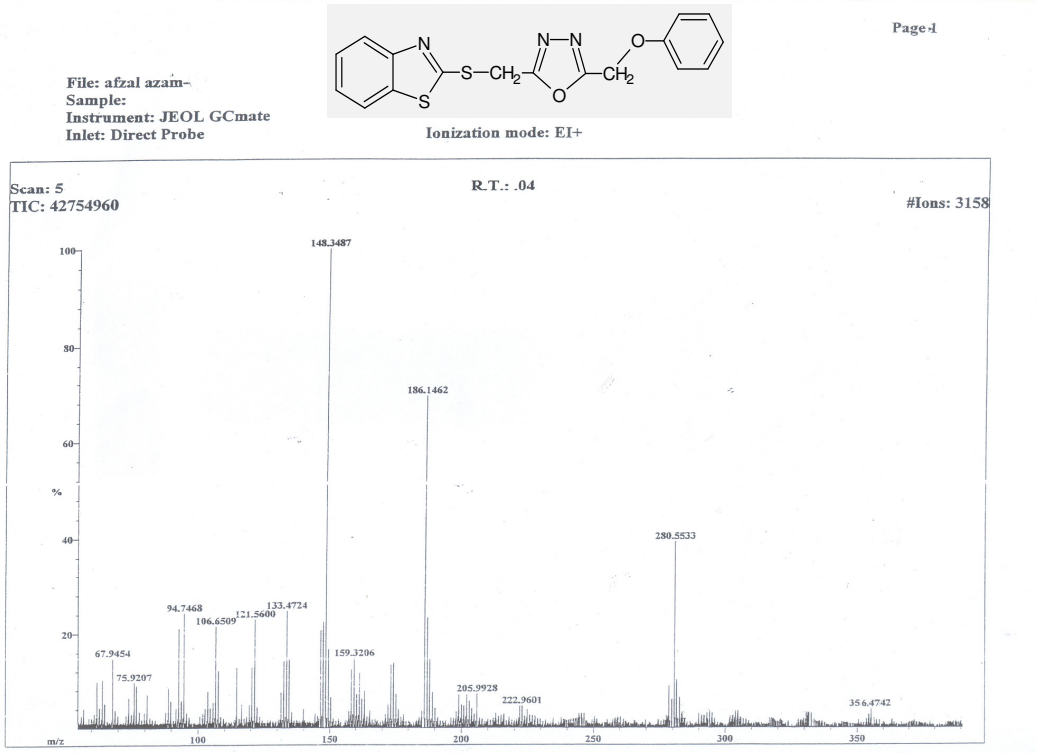


Figure 32. Mass spectrum of the synthesized compound **OXZ_1**

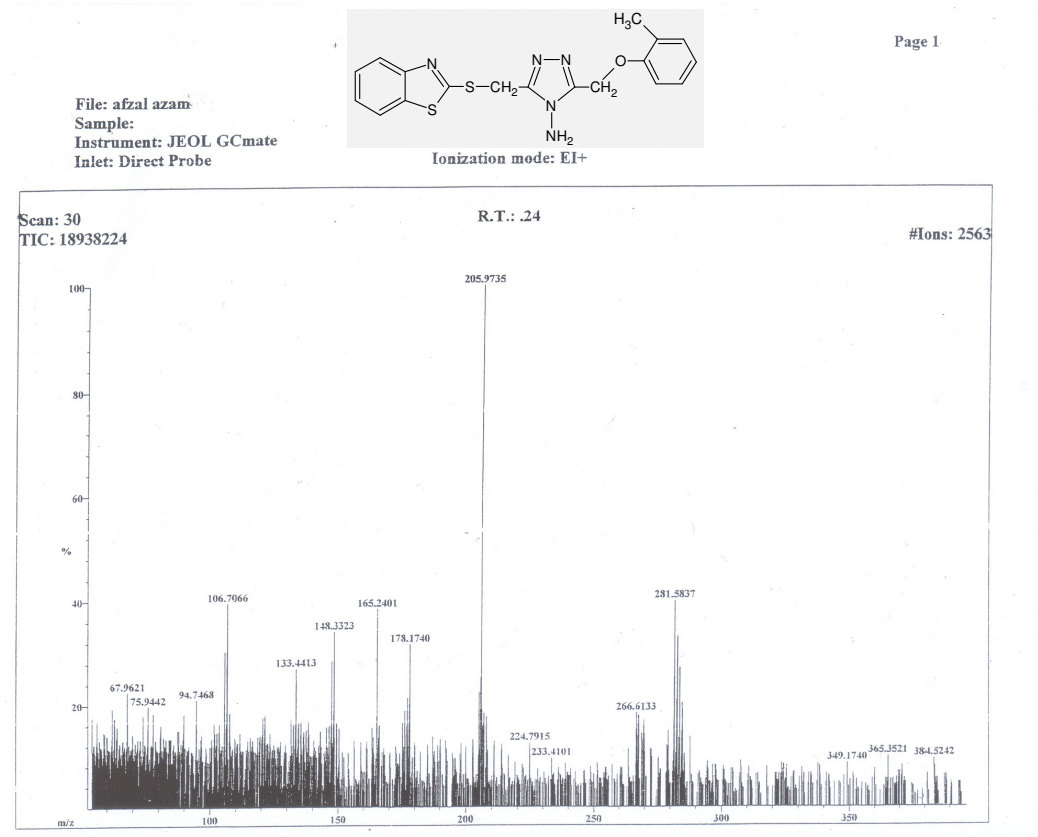


Figure 33. Mass spectrum of the synthesized compound **TRZ_3**

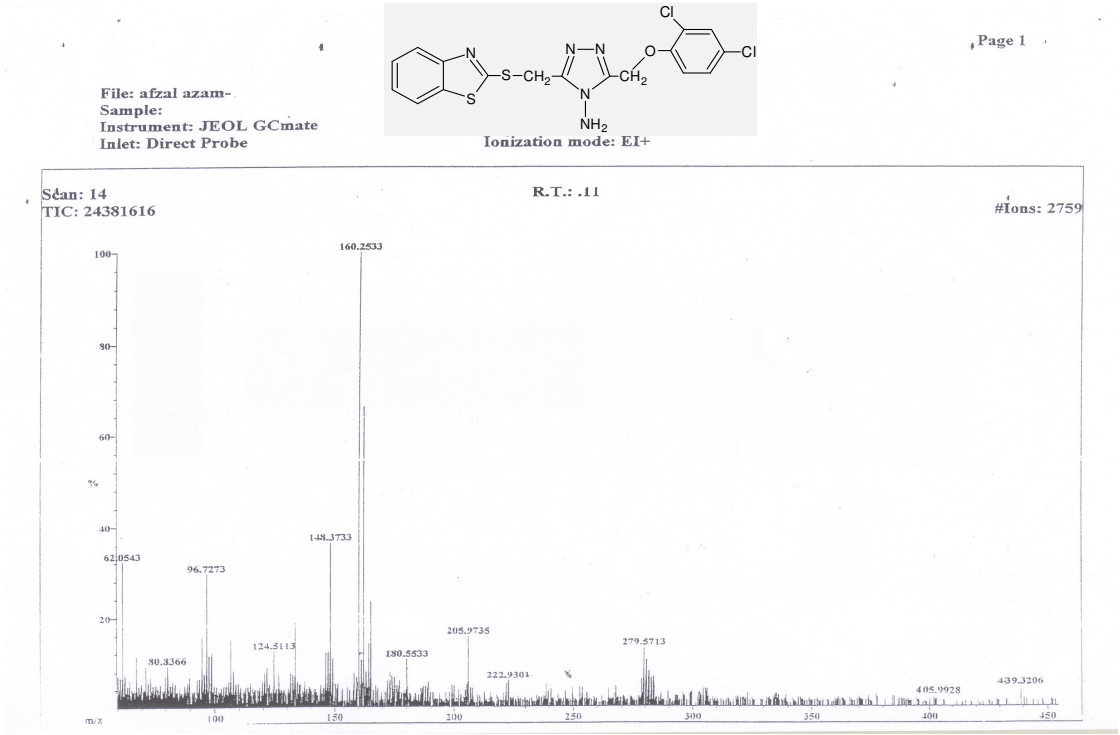


Figure 34. Mass spectrum of the synthesized compound **TRZ$_7$**

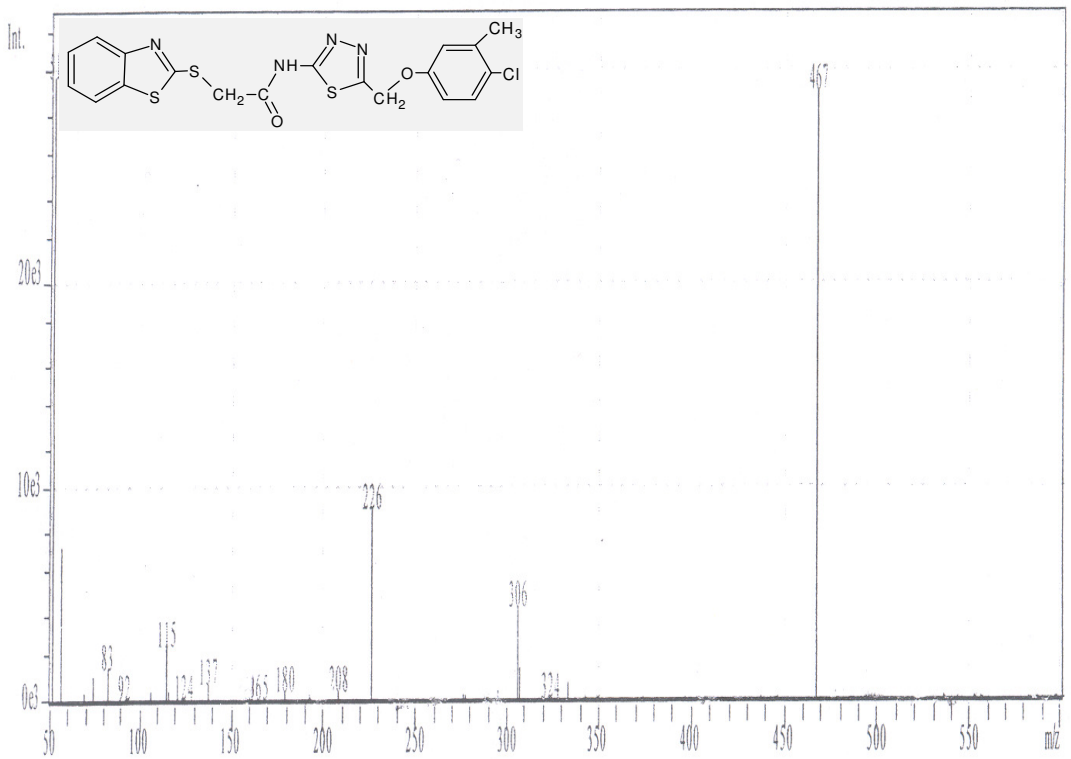
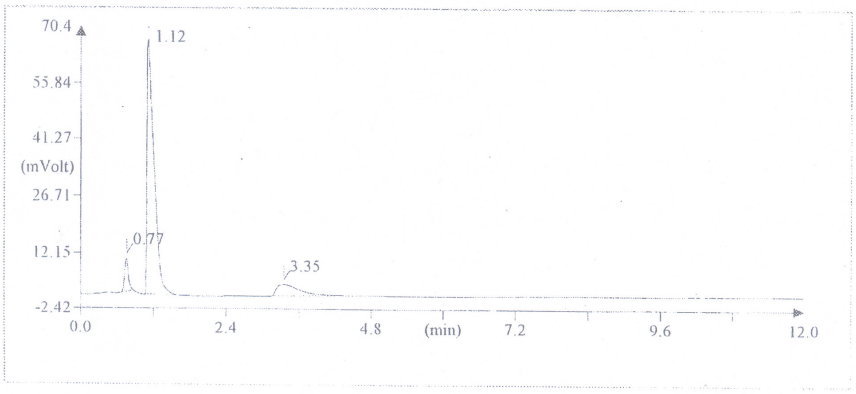


Figure 35. Electrospray ionization mass spectrum of the synthesized compound **TDZ$_{11}$**

FLASH EA 1112 SERIES CHNS REPORT
THERMO FINNIGAN

Company name:
Method filename: C:\Progam Files\Thermo Finnigan\Eager 300 for A1112\data\First Run\N C H S syster
Sample ID: J-12 (21) (# 239)
Analysis type: Unknown
Chromotogram filename: Azam-20032010-1.dat
Sample weight in mg: 1.339

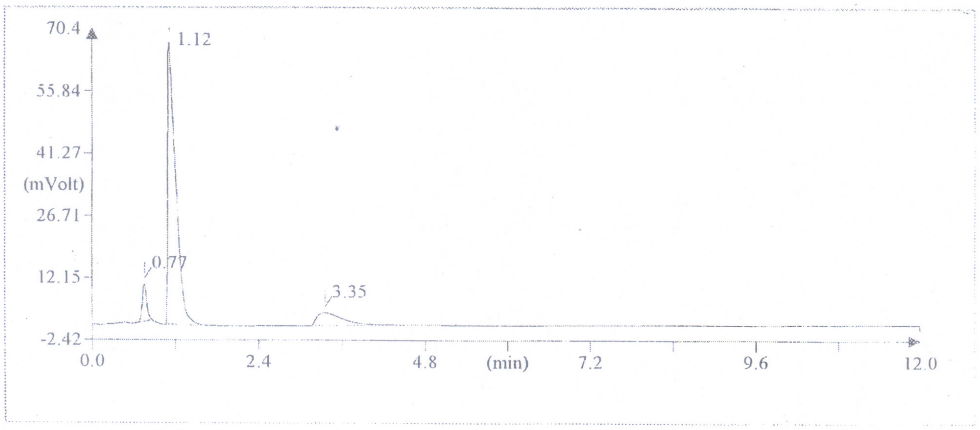


Peak Number (#)	Component Name	Elements %	Area (.1* uV* sec)
1	Nitogen	10.336	338196
2	Carbon	62.272	5479154
3	Hydrogen	3.670	721146
		76.278	6538496

Figure 36. CHN analysis spectrum of the synthesized compound **OXZ_7**

FLASH EA 1112 SERIES CHNS REPORT
THERMO FINNIGAN

Company name:
Method filename: C:\Progam Files\Thermo Finnigan\Eager 300 for A1112\data\First Run\N C H S syster
Sample ID: J-12 (21) (# 233)
Analysis type: Unknown
Chromotogram filename: Azam-20032010-1.dat
Sample weight in mg: 1.328



Peak Number (#)	Component Name	Elements %	Area (.1* uV* sec)
1	Nitogen	12.025	419070
2	Carbon	56.862	4861594
3	Hydrogen	3.411	656962
		72.298	5937626

Figure 37. CHN analysis spectrum of the synthesized compound **TDZ_7**

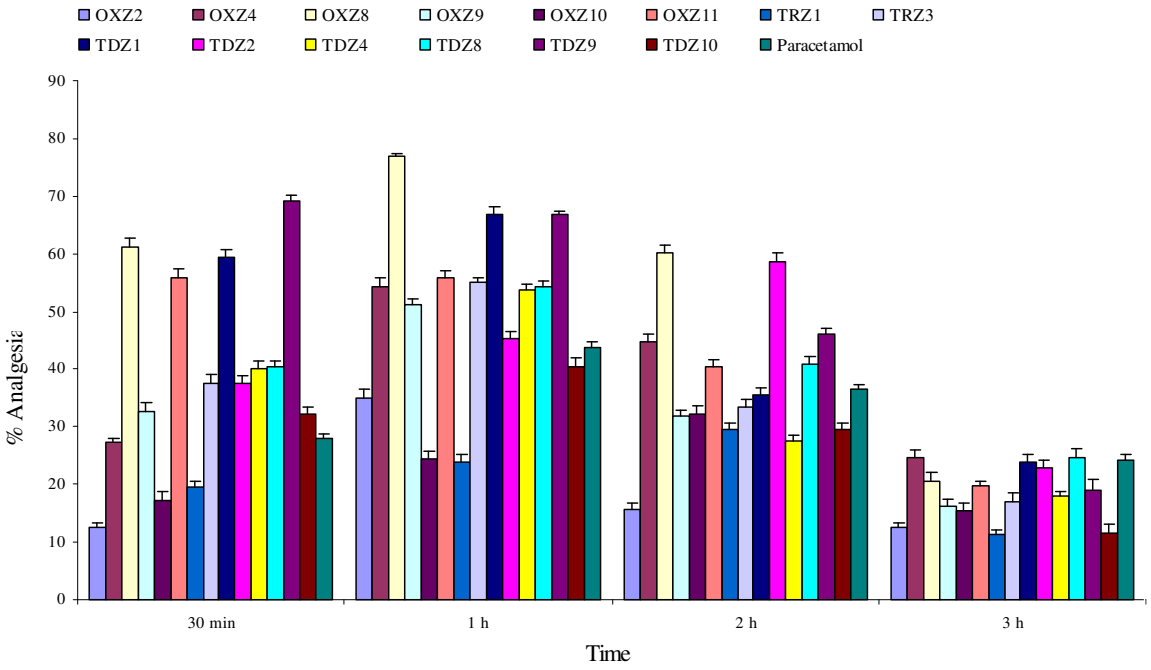


Figure 38. Analgesic activity of some selected 1,3,4-oxadiazole, 1,2,4-triazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles in mice by the tail immersion method

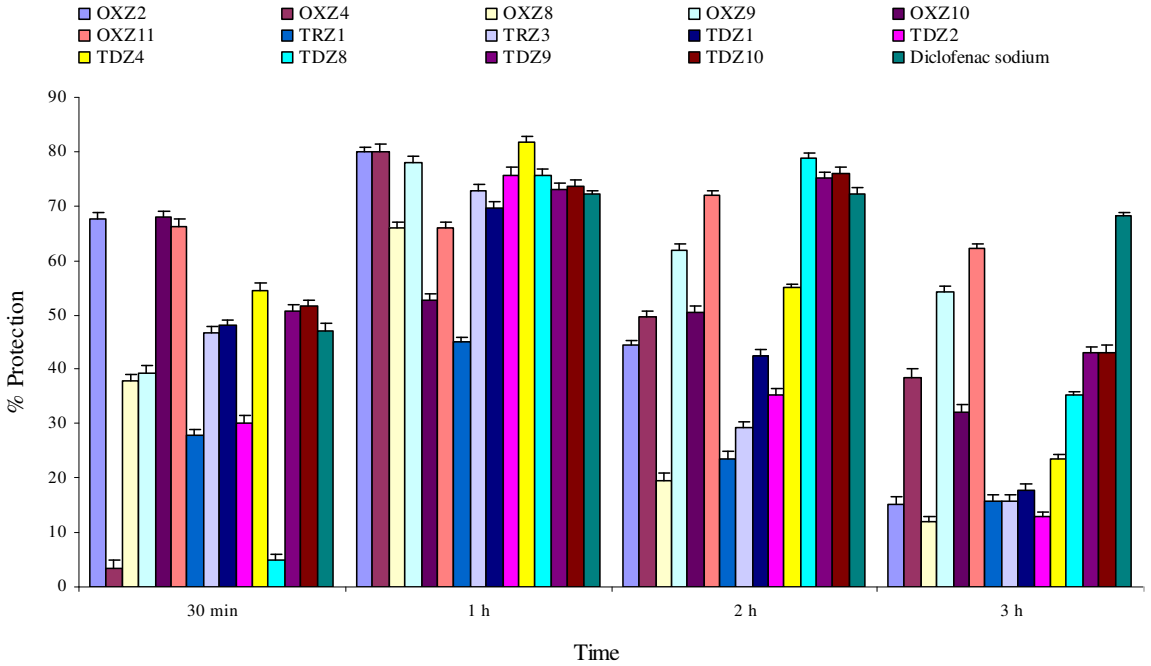
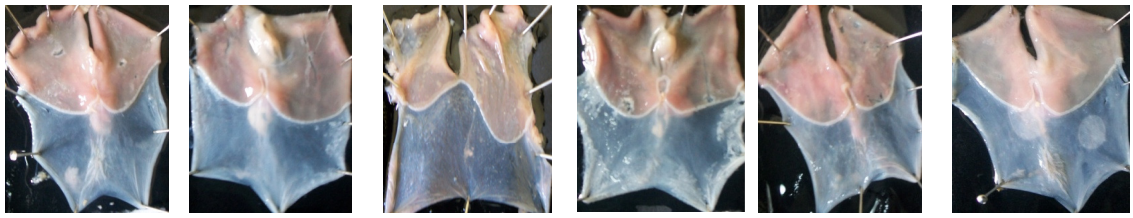
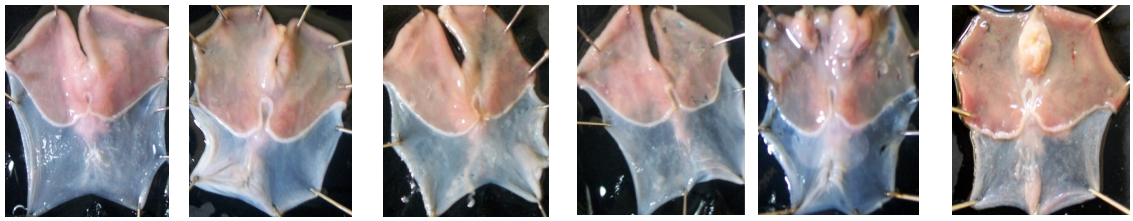


