"SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SUBSTITUTED (BENZOTHIAZOL-2-YL)-3-PHENYLTHIOUREA DERIVATIVES"

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BY

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UNDER THE GUIDANCE OF

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CERTIFICATE

This is to certify that ***Mr. NIKUNJ M. SUVAGIYA*** *has prepared his thesis entitled "Synthesis and Pharmacological evaluation of Substituted (Benzothiazole-2-yl)-3-phenylthiourea Derivatives" in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. He has carried out the work at the Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University.*

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DECLARATION

I declare that the thesis "Synthesis and Pharmacological evaluation of Substituted (Benzothiazole-2-yl)-3-phenylthiourea Derivatives" has been prepared by me under the guidance of Dr. Anuradha K. Gajjar, Professor, and Mrs. Jignasa K. Savjani, Assistant Professor, Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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.

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A B S T R A C T

A series of some novel (Benzothiazole-2-yl)-3-phenylthiourea Derivatives were synthesized and evaluated for anti-inflammatory activity. The titled compounds were synthesized from the substituted aromatic amines through the intermediate substituted 1-phenylthiourea oxidation by bromine water in acidic medium then reacted with Phenylisothiocyanate. The purity of the synthesized compounds were confirm by TLC and the structure was analyzed on the basis of IR and Mass spectral data. The anti-inflammatory activities of new compounds were determined by Carrageenan-induced rat paw edema method using diclofenac sodium as a standard. Among the compounds tested three compounds NP-x_2(1-(6-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea),NP-x_3(1-(5-methoxy-1,3-benzothiazol-2-yl)-3 phenylthiourea),NP-x_4(1-(6-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea) were the most active compounds in these series when compared with diclofenac sodium. In the SAR study, Methyl and Methoxy groups substituted 1-(benzthiazol-2-yl)-3-phenylthiourea more anti-inflammatory activity than halogen substituted 1-(benzthiazol-2-yl)-3-phenylthiourea derivative

1. **Introduction**

Pain is a fundamental and life experience, a counterbalance to pleasure, a warning of danger and a reminder to guard injured limbs and tissues while they heal. And yet, however beneficial pain may be to our physiological well-being, it is also the most acutely unpleasant of sensation and one of the primary reasons patients seek medical care. According to the definition of ISAP (International association for the study of pain), pain is "An unpleasant sensory and emotional experience with actual or potential tissue damage, or described in terms of such damages.

Over the past 20 years, the expansion of molecular and cellular biology knowledge has led to the creation of tools which have allowed scientist to develop a much more detail understanding of what causes pain and how it is transmitted, processed and perceived individual, including our haemostatics control mechanism. Most important, it has also helped to identify a large number of potential targets for drug discovery.

1.1. Pain types and distinguishing characteristics

Pain can be classified into three categories as follows

- **Nociceptive pain**
- **Neuropathic pain**
- **Inflammatory pain**

1.1.1 Nociceptive pain

Nociceptive pain may be somatic or visceral. Somatic pain receptors are located in skin, subcutaneous tissues, fascia, other connective tissues, periosteum, endosteum, and joint capsules. Stimulation of these receptors usually produces sharp or dull localized pain, but burning is not uncommon if the skin or subcutaneous tissues are involved. Visceral pain receptors are located in most viscera and the surrounding connective tissue. Visceral pain due to obstruction of a hollow organ is poorly localized, deep, and cramping and may be referred to remote cutaneous sites. Visceral pain due to injury of organ capsules or other deep connective tissues may be more localized and sharp.

Normal pain stimulus or nociceptive pain, resulting from tissue damage typically responds well to treatment with analgesic and trends to subside readily when noxious stimuli are removed or tissue damage is healed. In contrast neuropathic pain is characterized by a spontaneous hypersensitive pain response and can typically persist long after the original nerve injury is healed. This unusually heightened pain response could be observed as hyperalgesia (an increased sensitivity to noxious pain stimulus) or allodynia (an abnormal pain response to non –noxious stimulus, e.g. cold, warmth, touch).while noceceptive pain is typically acute in nature and diminishes upon healing, patients suffering from neuropathic pain is typically endure chronic, depilating episodes that are refractory to the current pharmacotherapies and profoundly affect their quality of life.

1.1.2 Neuropathic pain

Neuropathic pain may occur when there is either damage to or dysfunction of nerves in the peripheral or central nervous system. Faulty signals are sent to the brain and experienced as pain. Neuropathic pain can be either peripheral (outside the central nervous system) or central in origin. Examples of neuropathic pain include diabetic neuropathy, trigeminal neuralgia, post herpetic zoster pain (peripheral pains), and the thalamic pain syndrome (a central pain). Neuropathic pain frequently coexists with nociceptive pain. Examples include trauma that damages tissue and nerves, burns (that burn skin as well as nerve endings), and external nerve compression. Examples of the latter include tumor nerve compression and sciatica from herniated discs pressing on nerves.

1.1.3 Inflammatory pain

Inflammatory pain is the result when immune system releases white-blood cells and chemicals to fight foreign matter such as viruses and bacteria. Inflammation brings with it heat, redness, swelling and pain. It may also include fatigue, fever and chills, loss of appetite and muscle aches. Sometimes inflammation can result from autoimmune conditions, in which immune system damages healthy tissues. Inflammation can result in pain in any part of body.

1.2 Inflammation

The inflammatory response to injury is a normal host defense mechanism that serves to isolate and remove the damage. Early events in this process include release of mediators, such as histamine and serotonin that dilate the blood vessels and increase their permeability. Subsequently, fluid leaking into the surrounding tissue space causes inflammation (i.e., swelling, pain, redness, and heat). Included in this fluid exudates are proteins, such as fibrinogen, which, when converted to fibrin, helps to seal off the affected area. Also present are polymorphonuclear neutrophils (PMNs) and leukocytes that phagocytize the infectious or toxic agent [1].

The inflammatory process is triggered by several interrelated cascade systems in the body. These include:

- The Complement system
- The Clotting system
- The Plasmakinin system
- The Arachidonic acid cascade

1.2.1 Complement system

It represents a cascade of approximately 25 serum proteins. The complement system mediates lysis of antibody-coated targets (bacteria, viruses, cells), recruits inflammatory cells to the sites of inflammation, and increases the efficiency of phagocytosis through opsonisation.

1.2.2 Clotting system & Plasmakinin system

Activation and interaction of the clotting and plasmakinin systems of the body also contribute to the inflammatory process. Activation of the Hageman factor (clotting factor XII), in addition to its effects on the coagulation system of the host, in turn activates the circulating protein, prekallikrein., to its enzymatically active form, kallikrein.The subsequent cleavage of another plasma substrate, kinonogen, by kallikrein liberates bradykinin, a linear nonapeptide. This peptide is a potent vasodilator

that also has the ability to increase blood vessel permeability. In addition, bradykinin acts as a mediator of pain (elicits a pain response) [2,3].

1.2.3 Arachidonic acid cascade

It has been the most actively studied of all the physiologic components contributing to inflammation. Both cyclooxygenase and lipoxygenase pathway of arachidonic acid metabolism have been investigated for their effects on inflammation- Arachidonic acid is generated from cellular membrane phospholipids by the action of the enzyme phospholipase A_2. In the cyclooxygenase pathway, it is converted first to a short-lived endoperoxide and then to prostaglandins. In mammalian cells two cyclooxygenase (COX) enzymes exist which are encoded by different genes, but share a 60 % identity in amino acid sequence. COX-I is constitutively expressed as a "housekeeping" enzyme in most tissues and mediates physiological responses such as regulation of renal and vascular homeostasis and cytoprotection of the stomach. - In comparison, COX-2 is primarily considered as an inducible immediate-early gene product whose synthesis can be up regulated by various proinflammatory agents, including endotoxin, cytokines, and mitogenes. COX-2 is the major isoform expressed by inflammatory cells and has, accordingly, been shown to release the high levels of prostanoids present under pathological conditions such as acute and chronic inflammation. The role of prostaglandins in inflammation is profound. Those of the E type increase blood vessel permeability and have the ability to sensitize various tissues (e.g. blood vessels, pain receptors) to the effects of other mediators, such as bradykinin [4].