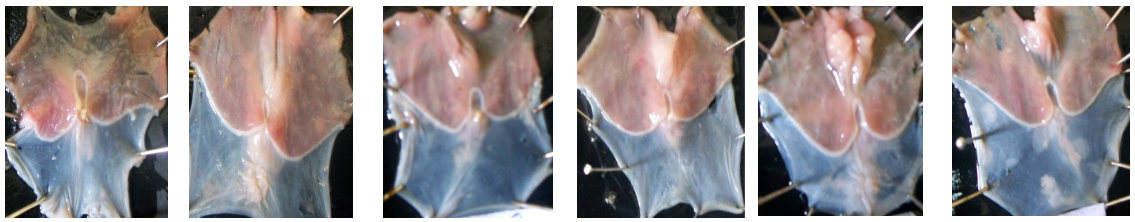
Figure 39. Anti-inflammatory activity of some selected 1,3,4-oxadiazole, 1,2,4-triazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles by the carrageenan induced rat paw oedema method



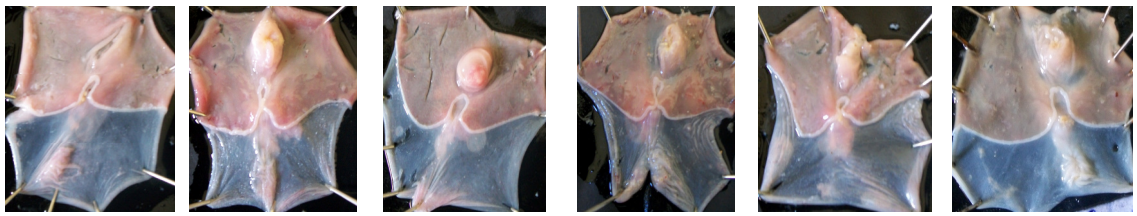
Control carboxymethyl cellulose



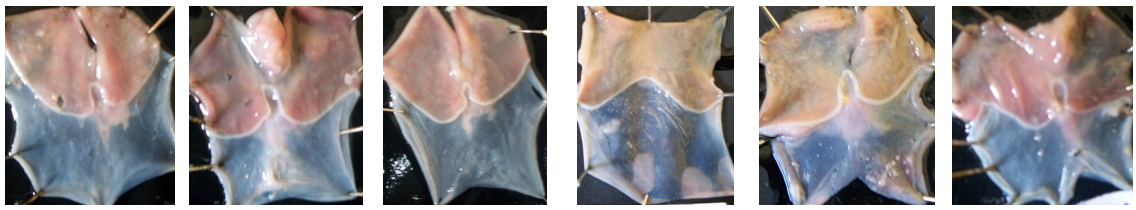
Compound **OXZ_4**



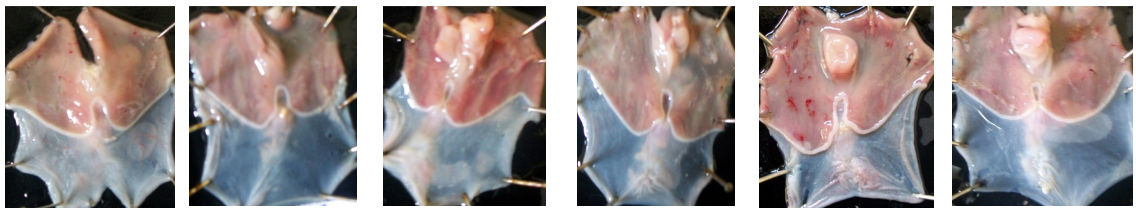
Compound **OXZ_9**



Compound **TDZ_4**

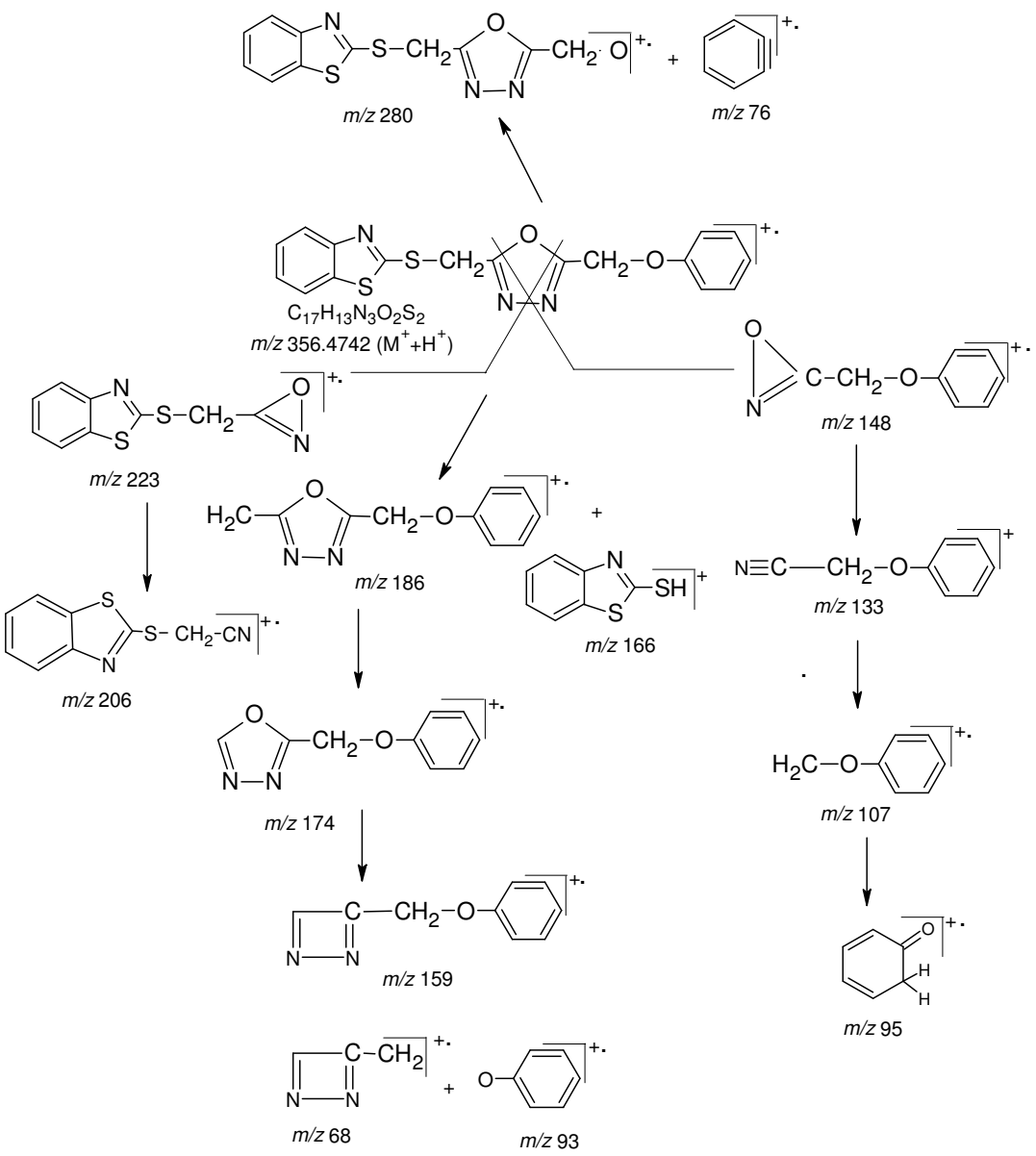


Compound **TDZ_9**

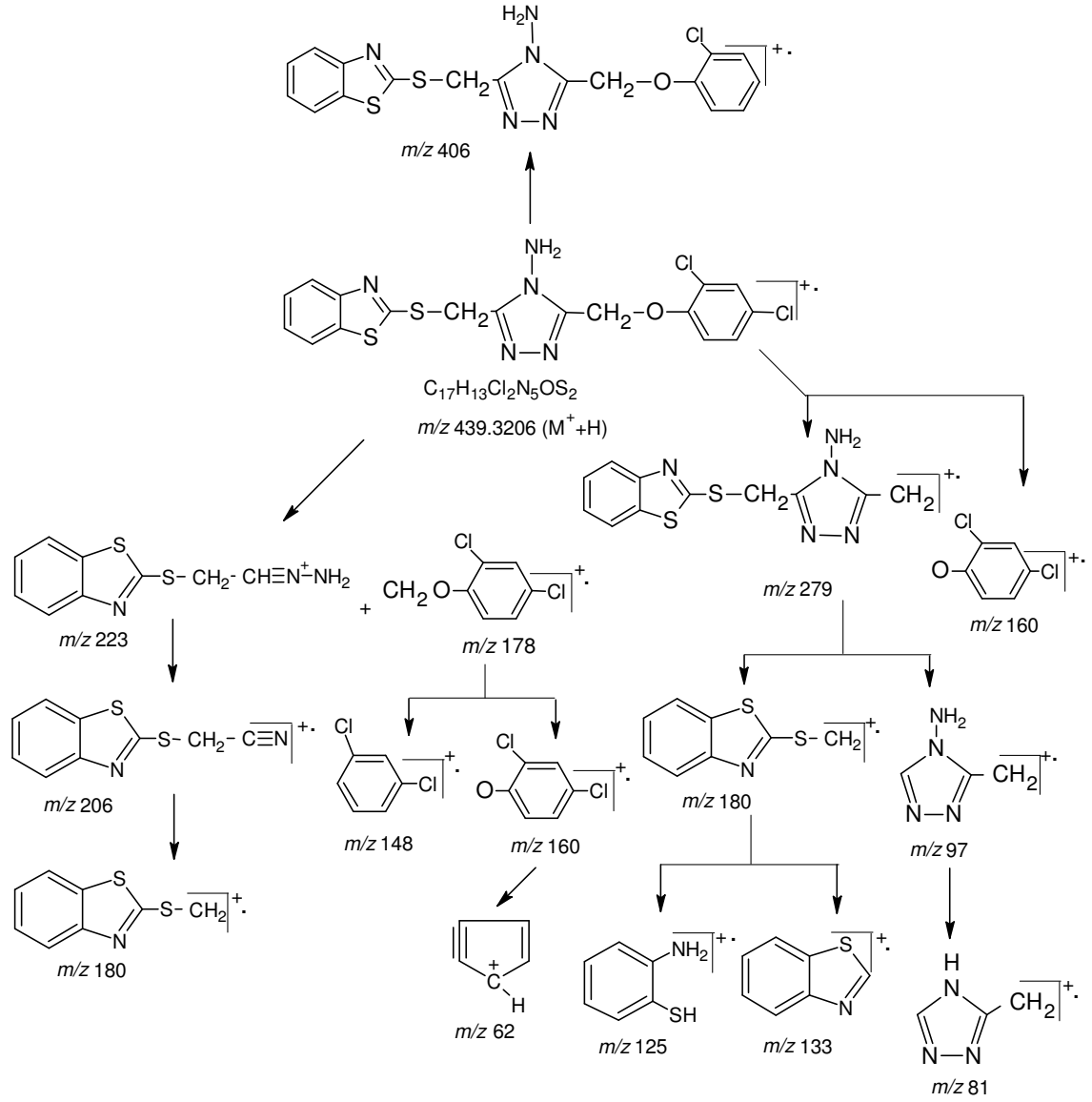


Diclofenac sodium

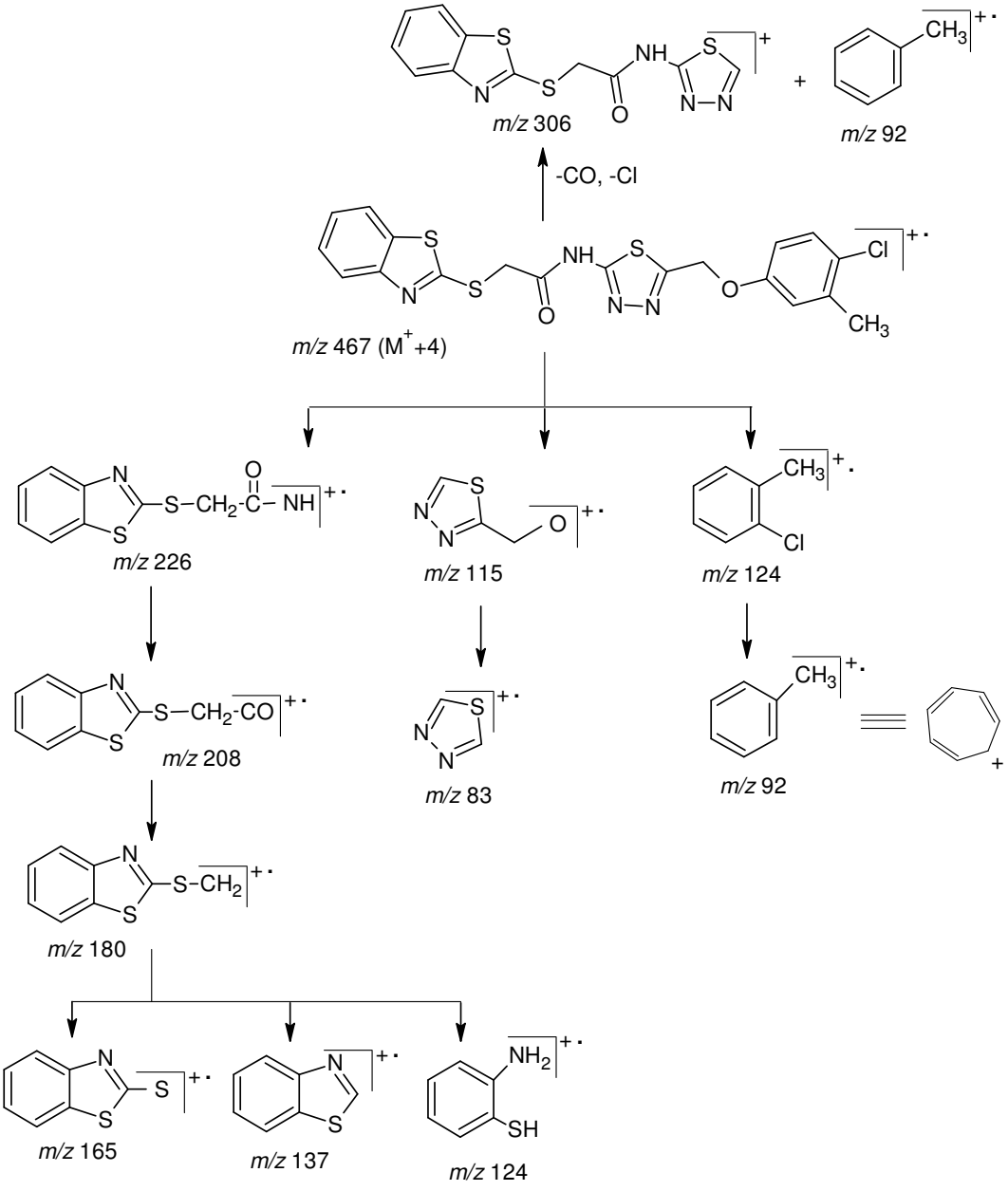
Figure 40. Ulcerogenic potential of some 1,3,4-oxadiazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles



Scheme 7. Possible mass fragmentation pattern of the synthesized compound **OXZ_1**



Scheme 8. Possible mass fragmentation pattern of the synthesized compound **TRZ$_7$**



Scheme 9. Possible mass framentation pattern of the synthesized compound **TDZ$_{11}$**

Table 11. Antimicrobial screening of some selected 1,3,4-oxadiazole, 1,3,4-triazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles by the cup plate method.

Compd.	Zone of inhibition (mm)[a,b]					
	P.a.	*S.d.*	*S. e.*	*E. f.*	*C.a.*	*A.n.*
OXZ_1	12	-	-	14	-	-
OXZ_2	17	-	-	-	-	-
OXZ_3	13	-	-	-	-	-
OXZ_4	18	-	-	-	-	-
OXZ_5	16	-	-	-	-	-
OXZ_6	-	-	-	-	-	-
OXZ_7	12	-	-	-	-	-
OXZ_8	20	-	-	-	-	-
OXZ_9	18	-	-	-	-	-
OXZ_{10}	19	-	-	-	-	-
OXZ_{11}	18	-	-	-	-	-
OXZ_{12}	-	-	-	-	-	-
OXZ_{13}	-	-	-	-	-	-
TRZ_1	23	-	-	-	-	-
TRZ_2	12	-	-	-	-	-
TRZ_3	18	17	19	-	-	-
ATZ_1	23	-	-	-	-	-
ATZ_2	14	-	-	-	-	-
TDZ_1	18	-	-	-	-	-
TDZ_2	19	-	-	-	-	-
TDZ_3	-	16	-	-	-	-
TDZ_4	20	-	-	-	-	-
TDZ_5	12	-	-	-	-	-
TDZ_6	13	-	-	12	-	-
TDZ_7	12	-	-	-	-	-
TDZ_8	21	-	-	-	-	-
TDZ_9	26	-	-	-	-	-
TDZ_{10}	24	-	16	-	-	-
DMSO	-	-	-	-	-	-
Ciprofloxacin	34	53	55	52	-	-
Ketoconazole	-	-	-	-	32	28

Test compounds, ciprofloxacin and ketoconazole were tested at 100, 10 and 20 µg mL^{-1} concentrations, respectively. [a]Average of three readings. [b]**-**indicates no activity. *P.a.: Pseudomonas aeruginosa; S. d.: Shigella dysenteriae; S. e.: Staphylococcus epidermidis*; *E. f.: Enterococcus faecalis*; *C. a.: Candida albicans*; *A. n.: Aspergillus niger*.

Table 12. Analgesic activity of some selected 1,3,4-oxadiazole, 1,3,4-triazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles in mice by the tail flick method.

Compd.	Percent analgesic activity			
	30 min	1 h	2 h	3 h
	%Analgesia ±SEM	%Analgesia ±SEM	%Analgesia ±SEM	%Analgesia ±SEM
OXZ_2	12.5 ± 0.8^b	34.9 ± 1.5^b	15.8 ± 0.9^b	12.5 ± 1.0^b
OXZ_4	27.2 ± 0.9^c	54.3 ± 1.4^b	44.7 ± 1.4^b	24.8 ± 1.1^b
OXZ_8	61.3 ± 1.4^b	76.8 ± 0.7^b	60.3 ± 1.2^c	20.7 ± 1.3^c
OXZ_9	32.7 ± 1.4^b	51.1 ± 1.1^b	31.8 ± 1.1^b	16.2 ± 1.2^b
OXZ_{10}	17.3 ± 1.4^b	24.5 ± 1.1^b	32.2 ± 1.5^c	15.4 ± 1.4^b
OXZ_{11}	55.9 ± 1.4^c	55.8 ± 1.2^c	40.5 ± 1.1^c	19.8 ± 0.7^d
TRZ_1	19.5 ± 1.2^b	23.8 ± 1.5^b	29.7 ± 0.9^a	11.4 ± 0.8^b
TRZ_3	37.5 ± 1.6^b	55.0 ± 0.7^c	33.5 ± 1.1^b	16.9 ± 1.5^c
TDZ_1	59.3 ± 1.4^b	66.9 ± 1.2^b	35.6 ± 1.3^b	23.8 ± 1.3^b
TDZ_2	37.5 ± 1.4^b	45.2 ± 1.3^b	58.7 ± 1.4^b	22.9 ± 1.3^b
TDZ_4	40.0 ± 1.3^b	53.7 ± 1.0^c	27.4 ± 1.2^c	18.1 ± 0.7^a
TDZ_8	40.3 ± 1.2^b	54.3 ± 1.0^c	40.9 ± 1.4^b	24.8 ± 1.4^b
TDZ_9	69.2 ± 0.9^b	66.8 ± 0.7^a	46.0 ± 1.1^b	19.0 ± 1.9^b
TDZ_{10}	32.1 ± 1.3^d	40.4 ± 1.6^b	29.7 ± 0.8^b	11.5 ± 1.5^b
Paracetamol	28.0 ± 0.8^a	43.8 ± 0.9^c	36.4 ± 1.0^b	24.1 ± 1.2^b

Test compounds and paracetamol were tested at 100 and 50 mg kg^{-1} body weight, respectively. Results are expressed in mean ± SEM (n=6); Significance levels $^aP<0.05$, $^bP<0.01$ and $^cP<0.001$ compared with the respective control.