The alternate pathway of arachidonic acid metabolism, the 5-lipoxygenase pathway; leads to the generation first of a hydro peroxide derivative, 5 HPETE, that stimulates histamine release from basophiles. Subsequent conversions lead to compounds known as leukotrienes that synergize with prostaglandins to cause increased blood vessel permeability and pain. One such leukotriene, LTB_4, is a potent chemotaclic agent and a hyperalgesic agent (pain sensitizer) that shows additive effects with prostaglandins in pain mediation [3, 4].

Much of the tissue destruction occurring during the inflammatory cycle can be attributed to substances released from the activated phagocytes that have been recruited to the site. These substances include free radicals and radical precursors, such as

hydroxyl radicals and superoxide anion. Free radicals are nonspecific in their action and can cause destruction of membrane components, degradation of connective tissue, and depolymerisation of hyaluronic acid leading to collagen damage. They may potentiate the action of proteolytic enzymes by oxidative destruction of naturally occurring inhibitors, such as -1-proteinase inhibitor. In addition they feed the inflammatory cycle by further stimulation of leukocyte and inacrophage functions [5].

1.2.4 Mediators of Inflammation

Once leukocytes have arrived at a site of infection or inflammation, they release mediators, which control the later accumulation and activation of other cells. However, in inflammatory reactions initiated by the immune system, the ultimate control is exerted by the antigen itself, in the same way as it controls the immune response itself. For this reason, the cellular accumulation at the site of chronic infection, or in autoimmune reactions (where the antigenic stimulus is rapidly cleared) [6].

There are four major plasma enzyme-systems, which have an important role in haemostasis and control of inflammation. These are the complement system, the clotting system, the fibrinolytic (plasmid) system and the kinin system.

Inflammatory mediators are soluble, diffusible molecules that act locally at the site of tissue damage and infection, and at more distant sites. They can be divided into exogenous and endogenous mediators.

Bacterial products and toxins can act as exogenous mediators of inflammation. Notable among these is *endotoxin,* or LPS (Lipopolysaccharide) of Gram-negative bacteria. The immune system of higher organisms has probably evolved in a veritable sea of endotoxin, so it is perhaps not surprising that these substance avokes powerful responses For example, endotoxin can trigger complement activation, resulting in the formation of anaphylatoxins C3a and C5a which cause vasodilation and increase vascular permeability [5].

Endogenous mediators of inflammation are produced from within the (innate and adaptive) immune system itself, as well as other systems. For example, they can be derived from molecules that are normally present in the plasma in an inactive form,

such as peptide fragments of some components of complement, coagulation, and kinin systems. Mediators of inflammatory responses are also released at the site of injury by a number of cell types that either contain them as preformed molecules within storage granules, e.g. histamine, or which can rapidly switch on the machinery required to synthesize the mediators when they are required, for example to produce metabolites of arachidonic acid [5,7].

Early phase mediators are produced by mast cells and platelets. They are especially important in acute inflammation and include mainly histamine, serotonin and other vasoactive substances. Platelets may contribute to inflammatory responses resulting as a consequence of tissue injury, through a variety of mechanisms including [7].

1. The release of vasoactive amines and other permeability factors,
2. The release of lysosomal enzymes,
3. The release of coagulation factors which lead to localized and generalized fibrin deposition, and
4. The formation of platelet aggregates or thrombi which result in the blocking of vessels and capillaries.

To the early phase mediators also belong chemoatractants (e.g. C5a) and cytokines such as 1L-1, 1L-6, and TNF-α late phase mediators are responsible for the regulation of vascular events occurring later- from about 6-12 hours after initiation of inflammation. The later vascular events are mediated, at, least in part, by products of arachidonic acid.

The chemical mediators of inflammation are summarized in Table 1 [10]. There is considerable functional redundancy of the mediators by inflammation. This explains why certain patients may have complete absence of a humoral component (e.g., complement component C3), yet minimal problems with increased susceptibility to infection [8].

Edema formation can be separated from phagocyte recruitment, Vasodilation in response to histamine, bradykinin, PGE_2 and PGI_2, and complement fragments C3a and C5a results from a direct action of these substances on endothelial cells and smooth muscle vasculature with resulting leakage of plasma. This is accompanied by release ofmediators, such as C5a, LTB4, and PAF that act directly on the phagocytec cells. In

addition N-forrnyl peptides are released from bacteria and mitochondria of damaged tissues, these mediators are potent chemoattractants that mobilize neutrophils

Function	Mediators
Increased vascular permeability Of blood vessels	Histamine, Serotonin, bradykinin, C3a, C5a, PGE, LTC, LTD2 prostacyclins, activated Hageman factor, high-molecular-weight kinino-gene fragments, fibrinopeptides
Vasoconstriction	TXA2, LTB,, LTC, LTD, C5a, N-formyl peptides
Smooth muscle contraction	C3a, C5a, histamine, LTB, LTC, TXA2, serotonin, PAF, bradykinin
Increased endothelial cell	1L-1, TNF-α, MCP, endotoxin,
Mast cell degranulation	C5a, C3a
Stem cell proliferation	1L-3, G-CSF, GM-CSF, M-CSF
Recruitment from bone marrow	CSFs, lL-l
Adherence/aggregation	IgG, fibronectin, lectins
Chemotaxis	C5a, LTB , 1 L-8 and other chemokines, PAF, histamine (for eosinophils). N-formyl peptides. collagen fragments, lymphocyte-derived chemo-tactic factor, fibrinopeptides
Lysosomal granule release	C5a, 1L-8, PAF, most chemo-attracta-attractants, phagocytosis
Production of reactive oxygen	C5a. TNF-ALFA, PAF, IL-8, phagocytic par
Intermediates	particles; IFN-β enhances
Phgocytosis	,C3b, iC3b, IgG (Fc portion), fibronectin; IFN-gema increases Fc recep factor expression
Granuloma formation	IFN-,TNF-ALPHA, 1L-1
Pyrogens	1L-1, TNF-alpha, 1L-1
Pain	PGE2, bradykinin

monocytes, and eosinophils, cause release of lysosomal contents, and activate the respiratory burst of the phagocytes with resulting production of toxic oxygen products [14].

The critical components of the inflammatory response- fever, neutrophil margination in the circularly vessels, and then mobilization from the bone marrow are associated with readily detected changes in circulating levels of certain mediators of inflammation. For example, and TNF-a peaks within two hours and is likely (he predominant pyrogen associated with the febrile response. Plasma levels of the chemoattractant IL-8-increase early and peak by four hours. Early increases in 1L-8 may relate to the transient decrease in the neutrophil count at 30 min (margination) [12].

Mediator accumulation at local inflammatory processes in skin blisters is somewhat different from the systemic effects following intravenous endotoxin. Mediators detected in blister fluid within 3 to 5 hr of the inflammatory response included LTB^4, C5a, 1L-8 and IL-6. In contrast IL-1b, GM-CSF, and TNF-a were not detected until after 8 hr in the blister. Thus the endotoxin and skin blister models of inflammation demonstrate that there are clear differences in the mediators that can be detected systemically and locally [12,13].