Table 13. Anti-inflammatory activity of some selected 1,3,4-oxadiazole, 1,3,4-triazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles by the carrageenan induced rat paw oedema method.

Compd.	Percent protection			
	30 min	1 h	2 h	3 h
	%Protection (Mean % protection ±SEM)	%Protection (Mean % protection ±SEM)	%Protection (Mean % protection ±SEM)	%Protection (Mean % protection ±SEM)
OXZ_2	67.6 ± 1.1^a	80.0 ± 0.8^a	44.3 ± 0.9^b	15.3 ± 1.3^b
OXZ_4	3.4 ± 1.4^b	80.0 ± 1.4^c	49.7 ± 1.1^c	38.5 ± 1.5^a
OXZ_8	37.8 ± 1.1^c	66.0 ± 1.1^a	19.6 ± 1.3^b	12.0 ± 0.8^b
OXZ_9	39.4 ± 1.3^b	78.1 ± 0.9^c	61.8 ± 1.2^b	54.2 ± 1.1^b
OXZ_{10}	67.9 ± 1.3^b	52.6 ± 1.2^c	50.5 ± 1.2^b	32.1 ± 1.3^b
OXZ_{11}	66.3 ± 1.3^b	65.8 ± 1.4^b	71.8 ± 1.0^b	62.1 ± 1.1^b
TRZ_1	27.8 ± 1.1^b	45.0 ± 0.8^a	23.5 ± 1.3^a	15.7 ± 1.2^c
TRZ_3	46.8 ± 1.0^b	72.8 ± 1.2^c	29.3 ± 1.0^b	15.8 ± 1.0^c
TDZ_1	48.1 ± 1.0^a	69.7 ± 1.0^b	42.3 ± 1.2^b	17.9 ± 1.1^c
TDZ_2	30.1 ± 1.4^b	75.7 ± 1.3^b	35.2 ± 1.1^b	12.8 ± 1.0^b
TDZ_4	54.5 ± 1.3^a	81.6 ± 1.3^b	54.9 ± 0.8^b	23.6 ± 0.9^b
TDZ_8	4.9 ± 1.1^c	75.7 ± 1.1^c	78.7 ± 0.9^c	35.2 ± 0.7^c
TDZ_9	50.6 ± 1.3^b	73.0 ± 1.3^b	75.2 ± 0.9^c	43.0 ± 1.1^b
TDZ_{10}	51.6 ± 1.2^b	73.7 ± 1.0^b	76.0 ± 1.1^b	43.1 ± 1.4^b
Diclofenac sodium	47.1 ± 1.2^b	72.1 ± 0.8^b	72.3 ± 1.1^b	68.1 ± 0.8^b

Test compounds and diclofenac sodium were tested at 100 and 20 mg kg^{-1} body weight, respectively; Result are expressed in mean ± SEM (n=6); Significance levels $^aP<0.05$, $^bP<0.01$ and $^cP<0.001$ compared with the respective control.

Table 14. Ulcerogenic effects of some selected some selected 1,3,4-oxadiazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles by the Cioli *et al*.'s method.

Compd.	Control 1% CMC	**OXZ_4**	**OXZ_9**	**TDZ_4**	**TDZ_9**	Diclofenac sodium
Severity Index	0.25 ± 0.2^{c}	3.2 ± 0.5^{b}	2.7 ± 0.2^{b}	2.2 ± 0.3^{b}	2.3 ± 0.3^{b}	4.4 ± 0.6^{b}

Test compounds and diclofenac sodium were tested at 200 and 20 mg kg^{-1} body weight, respectively. Results are expressed in mean ± SEM (n=6); Significance levels $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$ compared with the respective control.

4.3. Synthesis and Biological Evaluation of some Novel 2-Pyrazoline Incorporated 2-Mercaptobenzothiazoles

4.3.1. Chemistry

The synthesis of the target compounds **PYZ_{1-19}** and **PYS_{1-9}** is described in **Scheme 4**. Ethyl (benzothiazol-2-ylthio)acetate (**MBE_1**) and benzothiazol-2-ylthio acetic acid hydrazide (**MBZ_1**) were prepared according to the published procedures[117,118] (Karali *et al.*, 2004; Desai *et al.*, 2006). The intermediate chalcones (**CHA_{1-17}**) were prepared[183] (Furniss *et al.*, 2005) by the base catalyzed Claisen-Schmidt condensation of the aromatic ketones (**ACP_{1-3}**) with different aromatic aldehydes (**ALD_{1-7}**) The cyclo-condensation of prepared chalcones with hydrazide (**MBZ_1**) in absolute ethanol in the presence of few drops of glacial acetic acid furnished the corresponding novel 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-disubstituted-4,5-dihydro-1*H*-pyrazol-1-yl)ethanones (**PYZ_{1-19}**). Finally treatment of compounds **PYZ_2**, **PYZ_4**, **PYZ_{6-11}** and **PYZ_{14}** with 30% hydrogen peroxide and a catalytic amount of sodium tungstate in acetic acid afforded the corresponding novel sulfone derivatives **PYS_{1-9}**.

In the infrared (IR) spectra of synthesized compounds (**PYZ_{1-19}**) the carbonyl (C=O) absorption bands were observed in the region of 1689-1654 cm^{-1}. The shift in the frequency to lower values could be explained on the basis of the mesomeric effect. The C=N stretching observed at 1658-1612 cm^{-1} is due to the ring closure, which confirm the formation of the desired pyrazoline ring in all prepared compounds. In addition, compounds **PYZ_5**, **PYZ_{11}**, **PYZ_{17}** and **PYS_8** showed sharp bands in the region 1605-1622 cm^{-1} due to the aliphatic C=C stretching. Furthermore, appearance of SO_2 absorption bands in the regions at 1344-1305 and 1199-1126 cm^{-1} indicated the formation of compounds **PYS_{1-9}**.

The ^{1}H NMR spectra[172] (Bansal *et al.*, 2001) of compounds **PYZ_{1-4}**, **PYZ_{6-10}**, **PYZ_{12-16}**, **PYZ_{18}**, **PYZ_{19}**, **PYS_{1-7}** and **PYS_9** showed broad triplet signal in the region at δ 7.12-6.30 ppm for the CH proton at fifth position and doublet signal at δ δ 4.52-4.21 ppm for the CH_2 protons at third position of the pyrazoline ring. In the ^{1}H NMR spectra of compounds **PYZ_5**, **PYZ_{11}**, **PYZ_{17}** and **PYS_8** two multiplets in the region at δ 6.72-6.63 and δ 4.45-4.25 ppm were assigned to the CH and CH_2 protons, respectively (pyrazoline ring). The protons belonging to the aromatic rings and substitutent groups were observed within the expected chemical shift values[171,172] (Khode *et al.*, 2009; Bansal *et al.*, 2001). In the ^{1}H NMR spectrum (in DMSO-d_6) of **PYZ_9**, C_5 and C_3 protons of the pyrazoline ring resonated as triplet and doublet at δ 7.01 and 4.48 ppm, respectively (**Figure 43a-d**). The CH_2 protons of the acetyl group which is on first

position of the pyrazoline ring was observed as singlet at δ4.71 ppm and two multiplet signals at δ8.20-7.60 and 7.52-7.33 ppm were assigned to the eight and four aromatic protons.

The ^{13}C NMR spectra (see experimental) were recorded for a few members of the title compounds **PYZ$_{1-3}$**, **PYZ$_9$**, **PYZ$_{11}$** and **PYZ$_{17}$**. In the ^{13}C NMR spectrum (in DMSO-d_6) of **PYZ$_9$** (**Figure 46**) two signals observed at δ 166.01 and 163.52 ppm were ascribed to the azomethine carbon of the benzothiazole and pyrazoline ring systems, respectively. The carbonyl carbon (amide) displayed a signal at δ 169.18 ppm while the C_5 and C_4 carbons of the pyrazoline ring exhibited singlets at δ 55.22 and 35.57 ppm, respectively. The signal observed at δ 64.16 ppm was assigned to the CH_2 fragment of the acetyl group. The phenyl carbons appeared as sixteen signals at δ 152.52, 148.25, 147.59, 137.57, 137.22, 135.47, 134.68, 134.35, 133.93, 130.66, 130.06, 128.46, 126.30, 124.40, 123.53, 121.89, ppm. The electron impact (EI) mass spectrum of compound **PYZ$_2$** (**Figure 47**) showed (M^++1) peak at *m/z* 465 which is in agreement with its molecular formula $C_{24}H_{18}ClN_3OS_2$. The spectrum showed a base peak at *m/z* 166 and a peak at *m/z* 134, which corresponded to the fragments $[C_7H_4NS_2]^{+\cdot}$ and $[C_7H_5NS]^{+\cdot}$, respectively. The electron impact (EI) mass spectrum of compound **PYS$_4$** (**Figure 50**) displayed the molecular ion peak at *m/z* 496 which is consistent with the molecular formula $C_{24}H_{18}ClN_3O_3S_2$. Furthermore, the base peak at *m/z* 219 corresponds to the fragment $[C_9H_7N_3S_2]^{+\cdot}$ and the two strong intensity peaks at *m/z* 178 and 263 correspond to the fragments $[C_8H_6NS_2]^{+\cdot}$ and $[C_{12}H_{11}N_3O_2S]^{+\cdot}$, respectively. Proposed mechanism of fragmentations and the *m/z* of the fragments for compounds **PYZ$_2$** and **PYS$_4$** are given in **Schemes 10** and **11**, respectively.

4.3.2. Biological Evaluation

4.3.2.1. Antimicrobial Activity

The antimicrobial screening by the agar-plate method[177] (Barry ALJ, 1999) displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, while they were inactive against the two tested strains of fungi (**Table 17**). Compounds **PYZ$_8$**, **PYZ$_9$**, **PYZ$_{10}$** and **PYZ$_{14}$** having acetyl group as linker between 2-mercaptobenzothiazole and 2-pyrazoline moieties showed significant inhibitory activity against the Gram-negative bacteria *Pseudomonas aeruginosa* (inhibition zone 30-35 mm) whereas the same compounds were found to be moderately active against *Staphylococcus aureus* (inhibition zone 22-24 mm), *Bacillus subtilis* (inhibition zone 21-24 mm) and *Escherichia coli* (inhibition zone 25-28 mm). Furthermore, the maximum inhibitory activity against *Pseudomonas aeruginosa* (inhibition zone 35 mm) was observed in compound **PYZ$_{14}$** having 3-nitrophenyl

and phenyl groups respectively at third and fifth position of the pyrazoline ring. It is evident that in compound **PYZ_{14}** substitution of other groups in the phenyl ring present at fifth position of the pyrazoline ring either decreased or abolished the activity against the tested Gram-negative bacterial strains. On the other hand, compound **PYZ_{17}** having 3-nitrophenyl and 2-phenylethenyl groups at third and fifth position of the pyrazoline ring exhibited maximum inhibitory activity against the tested Gram-positive bacterial strains *Staphylococcus aureus* and *Bacillus subtilis* (inhibition zone 29 and 28 mm, respectively). In the sulfonyl series, compounds **PYS_{1-3}** and **PYS_{5-7}** showed significant inhibitory activity against the tested Gram-positive bacterial strains *Staphylococcus aureus* and *Bacillus subtilis* (inhibition zone 24-29 mm and 24-32 mm, respectively). It was also observed that conversion of the sulfanyl function of the derivatives **PYZ_2**, **PYS_4**, **PYS_{6-11}** and **PYS_{14}** into sulfone provided a remarkable increase in their activity against the tested Gram-positive bacteria. However, activity against the tested Gram-negative microorganism markedly decreased.

4.3.2.2. Pharmacological Activity

4.3.2.2.1. Acute Toxicity

No behavioral changes in animals were observed during the experiment and at the end hematological parameters were estimated and there was no observable change. In the present study, mortality was not observed even at 1000 mg kg^{-1} indicating that the tested compounds are nontoxic to animals.

4.3.2.2.2. Analgesic Activity

The tested compounds **PYZ_9** and **PYZ_{14}** exhibited fast analgesic activity (37.4 and 47.5%, respectively) as evident from observation at 30 min following oral administration (**Table 18**). At first hour compound **PYZ_{14}** showed significant activity (58.6% analgesia), while rest of the tested compounds were found to be either weakly or moderately active (17.4-40.9%). Compounds **PYZ_9**, **PYZ_{10}** and **PYZ_{14}** exhibited potent analgesic activity (55.9-69.8%) at second and third hour after oral administration, when compared to the reference drug (39.9 and 26.1%, respectively at a dose of 50 mg kg^{-1}). Rest of the investigated compounds showed variable degree of analgesic activity ranging between weak to moderate (8.3-35.9%). Moreover, maximum activity (69.8%) was observed at second hour in compound **PYZ_9** bearing 4-Cl and 3-NO_2 groups in the phenyl rings, respectively at third and fifth position of the pyrazoline ring. It is evident from results that conversion of the sulfanyl group of the compounds **PYZ_2**, **PYZ_4** and

PYZ_6 into sulfone **PYS_{1-3}** resulted in significant decrease in analgesic activity (**Figure 52**). It is also evident that substitution of 4-chlorophenyl or 3-nitrophenyl groups at third position and 3-nitrophenyl, 4-methoxyphenyl or phenyl groups at fifth position of the pyrazoline ring (**PYZ_9**, **PYZ_{10}** and **PYZ_{14}**, respectively) significantly enhanced the analgesic activity. It was observed that compounds bearing 2-Cl or 4-$(CH_3)_2N$ group in phenyl ring at fifth position of the pyrazoline ring resulted in a marked decrease in activity.

4.3.2.2.3. Anti-inflammatory Activity

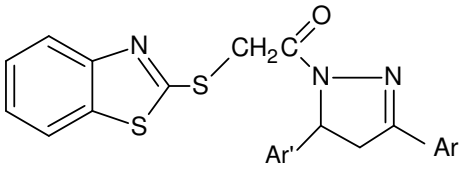
The anti-inflammatory activity results determined using the carrageenan-induced paw oedema method[179] (Winter *et al*., 1962) in rats are summarized in **Table 19**. It is clear that at 30 min after carrageenan injection, two compounds, namely **PYZ_9** and **PYZ_{14}** showed rapid onset of action (43.4 and 46.7% protection, respectively). At first hour tested compounds **PYZ_{8-10}** and **PYZ_{14}** showed significant protection (64.4-70.4%) against carrageenan induced oedema when compared to the reference drug (72.3% at a dose of 20 mg kg^{-1}). Whereas at second and third hour same compounds exhibited potent anti-inflammatory activity (58.9-76.9%) and the highest activity (76.9%) was observed at second hour in derivative **PYZ_{14}**. On the other hand, sulfonyl analogs (**PYS_{1-3}**) showed weak anti-inflammatory activity at all time interval and this could be attributed to the conversion of the sulfanyl group into sulfone (**Figure 53**). It is evident from results that substitution of 4-chlorophenyl (**PYZ_{8-10}**) or 3-nitrophenyl (**PYZ_{14}**) groups at third position and 2-chlorophenyl, 3-nitrophenyl, 4-methoxyphenyl or phenyl groups at fifth position of the pyrazoline ring (**PYZ_8**, **PYZ_9**, **PYZ_{10}** and **PYZ_{14}**, respectively) resulted in a marked increase in activity.

4.3.2.2.4. Ulcerogenic Effects

Compounds **PYZ_9**, **PYZ_{10}** and **PYZ_{14}** that exhibited higher analgesic and anti-inflammatory profiles in the pre-mentioned animal models and promising antibacterial activities were further evaluated for their ulcerogenic potential in rats according to the method reported by Cioli *et al.*[180] (1979). The results indicated low ulcerogenic activity of the tested compounds ranging from 2.0±0.3 to 4.0±0.8 whereas the standard drug diclofenac sodium showed high severity index of 4.9±0.5 (**Table 20**). The maximum reduction in ulcerogenic activity was observed in compound **PYZ_{14}** (2.0±0.3). The other tested compounds also exhibited better GI safety profile as compared to the standard drug diclofenac sodium (**Figure 54**).

In summary, various pyrazoline derivatives (**PYZ_{1-19}**) and (**PYS_{1-9}**) were prepared with the objective of developing dual acting antimicrobial-analgesic-anti-inflammatory agents with gastrosparing activity. It was interesting to note that derivative 2-(1,3-benzothiazol-2-ylsulfanyl)-1-[3-(3-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**PYZ_{14}**) was found to have significant inhibitory activity against the tested Gram-negative bacterial strains. When **PYZ_{14}** was subjected to analgesic activity by the tail immersion method in mice, it showed potent analgesic activity compared to the standard drug paracetamol. It also exhibited significant anti-inflammatory activity in carrageenan induced rat paw oedema method with a significant reduction in severity index than that of the standard drug. Therefore, compound **PYZ_{14}** represents a fruitful matrix for development of a new class of dual acting antimicrobial and analgesic-anti-inflammatory agents.