1.3 Biosynthesis of Prostaglandins

Prostaglandins are made by nearly every organ system, tissue, and cell in the body. Under normal conditions this is predominantly driven by COX-1. This enzyme is broadly expressed and is constitutively active Under homeostatic conditions, prostaglandin formation is controlled by substrate availability and perhaps peroxide tone Most cells and tissues tightly regulate free fatty acid concentrations to be very low. The chief enzymes responsible for this are the fatty acid transacylases, which rapidly reacylate free fatty acid into either phospholipids or triglycerides. Agents that stimulate production of prostaglandins from COX-1 have primarily been agents that cause a calcium spike inside cells. In contrast to COX-1, expression of COX-2 is normally limited in the body to specific cells in kidney, brain, and pancreas In general, COX-2 must be induced for substantial concentrations of prostaglandins to occur A plethora of inducers have been reported and fall into several classes including cytokines, growth factors, and hormones. COX-2-dependent prostaglandin production thus occurs over

hours rather than minutes, in contrast to COX-1-driven eicosanoid production that requires only seconds or a few minutes. Additionally the expression of COX-2 enzyme is usually the rate-limiting step for COX-2-driven eicosanoid formation, not substrate release. Several groups have explored the molecular details of how COX-2 expression is induced. It appears that agents that induce COX-2 expression do so by both inducing COX-2 mRNA production as well as stabilizing the mRNA. Recent work by Song et al. indicates that COX-2 expression is normally silenced through a hypermethylation mechanism. Induction of COX-2 mRNA has been reported to occur through IL-1 mediation, ceramide-dependent MAP kinases, p38 MAP kinase, and IKB kinases. Recently, the PEA3 family of transcription factors as well as NFKB p65 have been shown to increase COX-2 mRNA levels. PPAR *y* has been shown to suppress LPS induction of COX-2 in macrophages . In addition, p53 has been shown to be a transcriptional inhibitor, explaining the expression of COX-2 in tumor cells that in many cases lose p53. Associated with COX-2 expression in tumors is the report that k Ras**,** a protein associated with tumors, increases the stability of COX-2 mRNA. The anti-inflammatory activities of salicylate and corticosteroids are at least partly attributable to suppression of COX-2 expression by suppression of transcription and mRNA destabilization, respectively. In several cellular systems, higher levels of prostaglandin production have been associated with COX-2 expression. In some cells expression of both enzymes yields prostaglandin production from only COX-2, as shown by specific inhibitors. In addition COX-1 appears to prefer exogenousarachidonic acid, whereas with endogenous substrate COX-2 metabolism is predominant These phenomena have three possible explanations:

(1) Specific linkage of each enzyme with a specific phospholipase,

(2) linkage of COX-2 to an inducible PGE,

(3) Regulation by peroxide tone.

A number of workers have suggested specific phospholipase activation coupled to either COX-2 or COX-1. Very recently, two laboratories have made progress in identifying the enzymes responsible for PGE, production from PGH, Two enzymes have been identified. The first is a cytosolic, constitutively expressed enzyme and appears linked to COX-1. The other enzyme is microsomal in location, is inducible, and appears linked with COX-2 PGE, production. The importance of the inducible PGE, synthase in control of the relative contribution of COX-1 versus COX-2 PGE,

production remains to be thoroughly underhoodnbut has significant potential, perhaps even as a new drug target. Finally, a third molecular mechanism for the difference between the two COX enzymes turnover and activation rate has been proposed. Elegant kinetic studies show clearly that the two enzymes differ in the peroxide reaction kinetics of stabilization of the active cyclooxygenase species. The interpretation of these studies is that a lower level of peroxide is required for COX-2 turnover than that for COX-1. Practically, this means that under some cellular conditions COX-1 is silent, whereas COX-2 produces prostaglandins in significant amounts.

1.4 Nonsteroidal Anti-Inflammatory Drugs

Non Steroidal Anti-Inflammatory Drugs (NSAIDs) are medications which, as well as having pain-relieving (analgesic) effects, have the effect of reducing inflammation when used over a period of time.

Non-steroidal anti-inflammatory drugs, usually abbreviated to NSAIDs, are drugs with analgesic, antipyretic and anti-inflammatory effects - they reduce pain, fever and inflammation. The term "non-steroidal" is used to distinguish these drugs from steroids, which (among a broad range of other effects) have a similar eicosanoid-depressing, anti-inflammatory action. As analgesics, NSAIDs are unusual in that they are non-narcotic. NSAIDs are sometimes also referred to as non-steroidal anti-inflammatory agents/analgesics (NSAIDs) or non-steroidal anti-inflammatory medicines (NSAIDs). The most prominent members of this group of drugs are aspirin and ibuprofen. Paracetamol (acetaminophen) has negligible anti-inflammatory activity, and is strictly speaking not an NSAID [25].

1.4.1 Nonselective Cox Inhibitors:

A. Carboxylic acids:

1. Phenyl acetic acids

   e.g. Diclofenac, Fenclofenac, Alcofenac

2. Heterocyclic acids

   e.g. Indomethcin , Tolmetin, Etodolac ,Sulindac

3. Salicylic acids

   e.g. Aspirin,Diflunisal

4. Propionic acids

   e.g. Ibuprofen , Fenoprofen,Flubiprofen ,Ketoprofen, Naproxane , Oxaprozin

5. Fenamic acids

   e.g. Meclofenamic acids, Flufenamic acids,Mafenamic acids

B. Enolic acids:

1. Pyrazolones

   e.g. Butazones,Propazones

2. Oxicams

   e.g. Piroxicam,Tenoxicam, Isoxicams

Non-acidic:

e.g. Nabumetone

1.4.2 Selective Cox-2 Inhibitors:

Diaryl-substitutedfaranones : Rofecoxib

Diaryl-substituted pyrazoles : Celecoxib

Indole acetic acids : Etodolac

Sulfanilides : Nimesulides

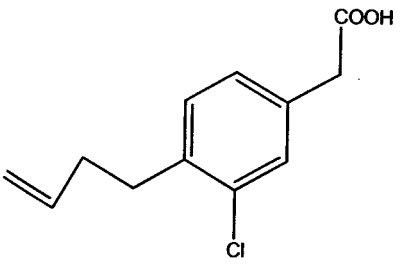
Others : Paracoxib, Meloxicam, Valdecoxib.

1.4.3 COX-1 Inhibitors:

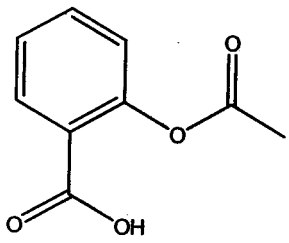
Indomethacin:

[1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid]

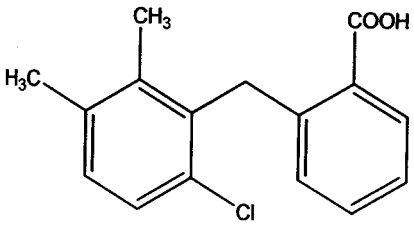
Indomethacin is used as anti-inflammatory drug.Dose:25mg bid. It is synthesized 1, 1-disubstituted hydrazine and levulinic acid.



Aclofenac



aspirine



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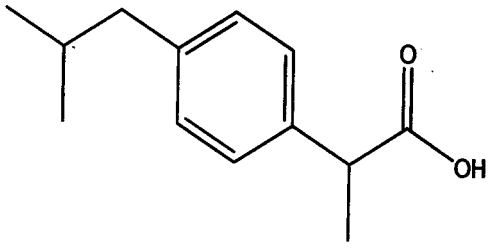
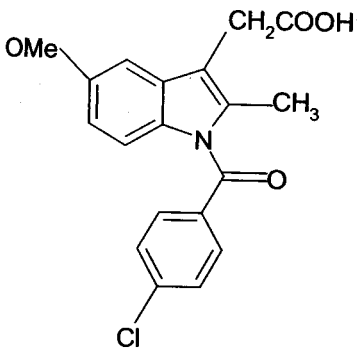
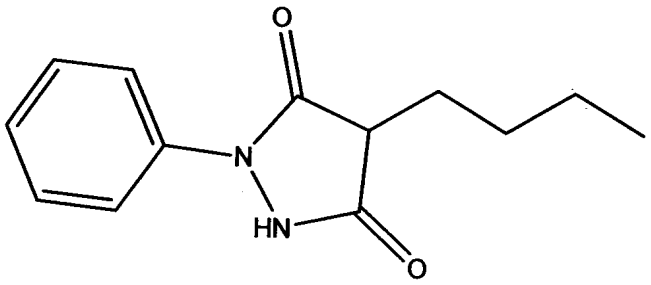


Ibuprofen

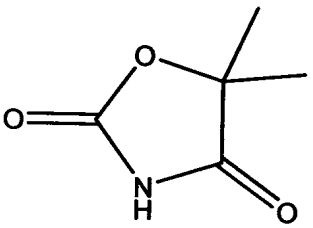


Indomethacine

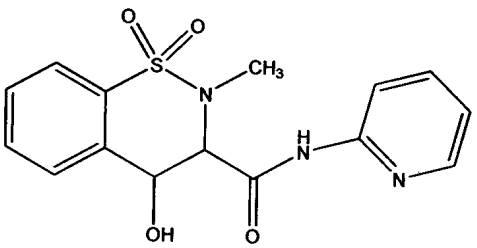
Structure of Nonselective Cox Inhibitors carboxylic acid derivatives



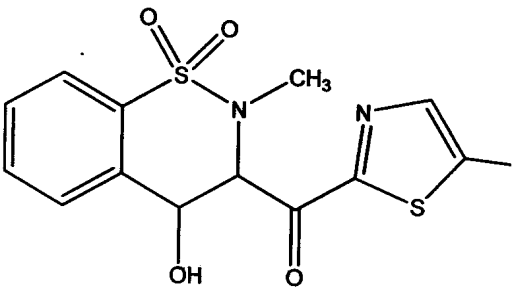
butazone



propazone

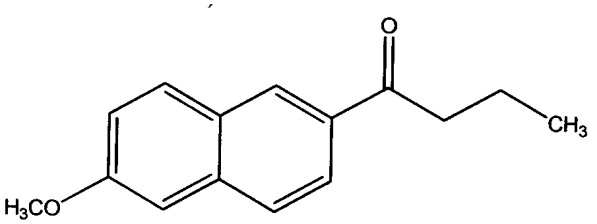


piroxicam



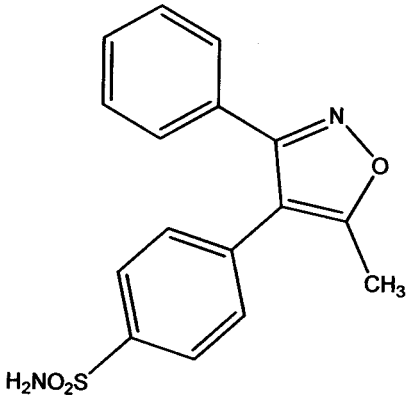
meloxicam

Structures of nonselective Cox inhibitors Enolic acid derivative



Nabumetone

Structure of non selective Cox inhibitor non-acidic derivative



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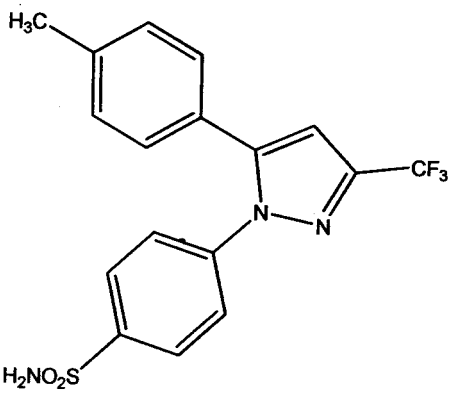
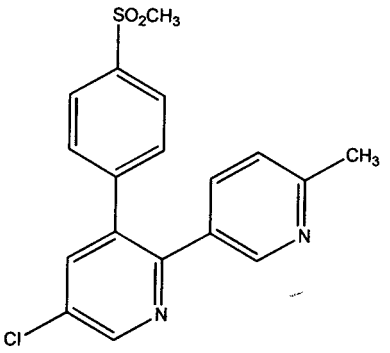


Celecoxib



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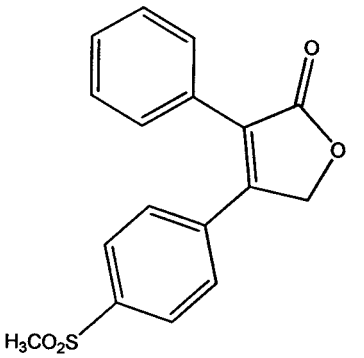


Rofecoxib

Structures of selective COX-2 inhibitor

Structures of selective COX-2 inhibitor

Beginning in 1829, with the isolation of salicin from the folk remedy willow bark, NSAIDs have become an important part of the pharmaceutical treatment of pain (at low doses) and inflammation (at higher doses). Part of the popularity of NSAIDs is that, unlike opioids, they do not produce sedation or respiratory depression and have a very low addiction rate. NSAIDs, however, are not without their own problems (see below). Certain NSAIDs, including ibuprofen and aspirin, have become accepted as relatively safe and are available over-the-counter without prescription.
The NSAIDs are among the most widely used of all therapeutic classes of drugs. These agents have been understood for many years to act peripherally to reduce the production of prostaglandins that sensitize nerve endings at the site of injury. This effect occurs due to inhibition of the cyclooxygenase (COX) enzyme that converts arachidonic acid liberated from the phospholipid membrane by phospholipases to prostanoids such as prostaglandins. Two forms of COX are well characterized; a constitutive form (COX1) that is normally expressed in tissues such as stomach and kidney and plays a physiological role in maintaining tissue integrity, and a form that is induced by inflammatory mediators (COX2) and plays a significant role in painand inflammation. The analgesic actions of NSAIDs can be dissociated from anti-inflammatory effects, and this may reflect additional spinal and supraspinal actions of NSAIDs to inhibit various aspects of central pain processing. Both COX isoforms contribute to spinal and supraspinal prostanoids production following tissue injury or inflammation. A major recent drug development that has occurred in an attempt to minimize Certain adverse effects with NSAIDs have been the development of selective COX2 inhibitors. This strategy targets the production of prostaglandins specifically involved in pain and inflammation while sparing constitutive prostaglandins that exert important physiological roles such as maintaining the integrity of the gastric lining and normal renal function. A further enzyme, COX-3, has recently been described; this has a prominent central distribution, is selectively inhibited by acetaminophen, and is potently inhibited by NSAIDs. Its identification has the potential to explain a number

of unresolved issues regarding the pharmacology of NSAIDs as analgesics. An additional strategy to try to minimize adverse effects has been the development of topical formulations of NSAIDs, as this can minimize plasma concentrations of drugs and lead to fewer adverse effects at sites remote from the area of application. Bioavailability and plasma concentrations following topical application are 5 to 15% of those achieved by systemic delivery. In human experimental pain paradigms, topical application of NSAIDs produces analgesia in models of coetaneous pain and muscle pain. In a clinical context, there have been three substantial reviews of the efficacy of topical NSAIDs.