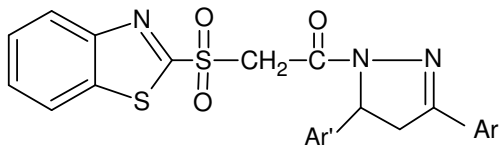
Tables 15. Physical data of the synthesized 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-disubstituted-4,5-dihydro-1H-pyrazol-1-yl)ethanones (**PYZ_{1-19}**)



Compd.	Ar	Ar′	Solv. Cryst.	M.p.(°C)	[a]Yield (%)
PYZ_1	C_6H_5	C_6H_5	acetone	168	57
PYZ_2	C_6H_5	2-Cl.C_6H_4	DMSO:ethanol (1:1)	200	69
PYZ_3	C_6H_5	4-NO_2.C_6H_4	methanol	178	56
PYZ_4	C_6H_5	4-CH_3O.C_6H_4	dichloromethane :acetone (1:1)	162	67
PYZ_5	C_6H_5	CH=CH.C_6H_5	ethanol	170	56
PYZ_6	C_6H_5	4-$(CH_3)_2N$.C_6H_4	DMF:ethanol (1:1)	210	47
PYZ_7	4-Cl.C_6H_4	C_6H_5	acetone	194	59
PYZ_8	4-Cl.C_6H_4	2-Cl.C_6H_4	THF	222	64
PYZ_9	4-Cl.C_6H_4	3-NO_2.C_6H_4	THF	210	72
PYZ_{10}	4-Cl.C_6H_4	4-CH_3O.C_6H_4	ethanol	184	66
PYZ_{11}	4-Cl.C_6H_4	CH=CH.C_6H_5	acetone	192	49
PYZ_{12}	4-Cl.C_6H_4	2-furyl	ethanol	168	64
PYZ_{13}	4-Cl.C_6H_4	4-$(CH_3)_2N$.C_6H_4	ethanol	210	61
PYZ_{14}	3-NO_2.C_6H_4	C_6H_5	ethanol	122	74
PYZ_{15}	3-NO_2.C_6H_4	2-Cl.C_6H_4	ethanol	102	69
PYZ_{16}	3-NO_2.C_6H_4	4-CH_3O.C_6H_4	ethanol	140	66
PYZ_{17}	3-NO_2.C_6H_4	CH=CH.C_6H_5	ethanol	206	70
PYZ_{18}	3-NO_2.C_6H_4	2-furyl	ethylacetate	136	66
PYZ_{19}	3-NO_2.C_6H_4	4-$(CH_3)_2N$.C_6H_4	ethylacetate	110	57

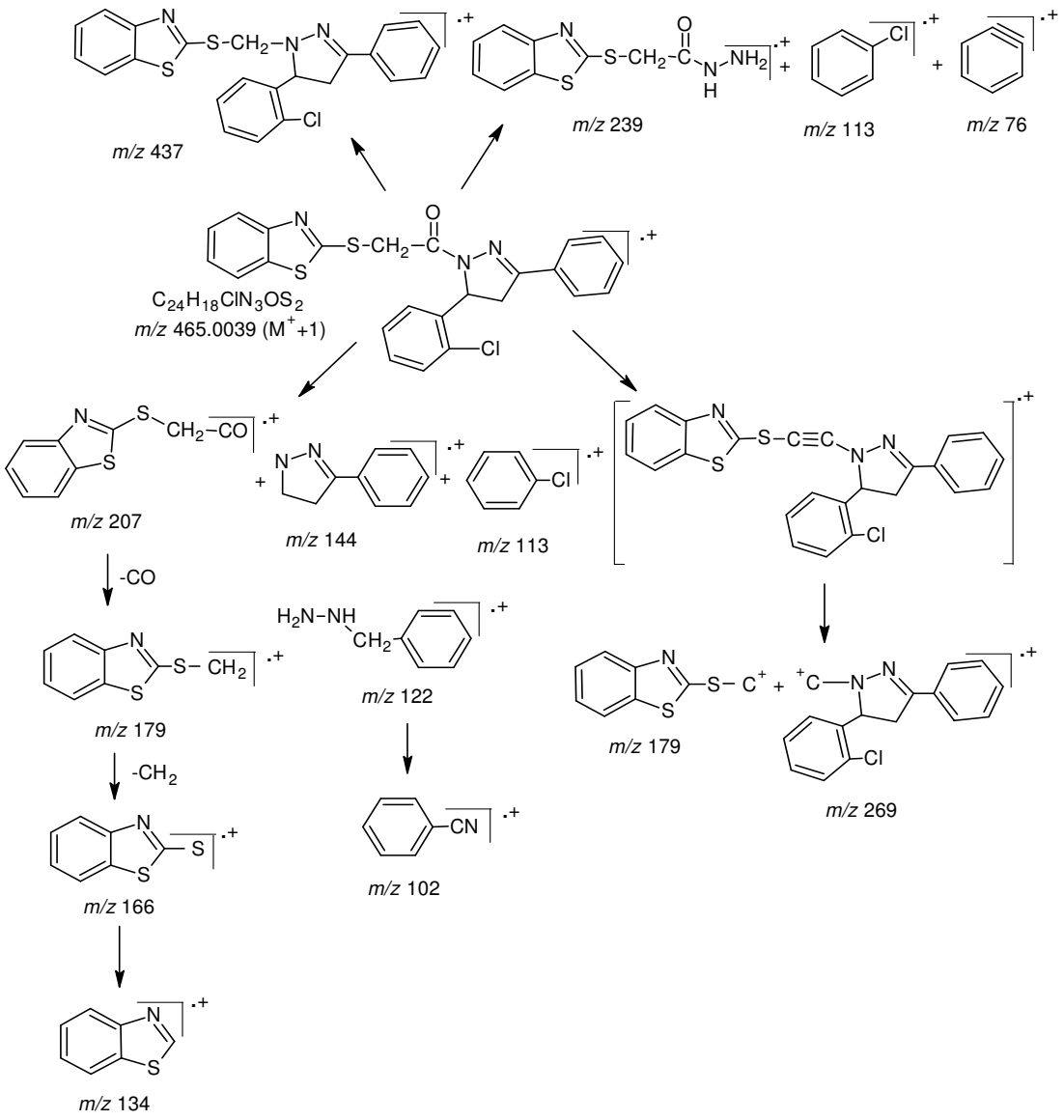
[a]Isolated yield

Tables 16. Physical data of the synthesized 2-(1,3-benzothiazol-2-ylsulfonyl)-1-(3,5-disubstituted-4,5-dihydro-1H-pyrazol-1-yl)ethanones (**PYS_{1-9}**)



Compd.	Ar	Ar′	Solv. Cryst.	M.p.(°C)	[a]Yield (%)
PYS_1	C_6H_5	2-Cl.C_6H_4	ethanol	190	59
PYS_2	C_6H_5	4-CH_3O.C_6H_4	ethanol	169	67
PYS_3	C_6H_5	4-$(CH_3)_2N$.C_6H_4	methanol	>300	69
PYS_4	4-Cl.C_6H_4	C_6H_5	DMF:ethanol (1:1)	195	62
PYS_5	4-Cl.C_6H_4	2-Cl.C_6H_4	ethanol	211	67
PYS_6	4-Cl.C_6H_4	3-NO_2.C_6H_4	DMF:acetone (1:1)	217	71
PYS_7	4-Cl.C_6H_4	4-CH_3O.C_6H_4	ethanol	191	56
PYS_8	4-Cl.C_6H_4	CH=CH.C_6H_5	ethanol	181	68
PYS_9	3-NO_2.C_6H_4	C_6H_5	ethanol	>300	57

[a]Isolated yield



Scheme 10. Possible mass fragmentation pattern of the synthesized compound **PYZ 2**

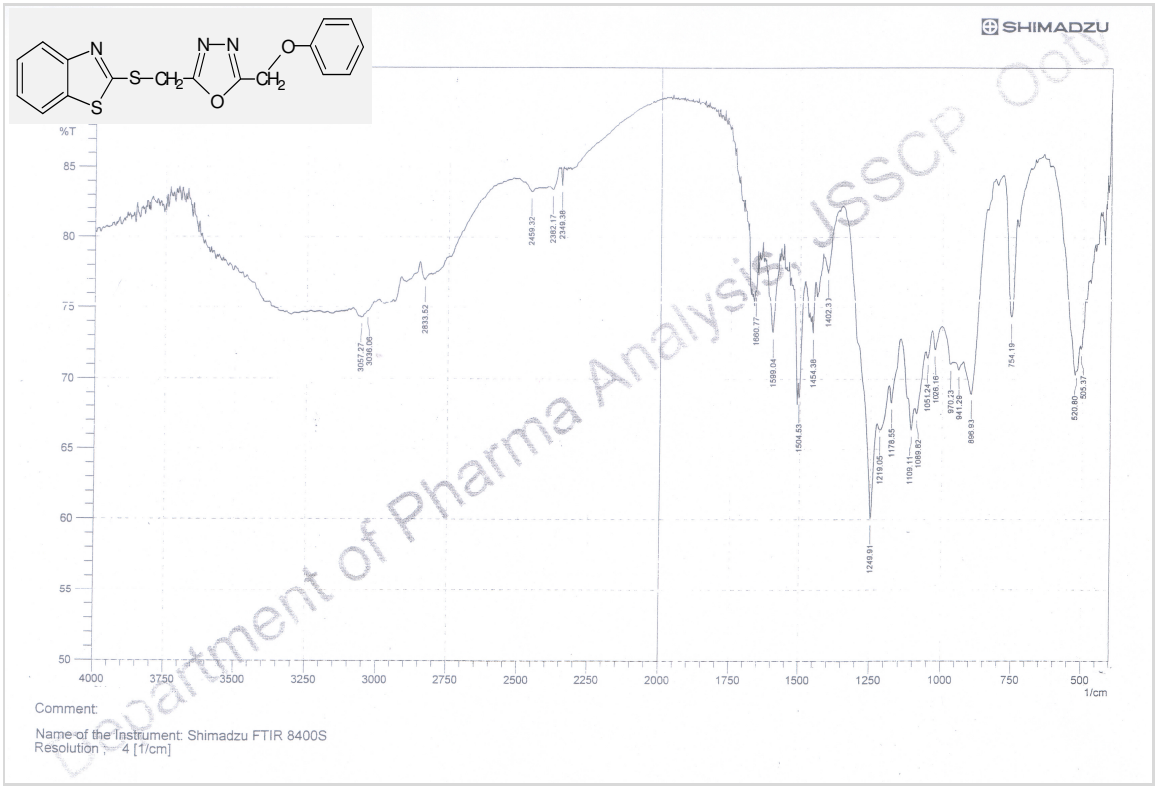


Figure 21. Infrared spectrum of the synthesized compound **OXZ_1**

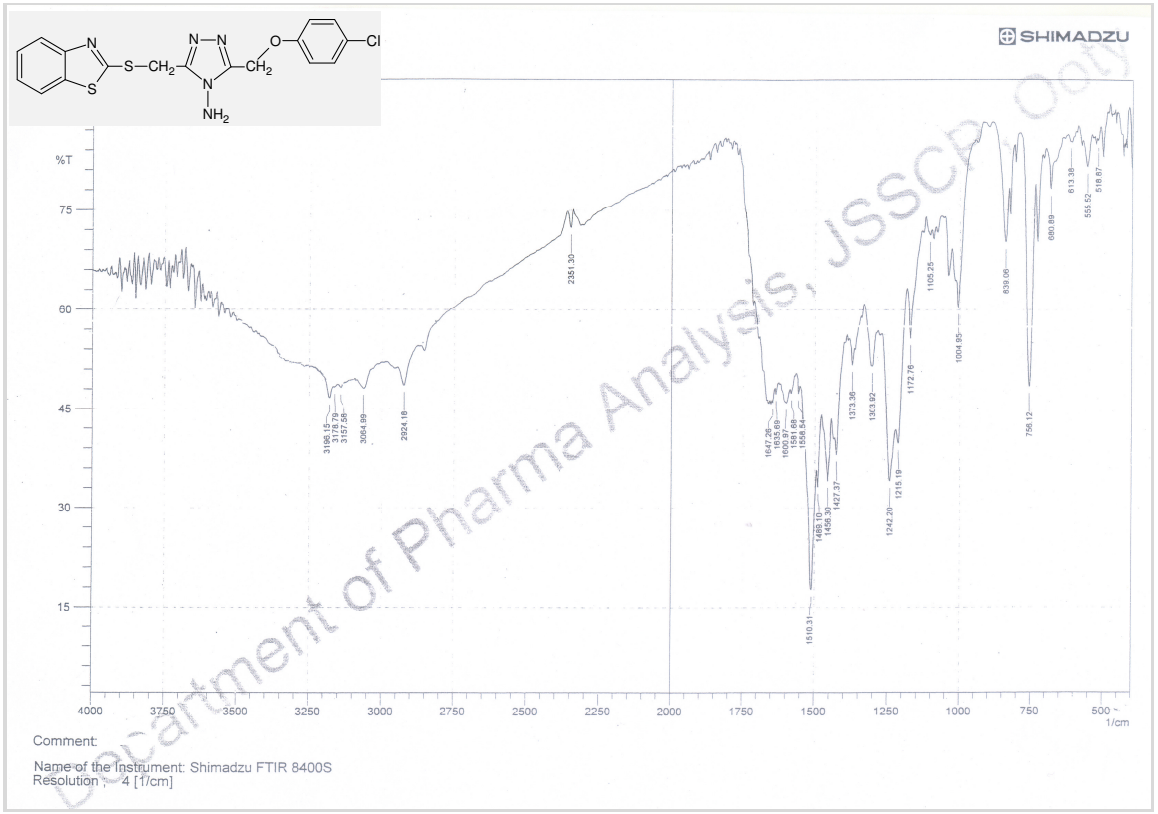


Figure 22. Infrared spectrum of the synthesized compound **TRZ_2**

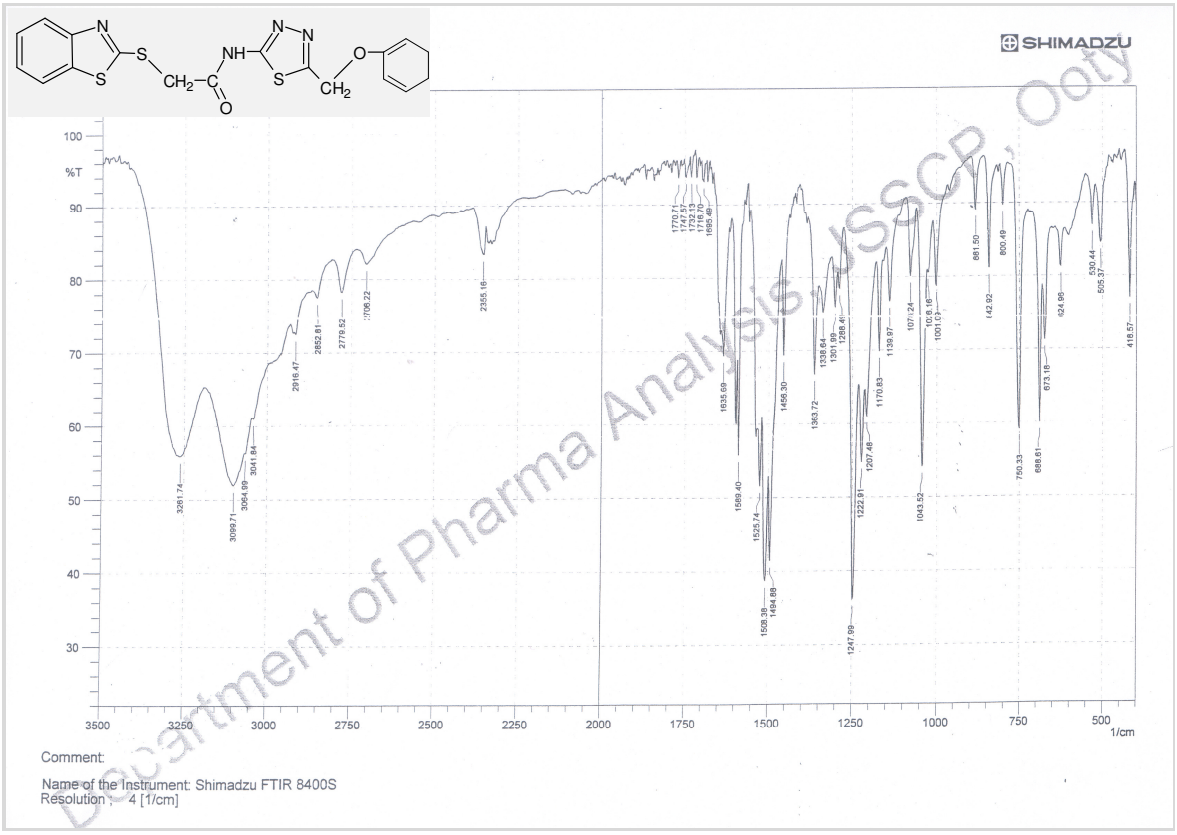


Figure 23. Infrared spectrum of the synthesized compound **TDZ_1**

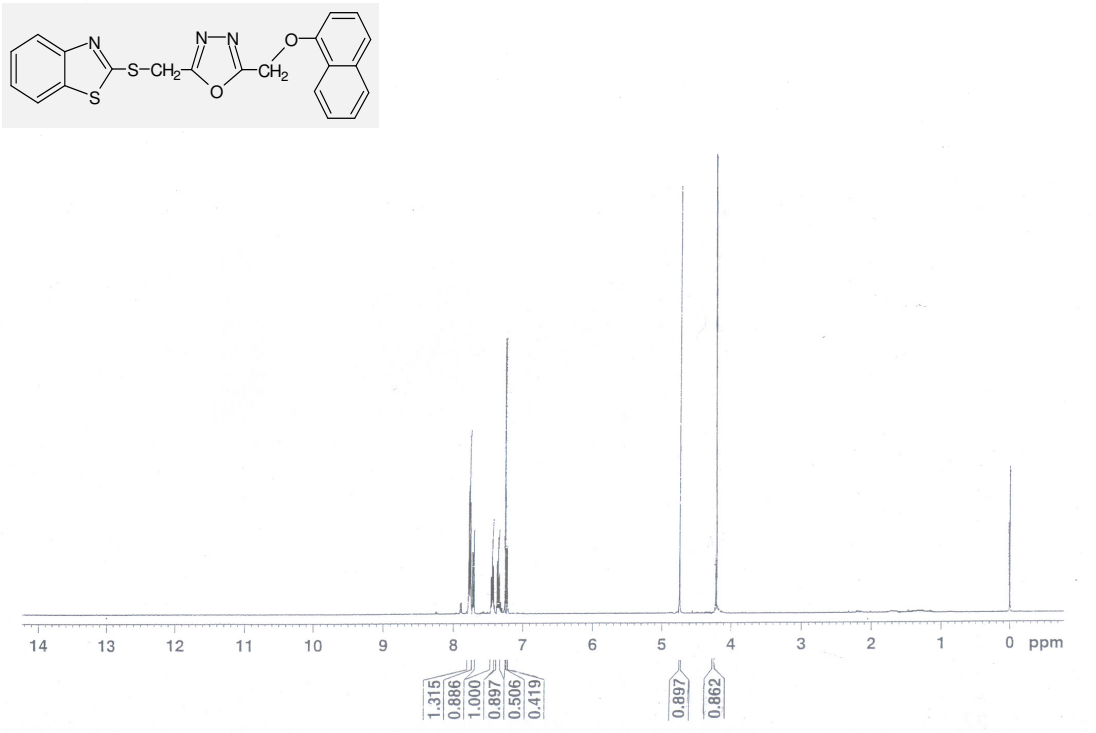


Figure 24a. ^{1}H NMR spectrum of the synthesized compound **OXZ_7**

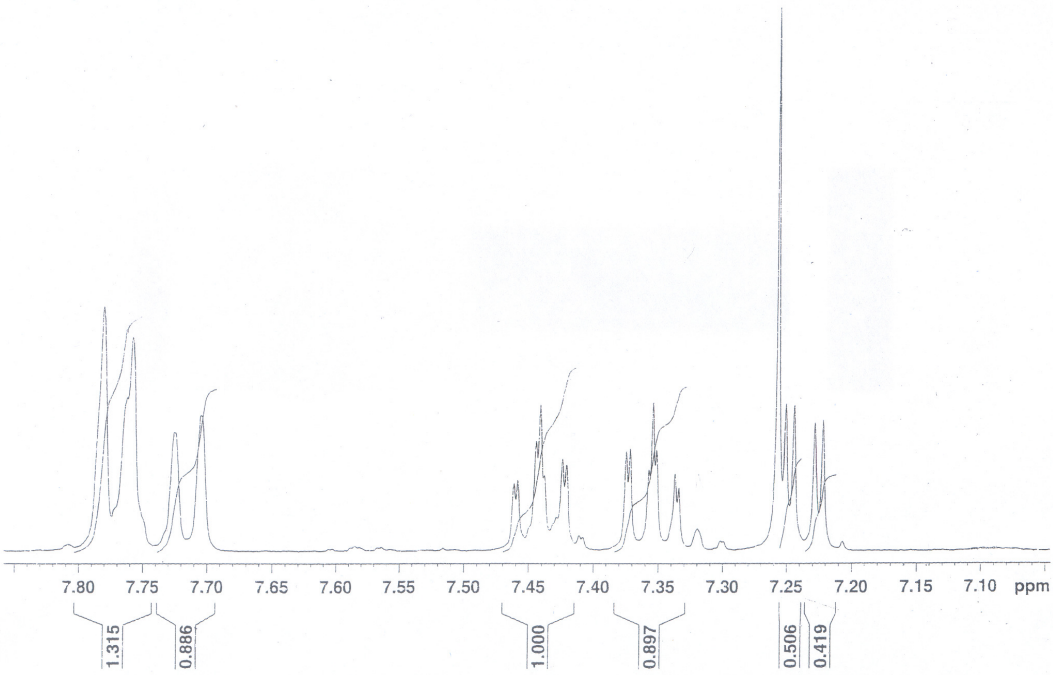


Figure 24b. Expansion of the [1]H NMR spectrum of the synthesized compound **OXZ_7**