NSAIDs potential risks and complications:

NSAIDs are cleared from the blood stream by the kidney, so it is very important that patients over 65 years of age or patients with kidney disease consult a physician prior to taking the medication. If patients take an NSAID for an extended period of time (six months or more), a blood test needs to be performed to check for early signs of kidney damage.

NSAIDs may also cause stomach upset or possibly ulcers. Patients with stomach ulcers or a history of stomach ulcers should first consult with their physician. Signs of stomach ulceration and intestinal bleeding typically include one or a combination of the following symptoms: abdominal pain, black tarry stools, weakness, or dizziness upon standing.

Most types of NSAIDs have a variety of other potential risks and complications associated with them. While most side effects are rare, it is important for patients to remain aware of them and under supervision by a health professional. As a general rule, patients with any of the following factors should be sure to meet with their doctor before taking any type of NSAID:

- Thyroid problems
- Diabetes
- Heart disease

- High blood pressure
- Allergy or reaction to aspirin, other NSAIDs or pain relievers
- Pregnant, about to become pregnant, or breast feeding
- Consume three or more alcoholic beverages a day
- About to have surgery or other invasive procedures (including dental surgery)

In order to ensure that NSAIDs are used safely, patients should meet with a physician to evaluate their individual risk factors (e.g. the patient's likelihood for developing certain health problems, including heart attack, stroke and gastrointestinal problems) and to determine the most appropriate dosages and treatment options. It is recommended that patients avoid taking over-the-counter NSAIDs for more than 10 days in a row without consulting their physician.

Warnings

NSAIDs cannot be used in the following cases:

- Allergy to aspirin or any NSAID
- Aspirin should not be used under the age of 16 years
- During pregnancy
- During breast feeding
- On blood thinning agents (anticoagulants)
- Suffering from a defect of the blood clotting system (coagulation)
- Active peptic ulcer

Controversies with COX-2 inhibitors

While it was hoped that this COX-2 selectivity would reduce gastrointestinal adverse drug reactions (ADRs), there is little conclusive evidence that this is true. The original study touted by Searle (now part of Pfizer), showing a reduced rate of ADRs for celecoxib, was later revealed to be based on preliminary data - the final data showed no significant difference in ADRs when compared with diclofenac.

Rofecoxib however, which has since been withdrawn, had been shown to produce significantly fewer gastrointestinal ADRs compared to naproxen[34]. This study, the VIGOR trial, raised the issue of the cardiovascular safety of the coxibs - a statistically insignificant increase in the incidence of myocardial infarctions was observed in patients on rofecoxib. Further data, from the Approve trial, showed a relative risk of cardiovascular events of 1.97 versus placebo - a result which resulted in the worldwide withdrawal of rofecoxib in October 2004 [23]

1.4.4 COX-3 inhibitors

Simmons also co-discovered COX-3 in 2002 and analyzed this new isozyme's relation to paracetamol (acetaminophen), arguably the most widely used analgesic drug in the world[35]. The authors postulated that inhibition of COX-3 could represent a primary central mechanism by which these drugs decrease pain and possibly fever.

The clinical ramifications and knowledge of COX isozymes are rapidly expanding and may offer significant hope for future treatments of pain, inflammation, and fever [(23)].

1.5 $TRPV_1$ receptor

The vanilloid 1 (TRPV1 or VR1) receptor is a member of a subgroup/super family of transient receptor potential (TRP) ion channels which subserve a whole host of cellular roles including many features of sensory transduction. The neuronally expressed TRPV1 is a non-selective, Ca2þ-preferring, cation channel. The TRPV1 channel is activated by a diverse range of chemical ligands such as capsaicin (the ‘hot’ component of chilli peppers) and other vanilloids (resiniferatoxin and the cannabinoid, anandamide), as well as acid (protons, Hþ), physical stimuli such as heat, certain arachidonic acid derivatives and direct phosphorylation
Via protein kinase C (PKC). In addition, TRPV1 is also activated (directly and indirectly) by a variety of mediators thought to contribute to neuroinflammation. Moreover, various endogenous mediators such as bradykinin, substance P, glutamate, prostaglandins, hydroperoxy fatty acids, and adenosine triphosphate (ATP) sensitise TRPV1.

1.5.1 Activation and sensitisation of TRPV1 receptors

Sequence analysis of the cloned capsaicin receptor VR1 revealed that it belongs to the TRP superfamily, characterised by having six transmembrane domains, and having a pore region between the fifth and sixth transmembrane domains. Once activated by vanilloid molecules the channel allows the influx of the cations Ca2þ and Naþ. TRPV1 mRNA is highly expressed in a subset of primary sensory neurones with Ad- and C-fibres that respond to chemical, mechanical and thermal stimuli and, therefore, they are classified as polymodal nociceptors. Recent studies have demonstrated that several endogenous chemical substances can activate TRPV1 in various tissues. The most prominent feature of TRPV1 is its responsiveness to physicochemical agents/noxious stimuli, such as temperature and protons. TRPV1 can be activated by acidic solutions with a pH of 5–6, which can be produced in tissues during pathological conditions with inflammation. TRPV1 is a thermosensor on afferent nerves, activated by temperatures between 42°C and 53°C, which coincides with the threshold temperature of thermal pain perception. The effect of temperature on airway afferent nerves has not been as widely studied as the cutaneous temperature sensors. However, it is probably unlikely that these temperatures are achieved in the lower airways, even in the inflamed lung. Whilst it is known that noxious cold air can induce cough, which may implicate TRPM8 receptors, there appears to be little or no evidence to show whether hot air can cause or sensitise the cough reflex. Recently, several members of the TRP family, including TRPV1 and TRPV4, have been implicated in sensory nerve mechanotransduction. Nonetheless, the molecular basis of mechanical transduction in the sensory terminals of the airways is little understood, but it would be fascinating to determine if TRPV1 receptors in airway sensory nerves can respond to mechanical stimuli that can cause cough. Additional stimuli of TRPV1 include elevated concentrations of the endocannabinoid, anandamide, the lipoxygenase metabolites of arachidonic acid, leukotriene B4 (LTB4), 12-(S) - and 15-(S)-hydroperoxyeicostetraenoic acid (12S- and 15S-HPETE), which can also sensitise TRPV1 receptors. Recently, N -arachidonoyl- dopamine (NADA) has been recognised as a TRPV1 stimulant, apparently more potent than anandamide. It is well known that bradykinin activates sensory neurones; however, the mechanism by which this occurs is not well understood, although possible sensitisationpathways have been suggested.

Bradykinin releases diacylglycerol (DAG), inositol-(1, 4,5)-triphosphate (IP3) and arachidonic acid from sensory neurones. Thus, it is likely that arachidonic acid, generated by bradykinin, would in turn activate phospholipase A2 (PLA2) and result in the production of lipoxygenase products from arachidonic acid.