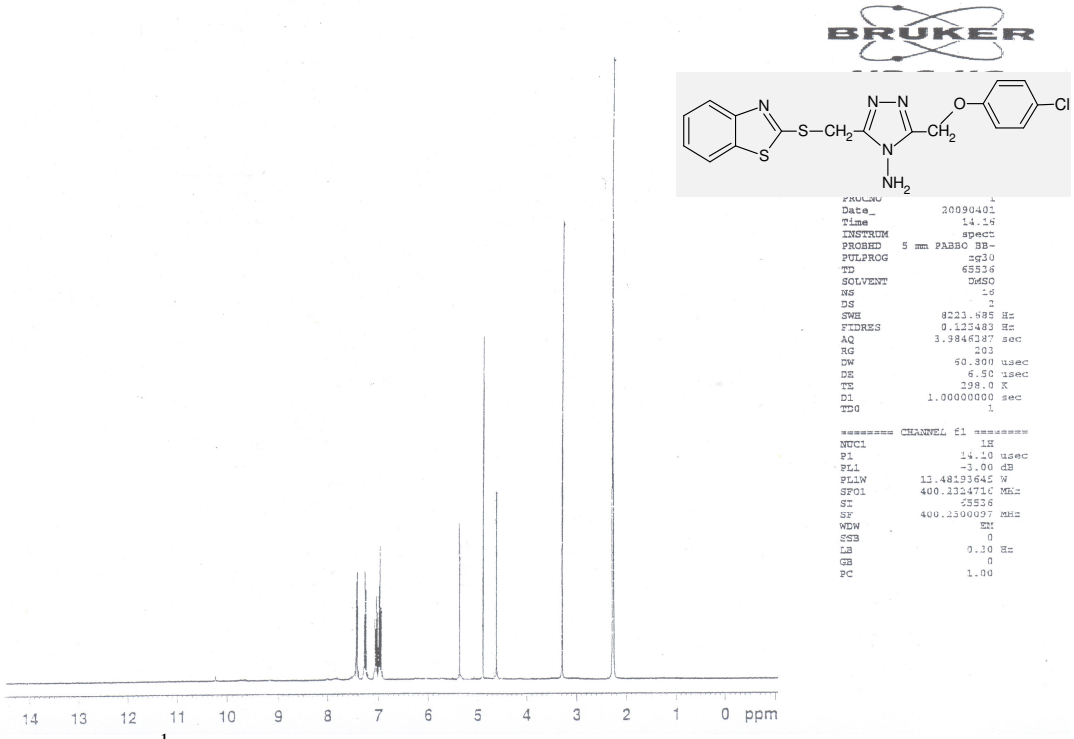


Figure 25a. [1]H NMR spectrum of the synthesized compound **TRZ_2**

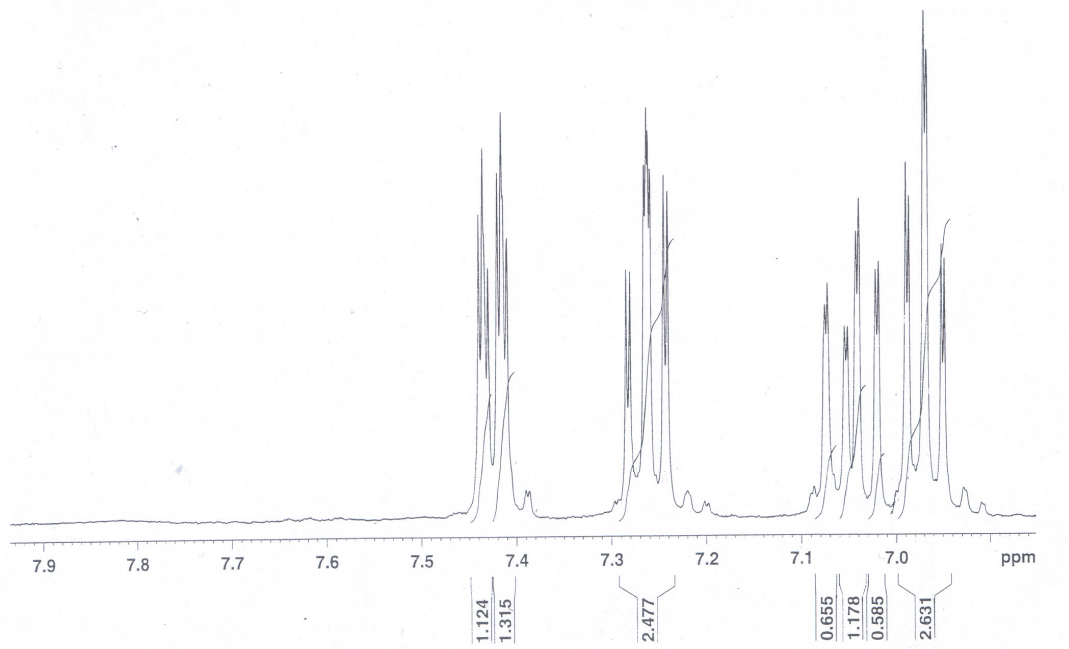


Figure 25b. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TRZ_2**

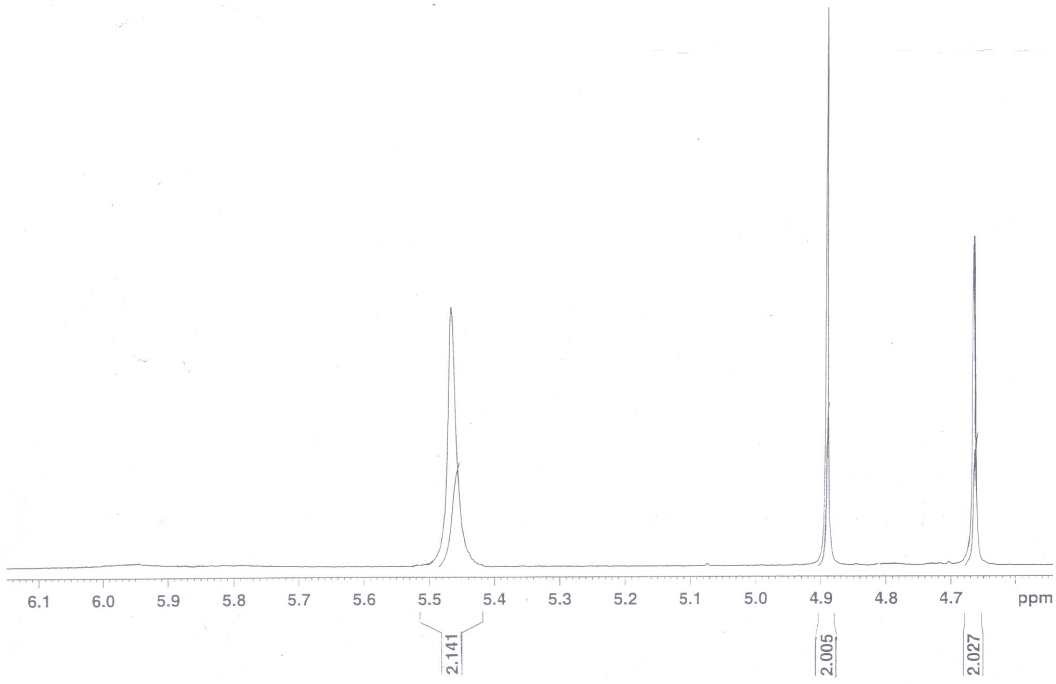


Figure 25c. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TRZ_2**

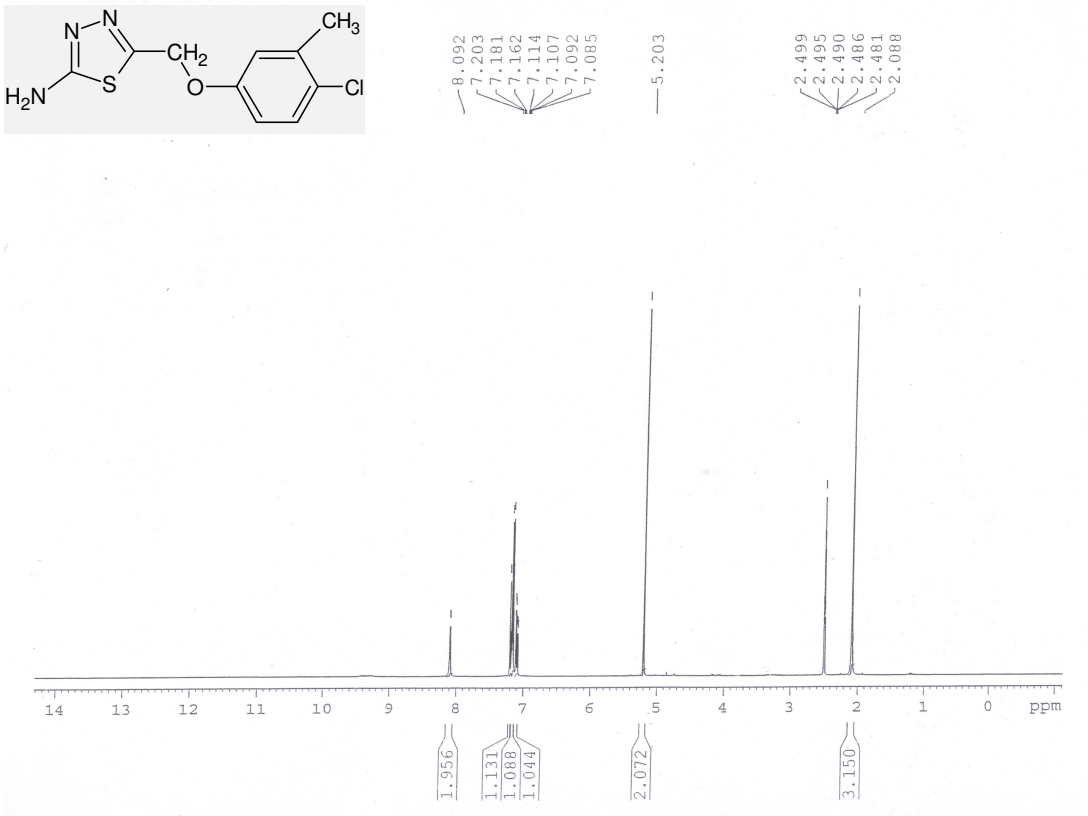


Figure 26. [1]H NMR spectrum of the synthesized compound **ATZ_{11}**

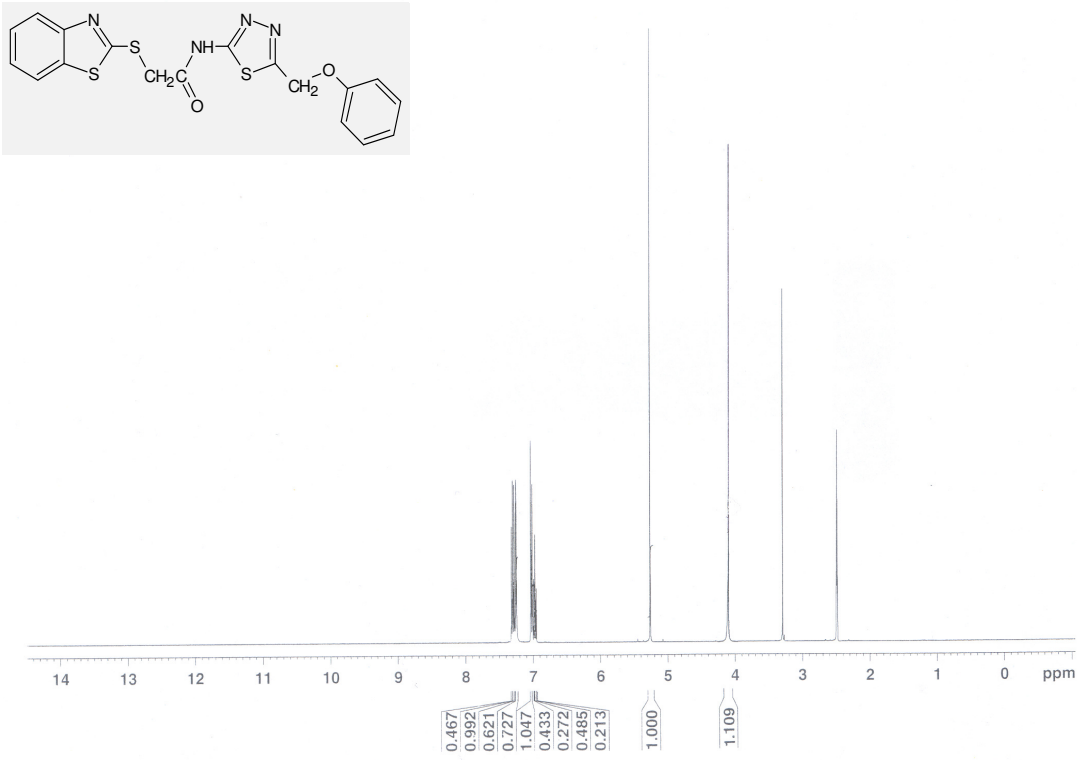


Figure 27a. [1]H NMR spectrum of the synthesized compound **TDZ_1**

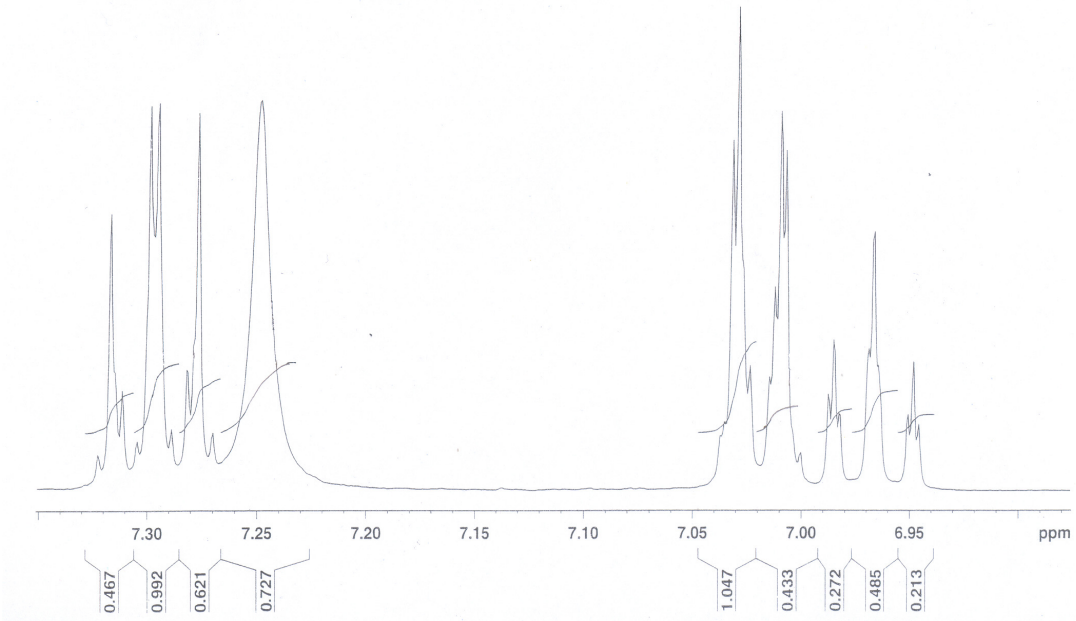


Figure 27b. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TDZ_1**

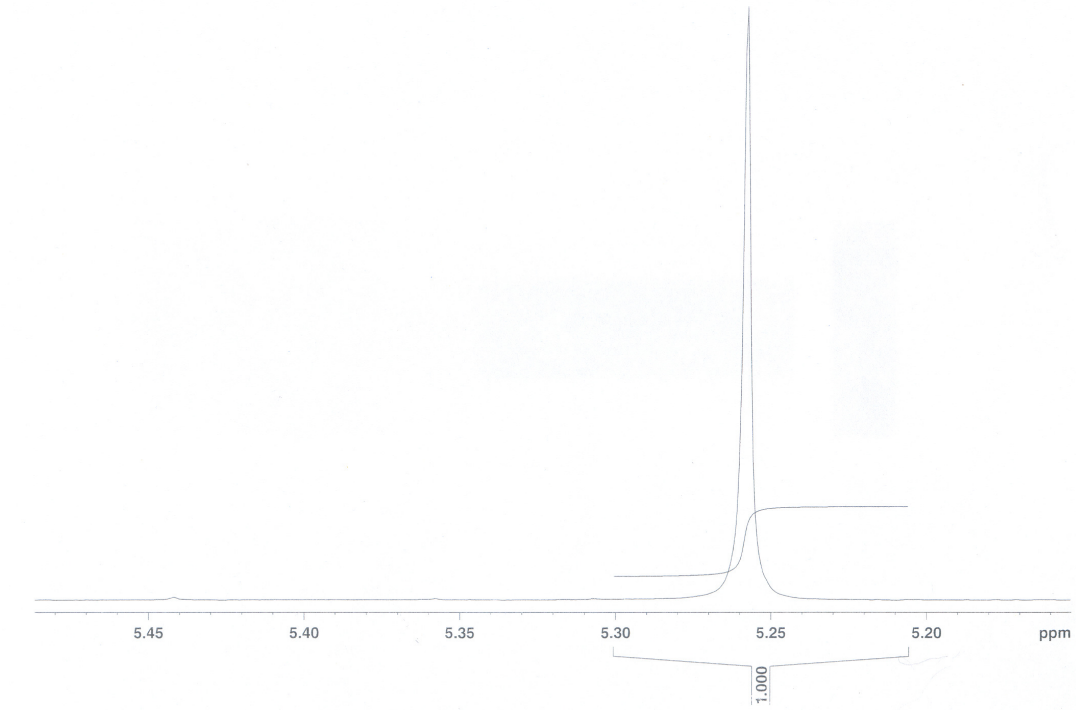


Figure 27c. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TDZ_1**

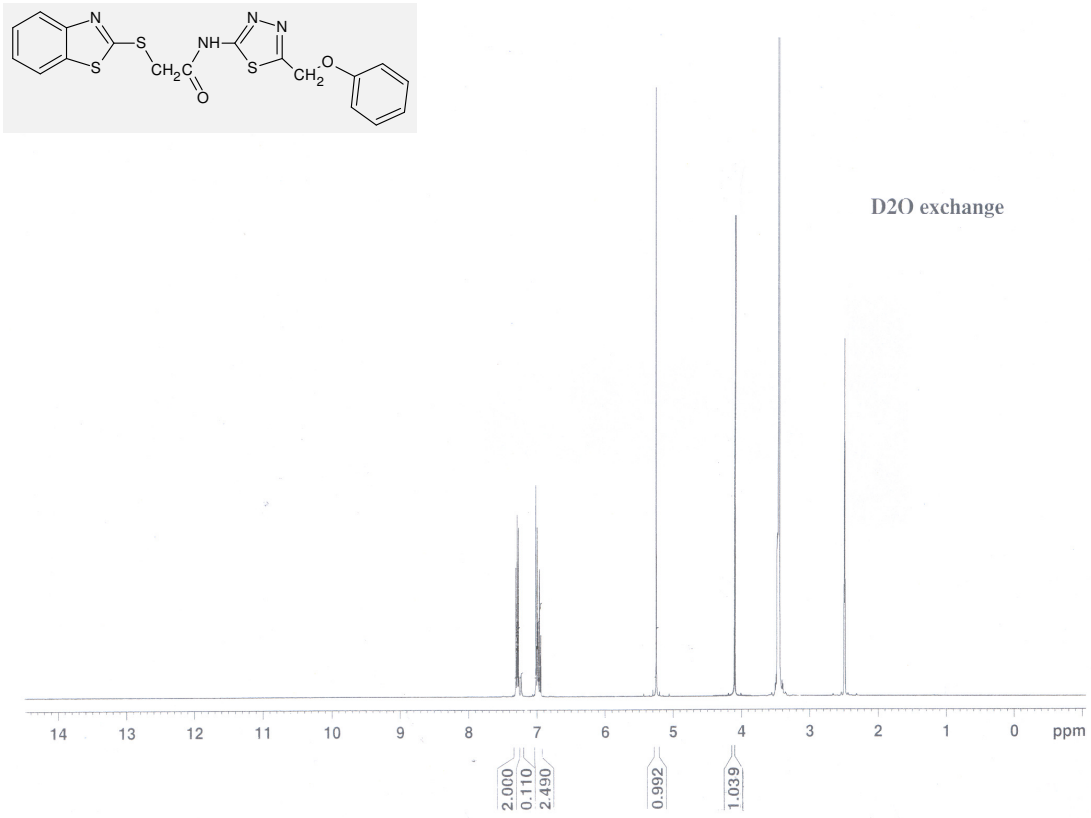


Figure 28a. ^{1}H NMR spectrum of the synthesized compound **TDZ$_1$** (D_2O exchange)

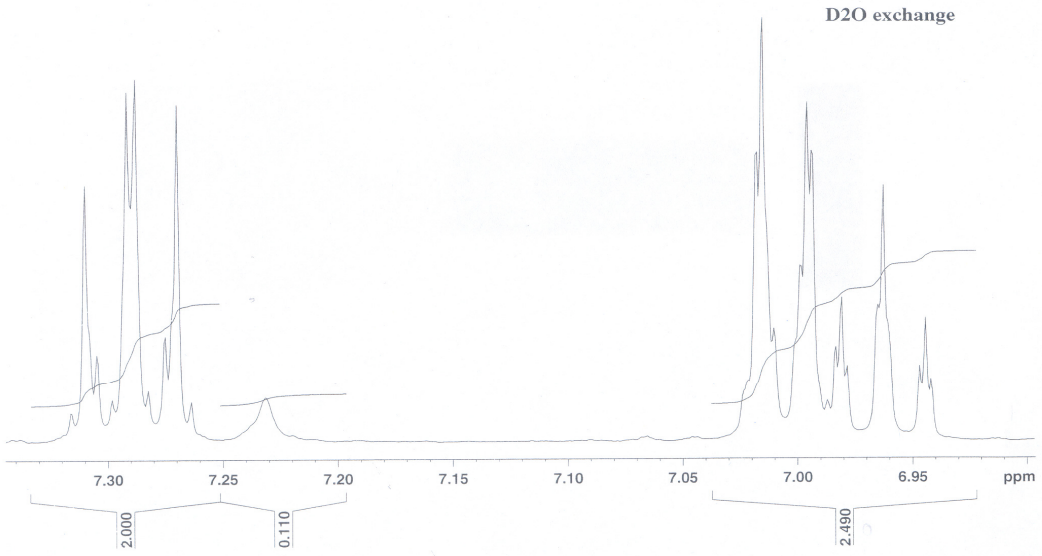


Figure 28b. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TDZ$_1$** (D_2O exchange)

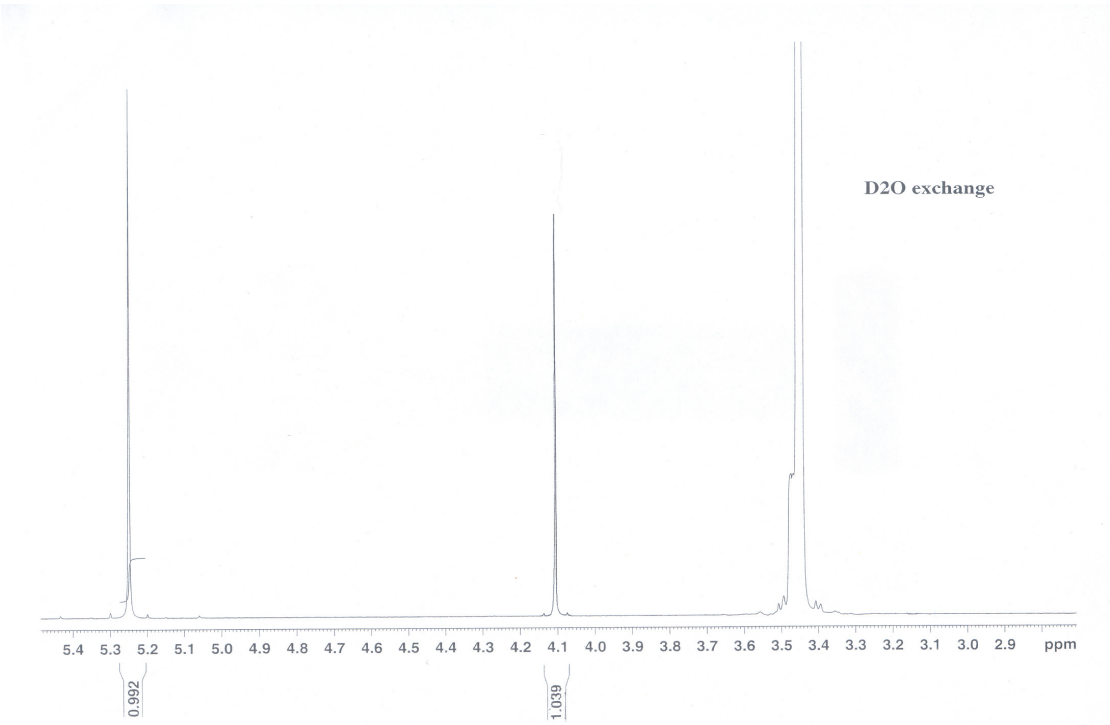


Figure 28c. Expansion of the ^{1}H NMR spectrum of the synthesized compound **TDZ_1** (D_2O exchange)