It is well documented that a number of endogenous inflammatory mediators can modulate the sensitivity of TRPV1 during tissue inflammation. The exact mechanisms underlying the sensitization of TRPV1 are not yet fully understood, but several signal transduction pathways are known to be involved. TRPV1 has several consensus phosphorylation sites that can be phosphorylated by protein kinases A, C, and G (PKA, C and G) and tyrosine kinase (Trk), which ultimately results in sensitisation of TRPV1 receptors. PKC activation increases neuronal current responses to noxious heat and the activation of PKC by phorbol esters enhances the responses of TRPV1 to capsaicin, anandamide, acid and heat. Thus, for example, bradykinin which, as already mentioned, could indirectly activate TRPV1 receptors via the production of arachidonic acid metabolites could also sensitise the TRPV1 receptor by an indirect action on PKC also via the production of lipoxygenase products such as 15 S-HPETE, which in turn activates PKC. Furthermore, bradykinin, via activation of the B2 receptor, is known to stimulate phospholipase C (PLC) and increase the production of DAG, which in turn activates PKC. Prostaglandin E2 (PGE2) also sensitises sensory neurones via an effect on TRPV1 receptors. Evidence suggests that PGE2 activates the Gs protein-coupled EP2 prostanoid receptor present on the membranes of these neurones, which upon activation increases the enzyme activity of adenyl cyclase. The resulting rise in cAMP may then stimulate PKA, which in turn increases the phosphorylation of TRPV1 and enhances its excitability. A further example is nerve growth factor (NGF): administration of NGF in somatic tissues induces a long-lasting increase in the sensitivity of TRPV1 receptors. This effect is believed to be mediated through the G-protein coupled TrkA receptors, which in turn activatesmitogen-activated protein kinase and the PLC signalling pathway,resulting in potentiation of the TRPV1 channel. Another pathway for sensitising TRPV1 involves the ‘disinhibition’ of the receptor. PLC cleaves phosphatidylinositol-(4, 5) - biphosphate (PIP2) to yield IP3 and DAG. PIP2 constitutively inhibits TRPV1, such that removal of PIP2 from TRPV1 results in disinhibition of the receptor. When PLC is activated by bradykinin or NGF, PLC sequesters PIP2 which release TRPV1 from the constitutive inhibition.

1.5.2 TRPV1 receptors modulators

Growing evidence suggests several members of the TRP superfamily are involved in the detection of acute noxious, mechanical and chemical as well as in neuropathic pain. The first evidence for the involvement of TRP channels in the pain pathway came with the cloning of the vanilloid receptor TRPV1, which is arguably the most extensively studied of the entire TRP superfamily. The appropriate expression of the receptor in target tissues and the unmistakable pungency of capsaicin and many other agonists at the vanilloid receptor clearly define TRPV1 as a key transducer in the pain pathway and as an important integrator of responses to inflammatory mediators. Moreover, sensitisation of TRPV1 receptors during chronic pain is believed to contribute to the transduction of noxious signaling for normally innocuous stimuli. Furthermore, TRPV1 has a unique expression profile in peripheral nociceptors and the ability to show polymodal activation. Thus the expression of TRPV1 in the dorsal root ganglion (DRG) and nodose ganglion neurones, particularly in association with nociceptive afferent fibers, together with its activation by heat, acid and pungent vanilloid compounds, strongly indicate that TRPV1 plays an important role in the detection and integration of noxious stimuli. In gene-based disruption experiments, analysis of TRPV1 gene-knockout mice revealed that the channel contributes to the detection of acute painful chemical and thermal stimuli. In addition to their normal role as detectors of harmful stimuli, several pathological conditions lead to changes in the expression level and/or sensitivity of ‘‘pain’’ TRP channels. This can lead to exaggerated pain, when the experienced pain overestimates the harmfulness of the stimulus, or chronic pain, when the pain persists after the noxious stimulus has terminated. Many pathological conditions are characterized by hyperesthesia, i.e. enhanced, sensitivity to sensory stimuli. With respect to pain a distinction can be made between allodynia, when pain is experienced in response to non-noxious stimuli, and hyperalgesia, when exaggerated pain is experienced in response to noxious stimuli. Mechanisms leading to allodynia and hyperalgesia are well described for TRPV1 The well-established role of TRPV1 in

the pain pathway has given rise to the development of TRPV1-selective antagonists as new therapeutic targets for the treatment of clinical pain. Recently, SB-705498 was reported as a potent selective TRPV1 antagonist with good oral bioavailability and effectiveness in reducing hyperalgesia and allodynia in animal models. Furthermore, encouraging pharmacodynamic effects, including an effect on heat pain threshold and a reduction in UV burn-induced flare in the skin, indicating on target activity of SB-705498 and activity versus inflammatory hyperalgesia, have been reported in Phase 1 healthy volunteer studies. This demonstrates that this compound is pharmacologically active in humans at the dose tested and provides further confidence in the progression and design of clinical trials to assess the efficacy of TRPV1 antagonists in patients. Similarly, AMG8562, a novel, second generation TRPV1 antagonist was shown to cause effective anti-nociceptive effects in several models of inflammatory and surgical pain. Importantly, this compound did not cause hyperthermia (increase in body temperature), an effect that has been observed previously with other TRPV1 antagonists in animal and human studies. These examples illustrate the potential of TRPV1 antagonists in the treatment of varied forms of pain in humans and with the development of even more selective agents further understanding of the role of TRPV1 in pain is within reach.

1.5.3 Capsaicin

Capsaicin is a natural constituent in pungent red chili peppers. Depending on the concentration used and the mode of application, capsaicin can selectively activate, desensitize, or exert a neurotoxic effect on small diameter sensory afferent nerves while leaving larger diameter afferents unaffected. Sensory neuron activation occurs due to interaction with a ligand-gated nonselective cation channel termed the vanilloid receptor (VR-1) and receptor occupancy triggers Na_ and Ca2_ ion influx, action potential firing, and the consequent burning sensation associated with spicy food or capsaicin- induced pain. VR1 receptors are present on both C and A_ fibers, and can be activated by capsaicin and its analogs, heat, acidification, and lipid metabolites. Desensitization occurs with repeated administration of capsaicin, is a receptor-mediated process, and involves Ca2_-and calmodulin-dependent processes and phosphorylation of the cation channel. Capsaicin induces release of substance P and calcitonin gene-related peptide from both peripheral and central terminals of sensory neurons, and

desensitization inhibits such release such inhibition may result from inhibition of voltage-gated Ca2_-currents. Neurotoxicity is partially osmotic and partially due to Ca2_ entry with activation of Ca2_-sensitive proteases in neonates, neurotoxicity can be lifelong, whereas in adult animals receiving a localized dose, reversible injury may occur as cell bodies capable of regeneration are left intact. Both desensitization and neurotoxicity lead to analgesia in rodent paradigms, with specific characteristics of analgesia depending on the dose of capsaicin, route of administration, treatment paradigm, and age of the animal. The topical skin application of capsaicin to rodents produces analgesia , but variability in outcome can occur due to the concentration, the number of applications, and the different vehicles used that can affect the rate and extent of skin penetration.

1.5.4 Current status of novel TRPV1 antagonists for drug Development

Several synthetic antagonists of the TRPV1 channel are being developed and are currently under investigation, focused primarily for use in pain, in particular dental pain and migraine. However, authoritative information regarding the exact progress of these molecules throughpre-clinical and early clinical development is often difficult to acquire. A number of pre-clinical, Phase I and Phase II clinical studies/trials are currently in progress emanating from various different pharmaceutical companies and collaborations. As discussed previously, encouraging pharmacodynamic effects have been obtained with SB-705498, demonstrating that this agent is pharmacologically active in humans. Likewise, Merck–Neurogen and Glenmark have also recently announced completion of successful Phase I clinical trials with MK-2295 (NGD-8243) and GRC6211, respectively, and are now in the process of assessing proof-of-concept studies in dental pain. Unfortunately, Amgen recently announced that their molecule AMG517 caused marked hyperthermia in humans and stated that this would prevent it from further development. Interestingly, hyperthermia has not been highlighted as a major issue in the other Phase I studies completed so far. Notwithstanding, Amgen have another, second-generation, TRPV1 antagonist (AMG8562) in pre-clinical development, which does not cause hyperthermia, but retains pharmacological efficacy in on-target (agonist) challenge models and rodent pain models. There are many other companies operational in this area and no doubt further clinical trials will soon be

underway. Indeed, Evotec AG very recently announced the initiation of a Phase I clinical trial of a TRPV1 antagonist under partnership with Pfizer Inc. To date the emphasis for TRPV1 antagonists from a clinical development viewpoint has been on pain, however, there is increasing preclinical and clinical evidence which suggests that TRPV1 antagonists may have potential for the treatment of cough as well as a variety of other human disorders.

1.5.5 $TRPV_1$ antagonists obtained by chemical modification of agonists

The very existence of TRPV1 predicted the existence of painful endogenous compounds, the so called endovanilloid. It can be argued that if endovanilloids are involved in the development of pathologic pain, competitive TRPV1 antagonists should be analgesic by blocking the access of pro-algesic endovanilloids to the receptor. This concept has gained strong experimental support by the absence of inflammatory thermal hyperalgesia in mice whose TRPV1 had been deleted by heterologous recombination The first competitive TRPV1 antagonists, as exemplified by capsazepine, were derived directly from structural modification of TRPV1 agonists by researchers at the Sandoz (now Novartis) Institute for Medical Research in an attempt to dissociate the intolerable irritant and pungent properties of capsaicin derivatives from their analgesic activity. Capsazepine is a conformationally constrained capsaicin analogue and extensive NMR and X-ray crystallographic studies gave rise to a proposal of different binding modes for agonist versus an antagonist agonists bind to
the TRPV1 receptor in an extended conformation, whereas the antagonists prefer an L-shaped orientation.Capsazepine is still the most widely used pharmacological tool in studies involving TRPV1 despite its many unfavorable properties, including low potency, metabolic instability, and interaction at receptors other than TRPV1 (e.g., nicotinic acetylcholine receptors and voltage sensitive calcium channels). One must use caution when interpreting positive or negative results with capsazepine. Since capsazepine is a class B TRPV1 antagonist, that is, it does not inhibit all types of TRPV1 activators, a lack of inhibition by capsazepine does not necessarily imply that TRPV1 was not involved in the response. Conversely; a block of response by capsazepine may be mediated by targets other than TRPV1. It was with serendipity that

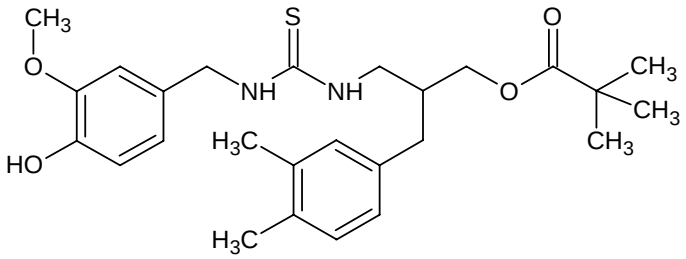
it was discovered by investigators at NovoNordisk that halogenation, and more specifically iodination, of TRPV1 agonist may provide potent antagonists. For example, iodination of RTX and nonivamide in the homovanillyl moiety results in potent TRPV1 antagonists. Interestingly, the position of iodine is critical for determining the pharmacological activity of the molecule. For instance, introduction of iodine at C-50 position in RTX or at C-0 position in nonivamide resulted in complete reversal, whereas the converse produced either less potent antagonist or partial agonists. It is unclear how halogenation works at the molecular level as iodination of vanillic- and dihydroferulic-RTX analogues has no impact on TRPV1 agonism. Halogenated TRPV1 antagonists are useful tool in in vitro assays, but not much is known about their in vivo efficacy, mostly because they are no considered as drug candidates.

1.5.6 Thiourea derivatives as TRPV1 antagonist

Capsazepine was the first TRPV1 antagonist to be reported in literature. The prototypical TRPV1 antagonist capsazepine, a compound related to capsaicin that has been Shawn to block capsaicin mediated behavior in rendts, has become an invaluable tool for studying the effect of TRPV1 antagonist in neuropathic pain models.

Attempts to improve on the poor physical properties associated with capsazepine scaffold have led to large number of thiourea structures as potent and useful RPV1 antagonists.

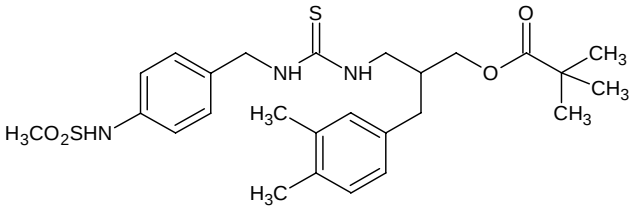
After the identification of resininferatoxin (RTX) as vanilloid receptor TRPV1 agonist with a binding potency approximately 4-ordes of magnitude better than that of capsaicin (CAP) on the base of these model to ultra potent TRPV1 agonist, JYL-79 and JYL-273, with kid values of 19 nm and 11nm respectively in a [3H] RTX binding assay, have been synthesized and characterized relative to capsaicin these compound appear to be approximately 300 and 500 times more potent.



JYL79

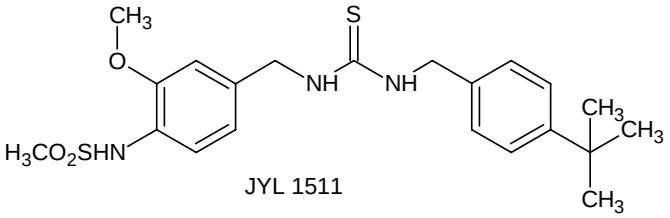
The isostearic replacement of phenolic hydroxyl groups in potent TRPV1 agonist JYL-79 and JYL-273 with alkyl sulfonamide group provide a series of compounds which are effective antagonists to the action of capsaicin on the rat TRPV1 .

As a result JYL-827 showed a high binding affinity with ki value of 29.3 nm for inhibition of [3H] RTX binding assay and potent antagonism with an IC_{50} value of 67nm for the inhibition of ca^{+} uptake in the response to capsaicin, displaying partial agonism .



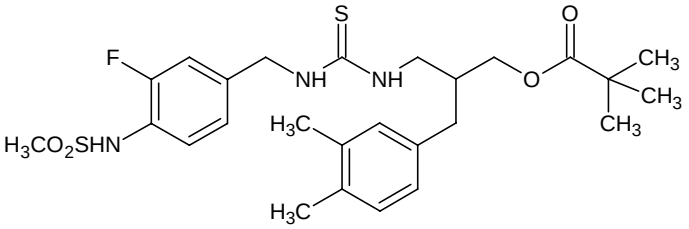
JYL 827

A similar compound N-[4-test]iupac of JYL-1511 has comparable potency relative to the lead molecule JYL-827 .



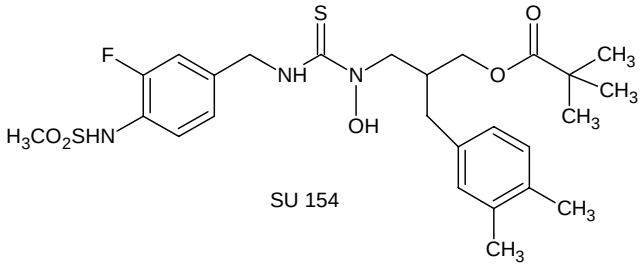
JYL 1511

The JYL-1433 (the 3-fluoro analogue of JYL-827) was found to be a full and potent TRPV1 antagonist with an IC_{50} value of 7.8nm.



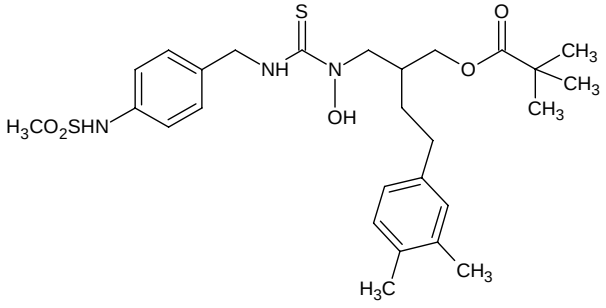
JYL 1433

Despite its relatively week in vitro potency Su-154, N- hydroxyl thiourea analogue, exhibit high analgesic potency in acetic acid writhing assay.