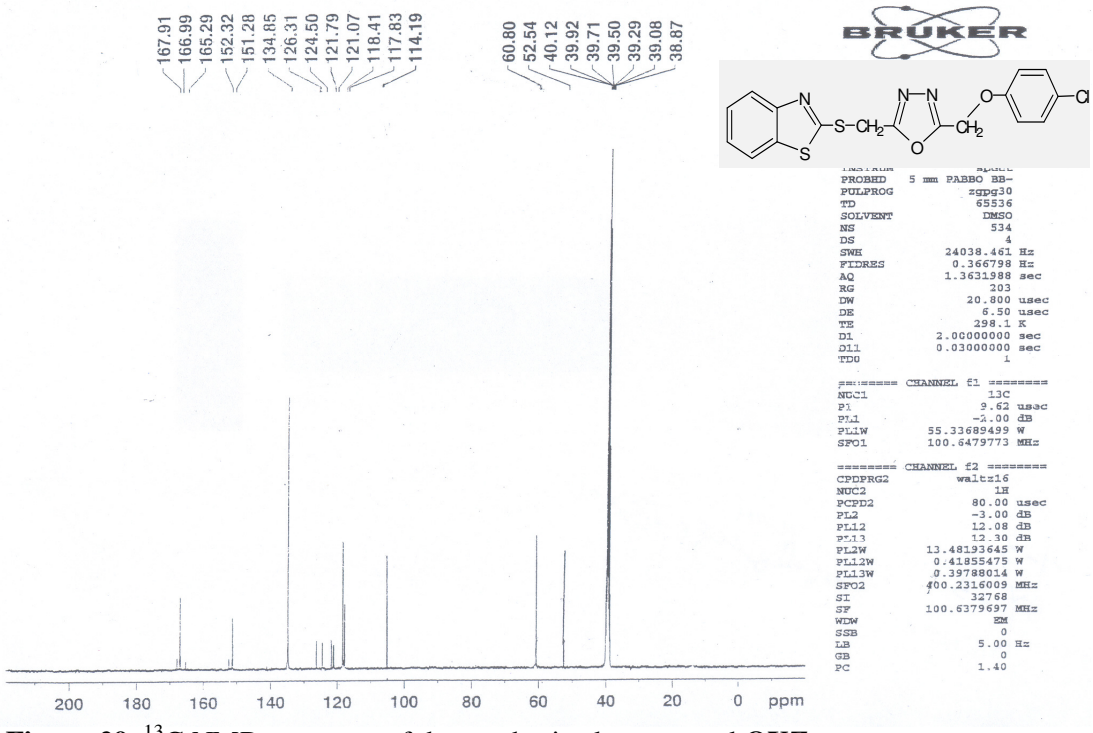


Figure 29. ^{13}C NMR spectrum of the synthesized compound **OXZ_3**

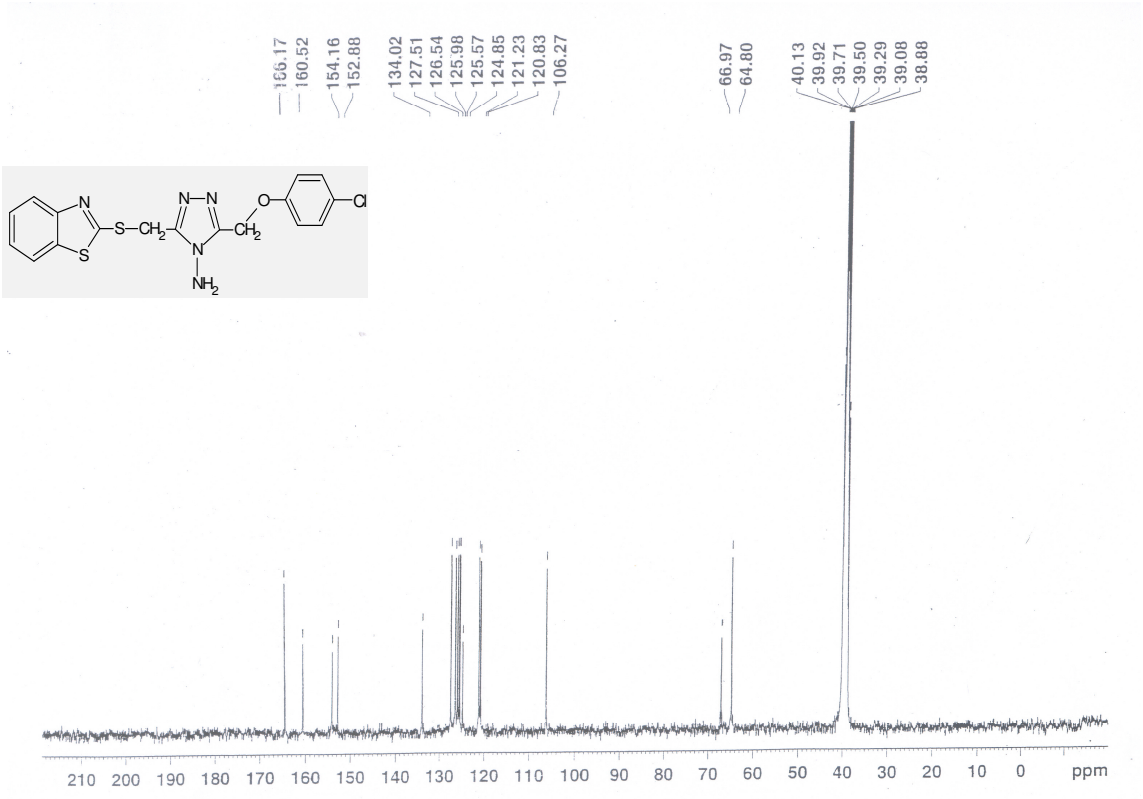


Figure 30. ^{13}C NMR spectrum of the synthesized compound **TRZ$_2$**

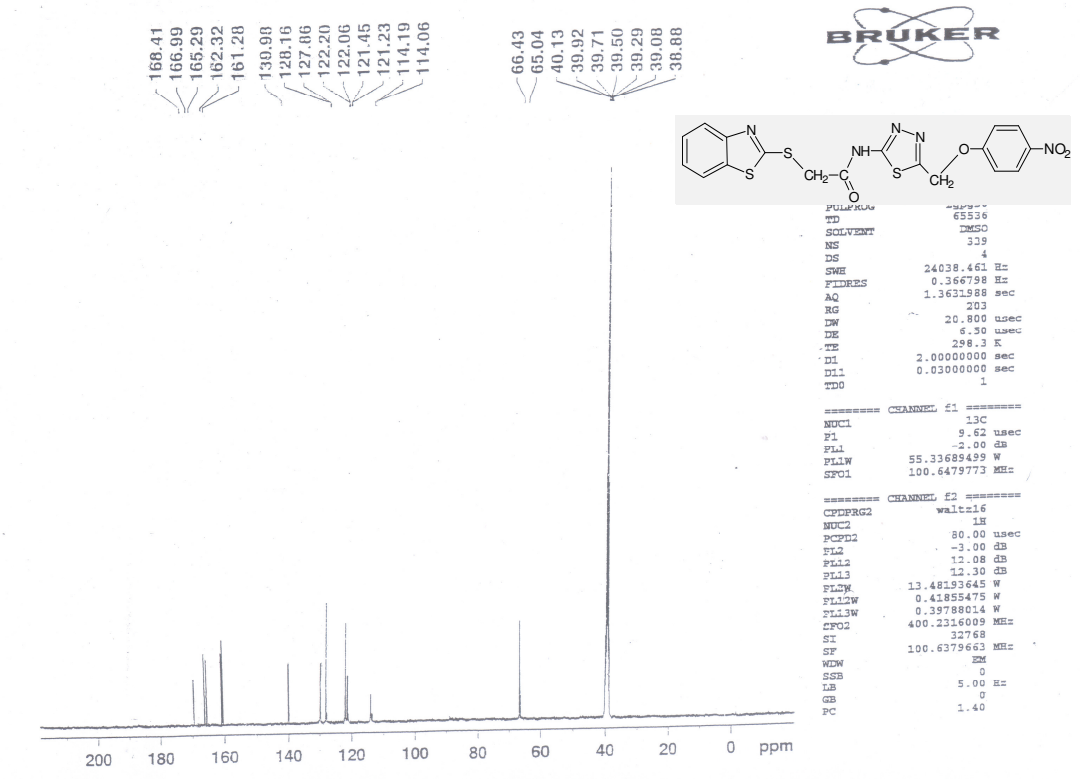


Figure 31. ^{13}C NMR spectrum of the synthesized compound **TDZ$_{10}$**

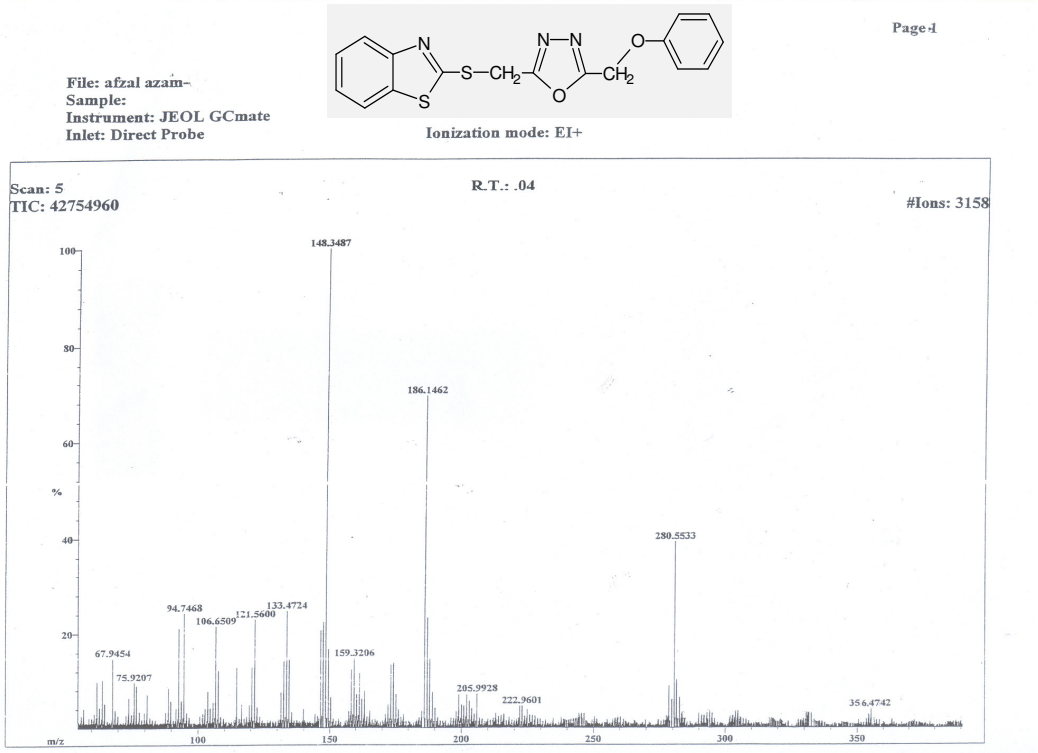


Figure 32. Mass spectrum of the synthesized compound **OXZ_1**

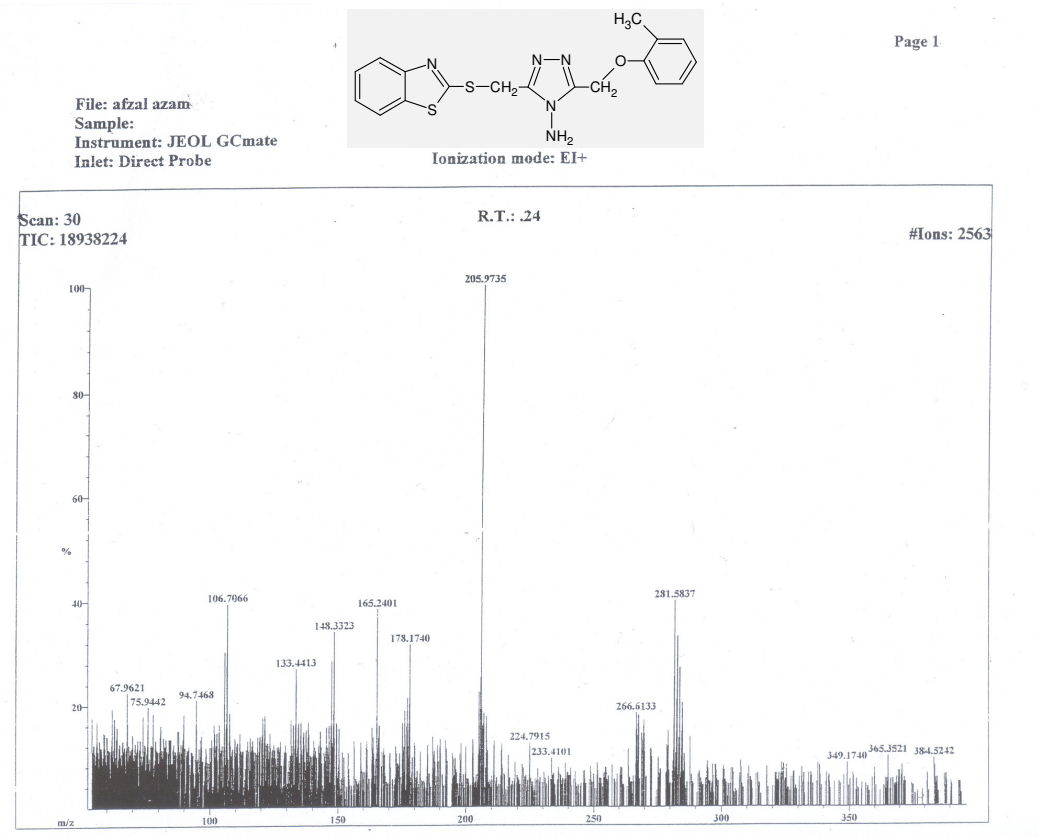


Figure 33. Mass spectrum of the synthesized compound **TRZ_3**

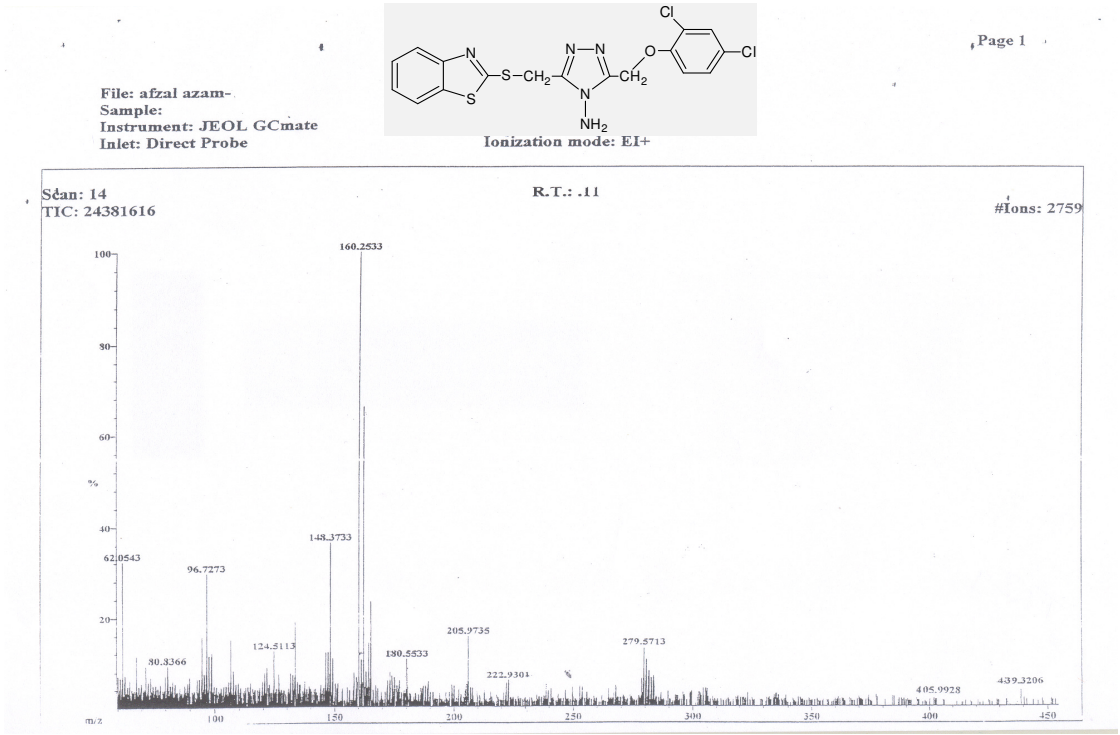


Figure 34. Mass spectrum of the synthesized compound **TRZ$_7$**

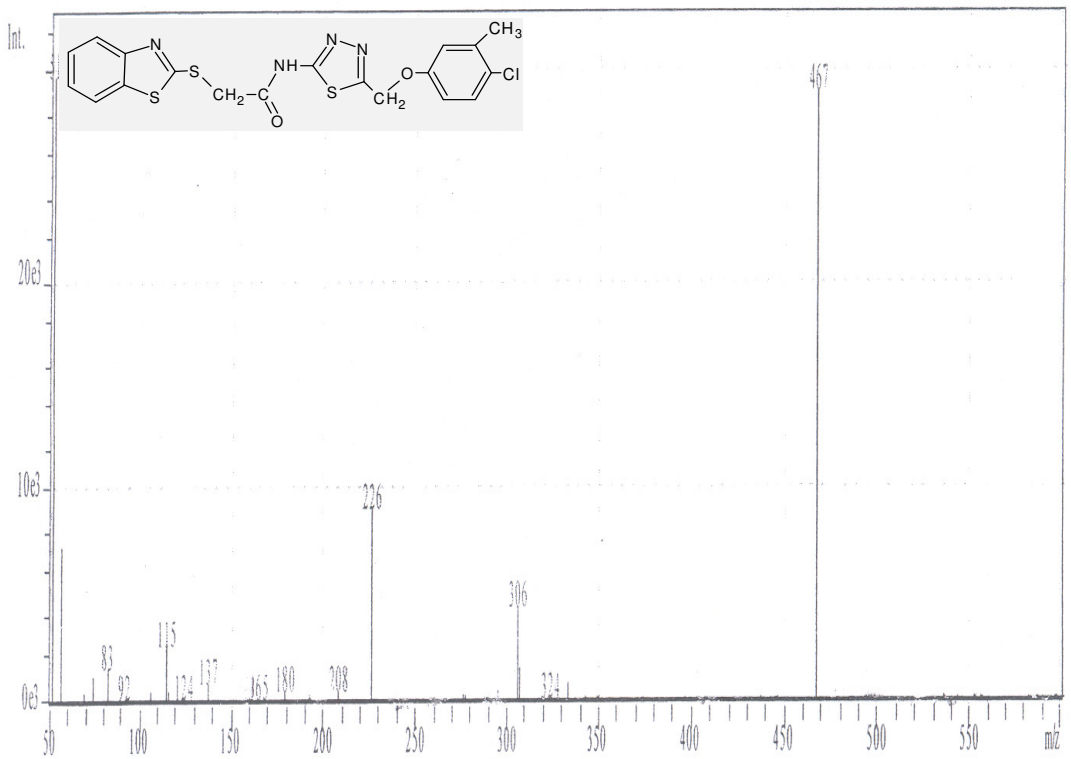
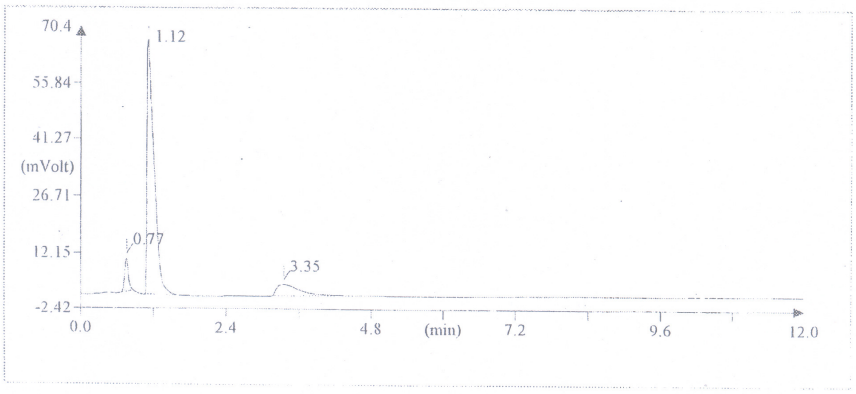


Figure 35. Electrospray ionization mass spectrum of the synthesized compound **TDZ$_{11}$**

FLASH EA 1112 SERIES CHNS REPORT
THERMO FINNIGAN

Company name:
Method filename: C:\Progam Files\Thermo Finnigan\Eager 300 for A1112\data\First Run\N C H S syster
Sample ID: J-12 (21) (# 239)
Analysis type: Unknown
Chromotogram filename: Azam-20032010-1.dat
Sample weight in mg: 1.339

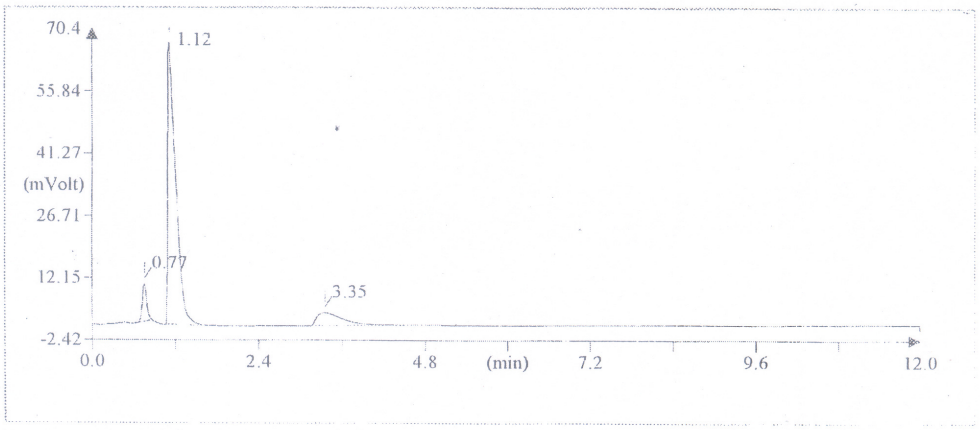


Peak Number (#)	Component Name	Elements %	Area (.1* uV* sec)
1	Nitogen	10.336	338196
2	Carbon	62.272	5479154
3	Hydrogen	3.670	721146
		76.278	6538496

Figure 36. CHN analysis spectrum of the synthesized compound **OXZ_7**

FLASH EA 1112 SERIES CHNS REPORT
THERMO FINNIGAN

Company name:
Method filename: C:\Progam Files\Thermo Finnigan\Eager 300 for A1112\data\First Run\N C H S syster
Sample ID: J-12 (21) (# 233)
Analysis type: Unknown
Chromotogram filename: Azam-20032010-1.dat
Sample weight in mg: 1.328



Peak Number (#)	Component Name	Elements %	Area (.1* uV* sec)
1	Nitogen	12.025	419070
2	Carbon	56.862	4861594
3	Hydrogen	3.411	656962
		72.298	5937626

Figure 37. CHN analysis spectrum of the synthesized compound **TDZ_7**

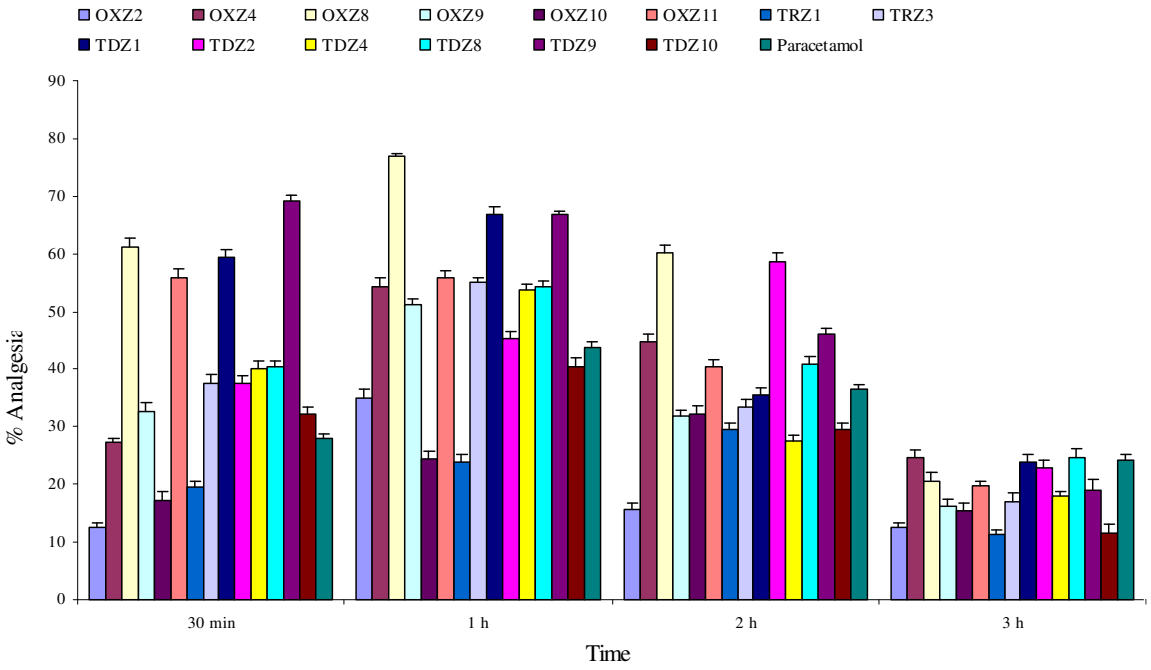


Figure 38. Analgesic activity of some selected 1,3,4-oxadiazole, 1,2,4-triazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles in mice by the tail immersion method

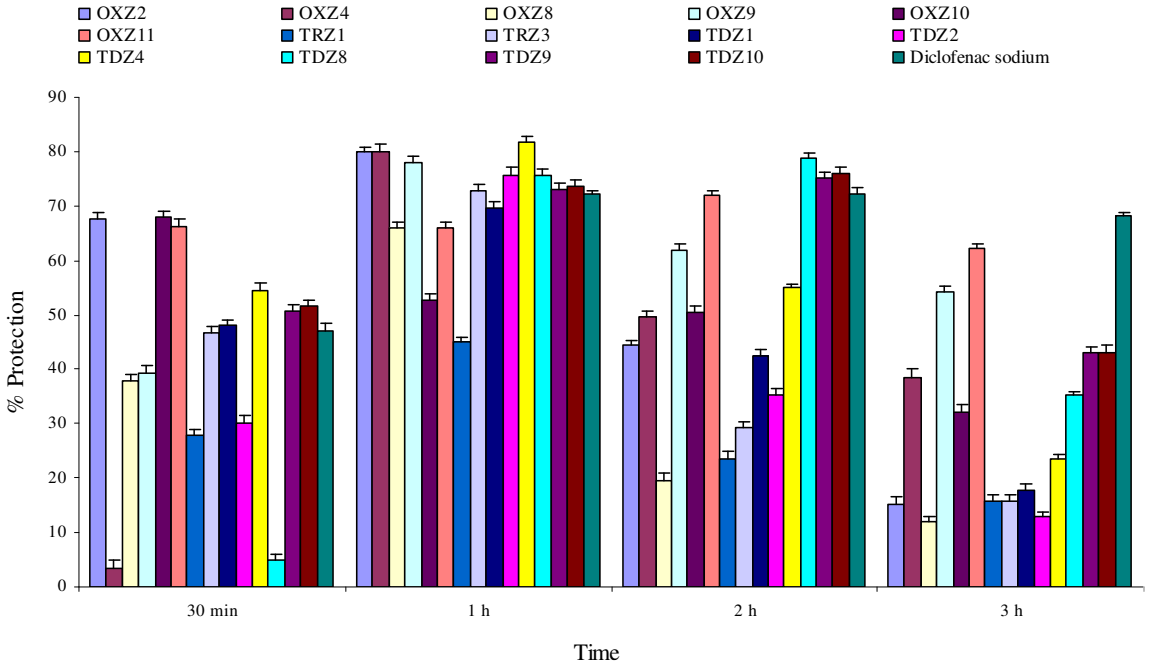
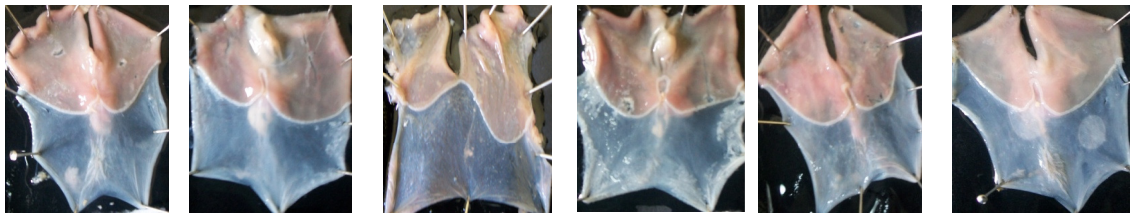
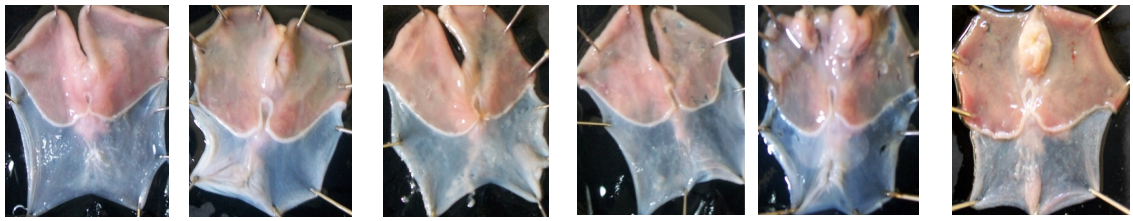


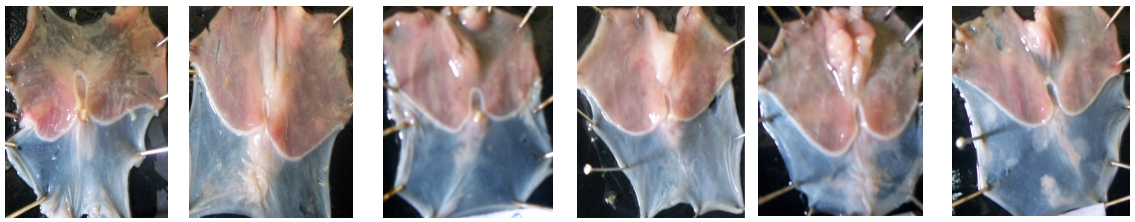
Figure 39. Anti-inflammatory activity of some selected 1,3,4-oxadiazole, 1,2,4-triazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles by the carrageenan induced rat paw oedema method



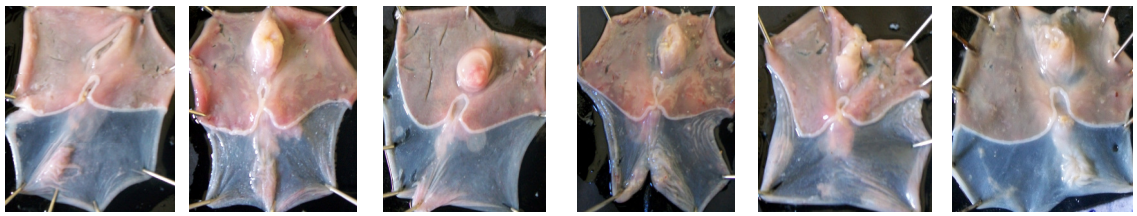
Control carboxymethyl cellulose



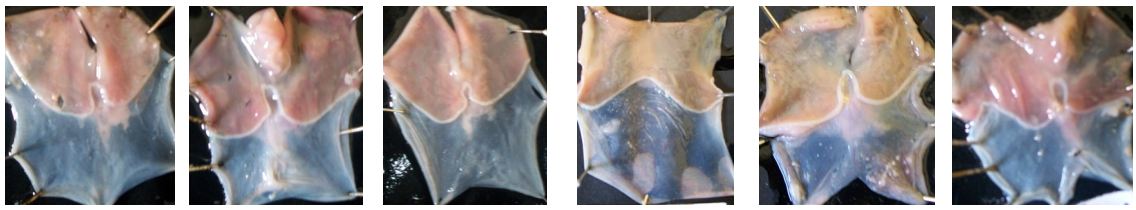
Compound **OXZ_4**



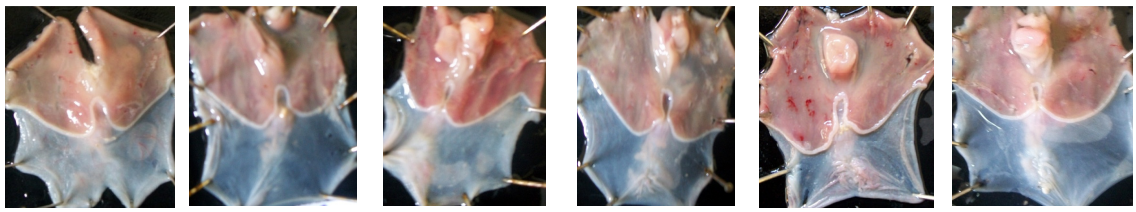
Compound **OXZ_9**



Compound **TDZ_4**

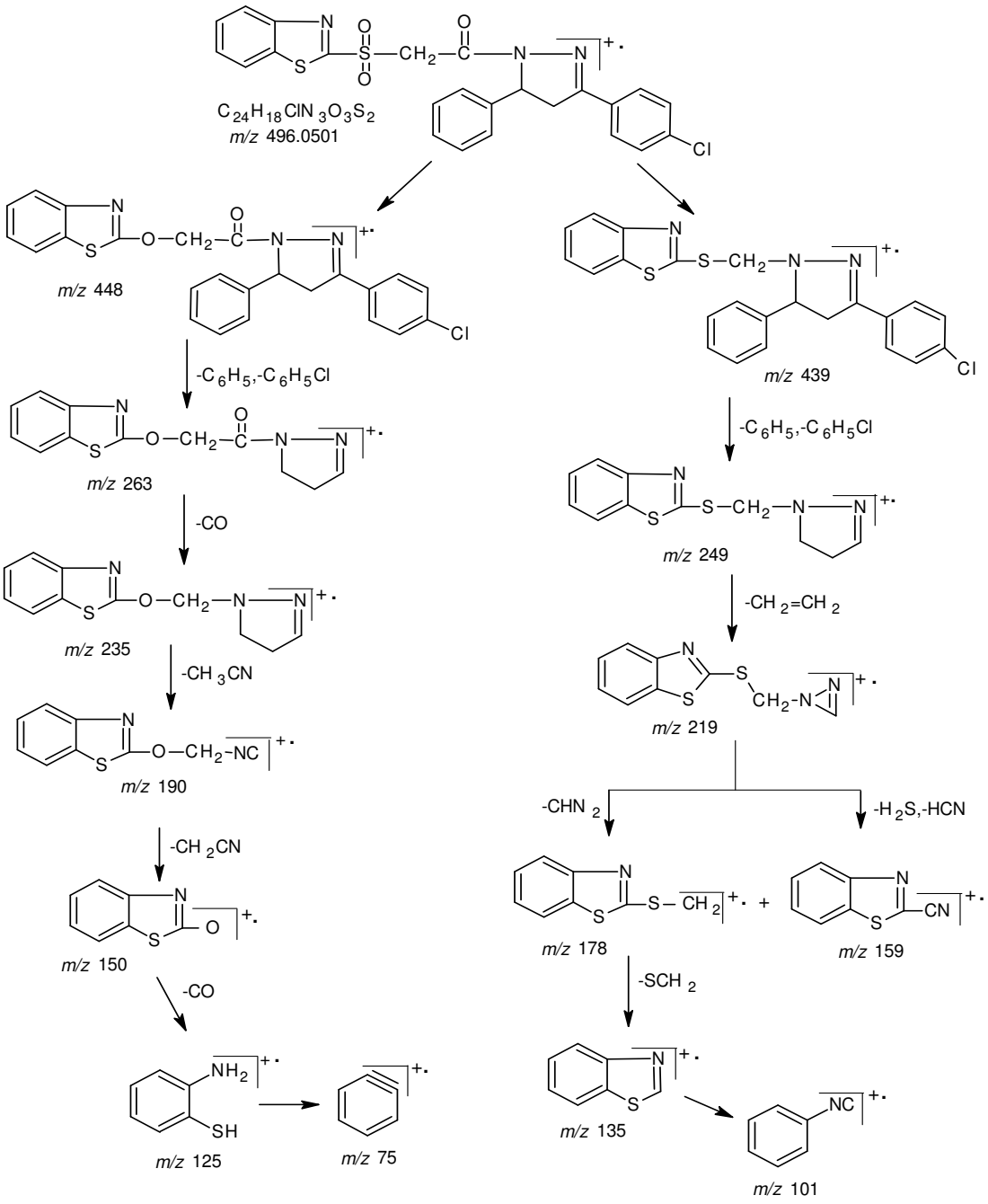


Compound **TDZ_9**



Diclofenac sodium

Figure 40. Ulcerogenic potential of some 1,3,4-oxadiazole and 1,3,4-thiadiazole incorporated 2-mercaptobenzothiazoles



Scheme 11. Possible mass fragmentation pattern of the synthesized compound **PYS 4**

Table 17. Antimicrobial screening of 2-pyrazoline incorporated 2-mercaptobenzothiazoles by the cup plate method

Compd.	Zone of inhibition (mm)[a,b]					
	S.a.	*B.s.*	*E. c.*	*P. a.*	*C.a.*	*A.n.*
PYZ_1	13	14	-	-	-	-
PYZ_2	26	24	14	15	-	-
PYZ_3	13	12	-	-	-	-
PYZ_4	21	23	33	27	-	-
PYZ_5	22	23	22	24	-	-
PYZ_6	27	22	21	26	-	-
PYZ_7	13	13	-	-	-	-
PYZ_8	23	24	25	30	-	-
PYZ_9	23	21	27	34	-	-
PYZ_{10}	22	24	29	33	-	-
PYZ_{11}	13	12	-	-	-	-
PYZ_{12}	14	12	-	-	-	-
PYZ_{13}	27	26	19	20	-	-
PYZ_{14}	24	23	28	35	-	-
PYZ_{15}	14	15	-	-	-	-
PYZ_{16}	12	14	-	-	-	-
PYZ_{17}	29	28	22	28	-	-
PYZ_{18}	23	22	13	14	-	-
PYZ_{19}	13	12	14	16	-	-
PYS_1	29	32	13	14	-	-
PYS_2	28	25	14	13	-	-
PYS_3	29	24	15	14	-	-
PYS_4	16	15	-	-	-	-
PYS_5	26	25	16	14	-	-
PYS_6	26	27	13	15	-	-
PYS_7	24	26	14	13	-	-
PYS_8	19	17	-	-	-	-
PYS_9	13	16	-	-		
DMSO	-	-	-	-	-	-
Ciprofloxacin	34	34	40	35	-	-
Ketoconazole	-	-	-	-	32	28

Test compounds, ciprofloxacin and ketoconazole were tested at 100, 10 and 20 µg mL^{-1} concentrations, respectively. [a]Average of three readings. [b]**-**indicates no activity. *S.a.: Staphylococcus aureus;B. s.: Bacillus subtilis; E.c: Escherichia coli*; *P.a.:Pseudomonas aeruginosa*; *C. a.: Candida albicans*; *A. n.: Aspergillus niger*.

Table 18. Analgesic activity of some selected 2-pyrazoline incorporated 2-mercaptobenzothiazoles in mice by the tail immersion method.

Compd.	Percent analgesic activity			
	30 min %Analgesia ±SEM	1 h %Analgesia±SEM	2 h %Analgesia ±SEM	3 h %Analgesia±SEM
PYZ_4	10.8±0.3[a]	40.9±0.3[b]	29.8±1.0[b]	17.9±0.6[a]
PYZ_5	13.5±0.3[a]	19.3±0.2[a]	56.6±0.7[b]	25.8±0.2[b]
PYZ_6	12.7±0.6[b]	19.4±0.5[b]	24.6±0.6[a]	12.5±0.9[b]
PYZ_8	14.3±0.8[c]	23.4±1.1[b]	34.6±0.4[a]	12.5±0.4[b]
PYZ_9	37.4±0.4[a]	40.2±1.2[c]	69.8±1.3[c]	55.9±1.1[b]
PYZ_{10}	19.2±0.8[c]	25.8±0.7[b]	66.2±0.4[a]	58.0±0.9[b]
PYZ_{13}	19.6±0.5[b]	29.4±0.8[b]	32.6±0.7[a]	15.4±0.9[b]
PYZ_{14}	47.5±0.6[c]	58.6±0.9[b]	57.2±0.5[b]	57.9±0.4[a]
PYZ_{17}	15.1±0.7[c]	17.4±0.3[a]	20.3±0.8[c]	8.3±0.6[c]
PYS_1	17.9±0.9[b]	22.2±0.6[c]	26.1±0.8[b]	10.3±0.5[c]
PYS_2	13.6±0.5[b]	23.1±0.4[c]	26.1±0.8[c]	13.2±0.3[a]
PYS_3	15.4±0.8[c]	27.4±0.3[b]	30.6±0.4[a]	10.5±0.5[b]
Paracetamol	29.2±0.6[c]	44.5±0.7[b]	39.9±0.9[b]	26.1±1.1[c]

Test compounds and paracetamol were tested at 100 and 50 mg kg^{-1}, respectively. Results are expressed in mean ± SEM (n=6); Significance levels [a]$P<0.05$, [b]$P<0.01$ and [c]$P<0.001$ compared with the respective control.

Table 19. Anti-inflammatory activity of some selected 2-pyrazoline incorporated 2-mercaptobenzothiazoles by the carrageenan induced rat paw oedema method.

Compd.	Percent protection			
	30 min % Pretection (Mean % protection ±SEM)	1 h %Protection (Mean % protection ±SEM)	2 h %Protection (Mean % protection ±SEM)	3 h %Protection (Mean % protection ±SEM)
PYZ_4	20.5 ± 1.2^b	31.9 ± 0.6^c	49.1 ± 0.5^a	35.7 ± 0.7^b
PYZ_5	35.3 ± 0.8^b	52.5 ± 0.9^b	58.4 ± 0.7^c	53.6 ± 1.1^c
PYZ_6	28.1 ± 0.9^c	58.0 ± 0.6^b	48.0 ± 0.5^b	26.1 ± 0.6^c
PYZ_8	35.0 ± 0.4^b	70.4 ± 0.7^a	75.4 ± 0.5^b	61.2 ± 0.2^a
PYZ_9	43.4 ± 0.6^a	68.3 ± 0.5^c	73.7 ± 0.4^b	65.0 ± 0.6^c
PYZ_{10}	36.8 ± 0.4^b	64.4 ± 0.3^b	72.0 ± 0.7^b	58.9 ± 0.4^b
PYZ_{13}	23.4 ± 0.4^c	45.3 ± 0.8^b	48.7 ± 0.7^b	22.3 ± 1.1^c
PYZ_{14}	46.7 ± 0.7^a	69.9 ± 0.5^c	76.9 ± 0.9^b	69.7 ± 0.6^b
PYZ_{17}	38.5 ± 0.6^b	59.3 ± 1.2^c	61.2 ± 0.5^a	41.2 ± 0.6^c
PYS_1	15.8 ± 0.6^c	36.8 ± 0.6^b	23.5 ± 1.1^a	11.4 ± 0.8^b
PYS_2	15.9 ± 0.8^b	29.7 ± 0.7^b	24.6 ± 0.3^a	9.3 ± 0.7^b
PYS_3	12.6 ± 0.8^b	26.8 ± 0.6^b	20.1 ± 0.4^a	8.8 ± 0.6^b
Diclofenac sodium	46.8 ± 1.2^c	72.3 ± 0.8^b	72.6 ± 1.1^b	67.8 ± 0.8^b

Test compounds and diclofenac sodium were tested at 100 and 20 mg kg^{-1} body weight, respectively; results are expressed in mean ± SEM (n=6); Significance levels $^aP<0.05$, $^bP<0.01$ and $^cP<0.001$ compared with the respective control.

Table 20. Ulcerogenic effects of some selected 2-pyrazoline incorporated 2-mercaptobenzothiazoles by the Cioli *et al.*'s method.

Compd.	Control 1% CMC	**PYZ_9**	**PYZ_{10}**	**PYZ_{14}**
Severity Index	0.00 ± 0.2^a	3.2 ± 0.7^c	4.0 ± 0.8^b	2.0 ± 0.3^b

Test compounds and diclofenac sodium were tested at 200 and 20 mg kg^{-1} body weight, respectively. Results are expressed in mean ± SEM (n=6); Significance levels $^aP<0.05$, $^bP<0.01$ and $^cP<0.001$ compared with the respective control.

CONCLUSION

Advances in molecular analyses of the information relay pathways for their constituents and principal ligands along with mechanisms utilized by the host for microbial recognition have stimulated interest in therapeutic agents with dual functionalities i.e. antimicrobial and anti-inflammatory effects. Clinical studies demonstrated therapeutic benefits of agents with dual functionality with their effects on microorganisms and the concomitant host inflammatory response and it will facilitate progress in this emerging area poised to be a significant milestone for therapeutics.

In the present study seven series of novel 2-mercaptobenzothiazole derivatives bearing 1,3,4-oxadiazole (**ODZ_{1-15}** and **OXZ_{1-13}**), acetohydrazide (**ACH_{1-5}**), 1,3,4-triazole (**TRZ_{1-13}**), 1,3,4-thiadiazole (**TDZ_{1-13}**), and 2-pyrazoline (**PYZ_{1-19}** and **PYS_{1-9}**) moieties at the second position were synthesized with the objective of developing dual acting antimicrobial-analgesic-anti-inflammatory agents with gastrosparing activity. Synthesized compounds were evaluated for their *in vitro* antimicrobial activity by the cup plate method. It was interesting to note that compounds **ODZ_4**, **ODZ_{10}** and **ODZ_{13}** with acetamide group as linker between 2-mercaptobenzothiazole and 1,3,4-oxadiazole moieties showed significant inhibitory activity (inhibition zone 28-32 mm) against the tested Gram-positive and Gram-negative bacterial strains. Whereas compounds **TDZ_9** and **TDZ_{10}** with acetamide group as linker between 2-mercaptobenzothiazole and 1,3,4-thiadiazole moieties showed specific inhibitory activity against the tested Gram-negative bacteria *Pseudomonas aeruginosa* (inhibition zone 26 and 24 mm, respectively). In general a significant decrease or loss in inhibitory activity against *Pseudomonas aeruginosa* was observed when methylene group was introduced as a linker between 2-mercaptobenzothiazole and 1,3,4-oxadiazole/triazole moieties (**OXZ_{1-13}** and **TRZ_{1-18}**). On the other hand 2-pyrazoline incorporated derivatives **PYZ_9**, **PYZ_{10}** and **PYZ_{14}** belonging to the sulfanyl series with acetyl group as linker between the 2-mercaptobenzothiazole ring and 2-pyrazoline ring showed significant inhibitory activity against the tested Gram-negative bacterial strains *Escherichia coli* (inhibition zone 27-29 mm) and *Pseudomonas aeruginosa* (inhibition zone 33-35 mm). Meanwhile they showed moderate inhibitory activity against the tested Gram-positive bacterial strains *Staphylococcus aureus* (inhibition zone 22-24 mm) and *Bacillus subtilis* (inhibition zone 21-24 mm). It was also observed that conversion of the sulfanyl

function of derivatives **PYZ_2**, **PYS_4**, **PYZ_{6-11}** and **PYZ_{14}** into sulfone (**PYS_{1-9}**) provided a remarkable increase in their activity against the tested Gram-positive bacteria. However, activity against the tested Gram-negative microorganism markedly decreased. From above results it is evident that antibacterial activity is dependent on both the nature of substituents group and basic skeleton of the molecules. In the present investigation synthesized compounds showed no activity against the tested *Candida albicans* and *Aspergillus niger*.

From the collective analgesic activity results of the tested compound, **ODZ_{10}** belonging to the 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethy)-1,3,4-oxadiazol-2-yl]acetamide series showed potent activity at second and third hour (70.7 and 76.5% analgesia, respectively). Whereas compounds **OXZ_8** and **TDZ_9** exhibited significant analgesic activity (46.0-76.8%) upto 2 h compared to the standard drug paracetamol (28.0-43.8% at a dose of 50 mg kg^{-1}). On the other hand, 2-pyrazoline incorporated 2-mercaptobenzothiazoles **PYZ_9**, **PYZ_{10}** and **PYZ_{14}** exhibited potent analgesic activity (55.9 to 69.8%) at second and third hour after oral administration. In general a sharp decline in analgesic activity was observed at 3 h following oral administration except for derivatives **ODZ_4**, **ODZ_6**, **ODZ_{10}**, **ODZ_{13}**, **PYZ_9**, **PYZ_{10}** and **PYZ_{14}** (55.9-76.5% analgesia). From above results it is evident that analgesic activity is dependent on both the nature of substituents group and basic skeleton of the molecules.

Collective anti-inflammatory activity results indicated that at 2 and 3 h compound **ODZ_{10}** was nearly effective in inhibiting the paw oedema (68.2 and 64.6% protection, respectively), when compared with the reference drug (69.3 and 66.2% protection, respectively at 20 mg kg^{-1}). In case of 2-{(benzo[d]thiazol-2-ylthio)methyl}-5-(aryloxymethyl)-1,3,4-oxadiazole/triazole and 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl]acetamide series compounds **OXZ_2**, **OXZ_4**, **OXZ_9**, **TRZ_3**, **TDZ_2**, **TDZ_4**, **TDZ_8**, **TDZ_9** and **TDZ_{10}** were effective in inhibiting the paw oedema (72.8-81.6%), when compared with the reference drug diclofenac sodium (72.1% at a dose of 20 mg kg^{-1}) at 1h after carrageenan injection and in this regard highest activity (81.6%) was found in derivative **TDZ_4** having 2-methylphenyloxymethyl group at fifth position of the thiadiazole ring. At second and third hour compounds **PYZ_8**, **PYZ_9**, **PYZ_{10}** and **PYZ_{14}** belonging to the 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-disubstituted-4,5-dihydro-1H-pyrazol-1-yl) ethanone series showed significant protection (58.9-76.9%) against carrageenan induced paw oedema in rat and the highest activity (76.9%) was observed at second hour in derivative **PYZ_{14}**. On the other hand, sulfonyl analogs (**PYS_{1-3}**) showed weak anti-inflammatory activity at all time interval and this could be attributed to the conversion of the sulfanyl group into sulfone.

The synthesized compounds **ODZ_{10}**, **TDZ_9** and **PYZ_{14}** exhibited promising *in vitro* antibacterial activity; significant *in vivo* analgesic and anti-inflammatory activities and minimal ulcerogenic effects as compared to the reference drug. Hence it is concluded from the present study that analogs **ODZ_{10}**, **TDZ_9** and **PYZ_{14}** can be considered as one of the scaffolds for design and development of dual acting antimicrobial and analgesic-anti-inflammatory agents. This requires further derivatization and investigation to improve the antimicrobial, analgesic-anti-inflammatory and ulcerogenic profiles.

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LIST OF PUBLICATIONS

1. Md. Afzal Azam, Suresh B, Kalsi SS, Antony AS. Synthesis and Biological Evaluation of some Novel 2-Mercaptobenzothiazoles Carrying 1,3,4-Oxadiazole, 1,3,4-Thiadiazole and 1,2,4-Triazole Moieties. S Afr J Chem 2010;63:114-122.
2. Md. Afzal Azam, Suresh B, Thomas A. Synthesis and Biological Evaluation of some 1,3,4-Oxadiazole Incorporated 2-Mercaptobenzothiazoles. Indian J Heterocycl Chem 2010;20:77-80.
3. Md. Afzal Azam, B. Suresh. Synthesis and Biological Evaluation of some Acetohydrazide Incorporate 2-Mercaptobenzothiazoles. Indian Drugs 2011;48(03):9-15.

LIST OF PRESENTATIONS

1. "Synthesis and Biological Evaluation of some Novel 2-Mercaptobenzothiazoles Carrying 1,3,4-Oxadiazole, 1,2,4-Triazole and 1,3,4-Thiadiazole Moieties". Presented at Scientific Poster Session of 62nd Indian Pharmaceutical Congress 2010, held at Manipal University, Manipal (Karnataka) during 17-19 December 2010.
2. "Synthesis and Biological Evaluation of some 1,3,4-Oxadiazole Incorporated 2-Mercaptobenzothiazoles." Presented at Scientific Poster Session of CSIR and ICMR Sponsored National Conference on Evolving Paradigms in Molecular Pharmacology and Drug Design, Organised by the Ultra College of Pharmacy, Madurai (Tamil Nadu). 17-18 March 2011.
3. "Synthesis and Biological Evaluation of some Acetohydrazide Incorporated 2-Mercaptobenzothiazoles". Presented at Scientific Poster Session of Indian Pharmaceutical Association Sponsored National Seminar on Emerging Trends and Career Opportunities in Pharmacy Organized by the Seven Hills College of Pharmacy and Indian Pharmaceutical Association, Tirupati Branch, Tirupati (Andhra Pradesh), 28 March 2011.
4. "Synthesis and Biological Evaluation of some Novel 2-Pyrazoline Incorporated 2-Mercaptobenzothiazoles". Presented at Scientific Poster Session of International Indo-US Symposium on Frontiers in Medicinal Chemistry and Drug Discovery, April 21-23, 2011; Organised by the JSS University Mysore and Indian Institute of Chemical Technology, Hyderabad; Supported by the Indo-US Science and Technology Forum, American Chemical Society.

Annexure I

J.S.S. College of Pharmacy, Ootacamund, Tamil Nadu, India.
Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics committee (IAEC).

CERTIFICATE

Title of the Project: Synthesis and Biological Evaluation of some newer 2-mercaptobenzothiazole derivatives.

Proposal Number: JSSCP/IAEC/PH.D/PH.CHEM/04/2009-10

Date received after modification (if any):

Date received after second modification: 30.08.09

Approval date: 30.09.09.

Animals:
Wistar Rats: ✓
Albino mice: ✓
Rabbits:
Guinea pigs

No. of animals sanctioned: 36+36 Male/Female ✓ ✓

Expiry date (Termination of the Project): ONE MONTH.

Name of IAEC/CPCSEA chairperson: **Prof. K. Elango**

Signature of Chairperson

Date: 30.09.09.

(Prof. K. Elango)
Chairperson
Institutional Animal Ethics Committee
JSS College of Pharmacy
Rocklands, Ooty-643 001

Annexure II

J.S.S. College of Pharmacy, Ootacamund, Tamil Nadu, India.
Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics committee (IAEC)

CERTIFICATE

Title of the Project: **Synthesis and biological evaluation of some 2- mercaptobenzothiazole derivatives**

Proposal Number: **JSSCP/IAEC/Ph.D/Ph.Chem/05/2009-10**

Date received after modification (if any): 16.09.2009

Date received after second modification:

Approval date: **30.09.2009**

Animals: **Wistar rats/ Albino mice**

No. of animals sanctioned: **Wistar rats** **102 both sex**
Swiss albino mice **102 both sex**

Expiry date (Termination of the Project): 15.03.2010

Name of IAEC chairperson: **Dr.K.Elango**

Signature of chairperson

Date: 30.09.2009

Chairperson
Institutional Animal Ethics Committee
JSS College of Pharmacy
Rocklands, Ooty-643 001

Annexure III

J.S.S. College of Pharmacy, Ootacamund, Tamil Nadu, India.
Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics committee (IAEC).

CERTIFICATE

Title of the Project: Synthesis and Biological Evaluation of some Newer 2-Mercaptobenzothiazole Derivatives.

Proposal Number: JSSCP/IAEC/PH.D/PH.CHEM/04/2009-10

Date received after modification (if any):

Date received after second modification: 20.09.09.

Approval date: 30.09.09.

Animals:

Wistar Rats: ✓

Albino mice: ✓

Rabbits:

Guinea pigs

No. of animals sanctioned: 72 + 72. ✓/✓ Male/Female

Expiry date (Termination of the Project): ONE MONTH.

Name of IAEC/CPCSEA chairperson: **Prof. K. Elango**

Signature of Chairperson

Date: 30.09.09.

(Prof. K. Elango)
Chairperson
Institutional Animal Ethics Committee
JSS College of Pharmacy
Rocklands, Ooty-643 001