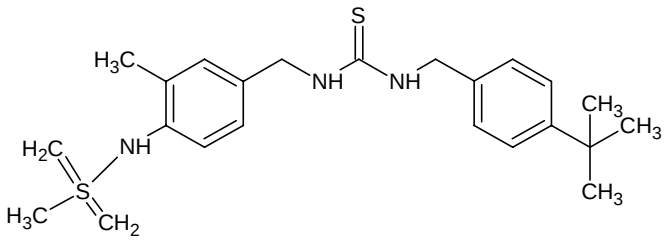
SU 154

Among a series of in which lead compound JYL-827 have been varied, JYL-1413 has shown to be a better TRPV1 antagonist than the lead compound (although the binding affinity is lower) it is assumed that JYL-1413 block TRPV1 on membrane most selectively rather than in internal stores.



JYL 1413

IBTU is a potent TRPV1 antagonist with marked selectively for the calcium entry-linked receptor subpopulation of TRPV1.



Pacific Corporation W 02002016318

Dibenzylthiourea from pacific corporation TRPV1 antagonist has recently been introduced in face clinical trial for painful condition.

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2.1 Aim and Scope of the present work

The design and development of new molecules potentially useful in the control of pain is a very important area today Over the last few years, the amount of information from studies on pain transmission by transient receptor potential channel activity relationships of 1, 3-diarylalkyl thioureas 3 possessing vanilloid subfamily member 1 (TRPV1) binding ligands has dramatically increased, thus revealing novel targets for the advent of new pain therapies. Furthermore, a gigantic step came with the identification of a protein called TRPV1, cloned in 1997, which is a ligand-gated nonselective cation channel vanilloid receptor with high Ca2þ permeability [1]. TRPV1 is activated not only by vanilloid ligands such as capsaicin 1 pH < 6) but also by endogenous mediators of inflammation noxious heat and protons (extracellular such as cannabinoid anandamide and arachidonic metabolites. Capsazepine ,which has been extensively characterized, was rhe first reported competitive VR1antagonist. However, its drawback is its modest potency and poor metabolic[2,3] and pharmacokinetics properties[4]. Recently the structure activity relationship of 1,3-diarylalkyl thiourea possessing new vanilloid equivalents have been reported[5]. Accordingly, the idea that TRPV1 function as an integrator of multiple pain producing stimuli implied that TRPV1 antagonist should have profound anti-inflammatory pain model.

Chemical modification of Synthesis and Pharmacological evaluations of 1-(benzothiazol-2-yl)-3-phenylthiourea may provide anti-inflammatory activity. Benzothiazolyl derivatives and thiourea derivatives are mostly reported as anti-inflammatory agents. The vanilloid receptor (TRPV1, formerly VR1) is a member of the transient receptor potential (TRP) family of ion channels, a large group of proteins involved in the detection of integration of sensory stimuli and regulation of cellular calcium [2]. TRPV1 is closely related to 3 other TRP channels all of which are thought to be involved in temperature sensation . TRPV1 is a cation channel that is highly expressed in a subset of primary afferent neurons and is directly activated by a wide range of stimuli including noxious heat (less than 42^0 C), protons, endogenous lipoxygenase products and fatty acid amides as well as an array of plant-derived chemicals such as capsaicin, gingerols, eugenol, resiniferatoxin, piperine and camphor. Activation of TRPV1 is potentiated by endogenous pro-nociceptive mediators such as prostaglandins, bradykinin and ATP, and together these properties make TRPV1 the

principle integrator of noxious information in many polymodal primary afferent neurons and is therefore a major potential target for novel analgesics.

2.2 Literature Review for the Synthetic Methods Available For Synthesis of Target Molecules

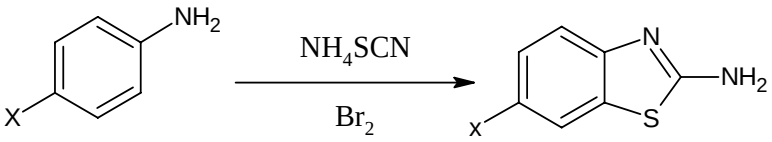
In bioorganic and medicinal chemistry 2-aminobenzothiazoles derivatives are broadly found with applications in drug discovery and development of the treatments of diabetes, epilepsy, inflammation, amyotrophic lateral sclerosis, analgesia, tuberculosis, and viral infection [8,9].

The various methods have reported in the literature to date are discussed below.

2.2.1 Synthesis of Benzthiazole derivatives

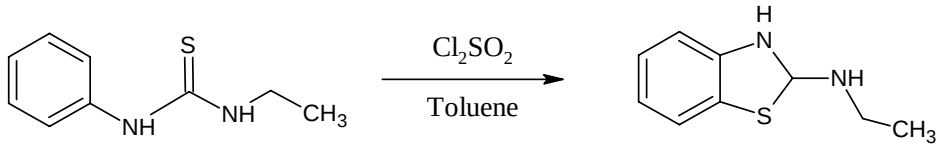
2.2.1.1 From 2-aminophenol

In 1887,Hoffmann first reported the cyclizations of 2- aminothiophenol to 2-aminobenzothiazoles. Hofmann noted only formation of 2- anilinobenzothiazole from the reaction of 2-aminothiophenol and phenyl isothiocyanate. Investigations into the preparation of 2-aminobenzothiazoles can also be traced to the early 1900s with work of Hugerschoff, who found that an arylthiourea can be cyclized with liquid bromine in Chloroform to form a 2-aminobenzothiaozles[12.13]. The reaction of molecular bromine (Br_2) with arylthioureas is known as Hugerschoff Reaction



2.2.1.2 From N'-acetylacetohydrazide

Castro and Martinez were synthesized 2-aminoethylbenzothiazole by intramolecular oxidations in N-ethyl-N'-phenylthiourea in presence of sulphuryl chloride and toluene

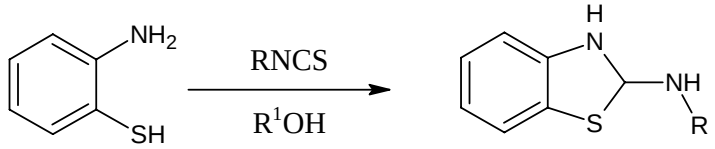


1-ethyl-3-phenylthiourea

N-ethyl-2,3-dihydro-1,3-benzothiazol-2-amine

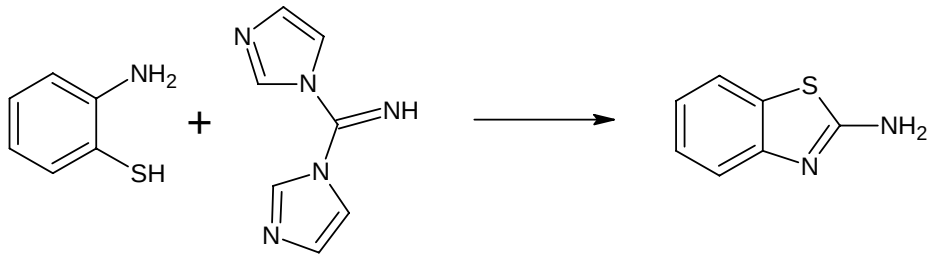
2.2.1.3 From Isothiocyanate

Tweit was done cyclizations of isothiocyanates to 2-aminobenzothiazole in presence of benzene



2.2.1.4 From Di(imidazole-1-yl)methanimine

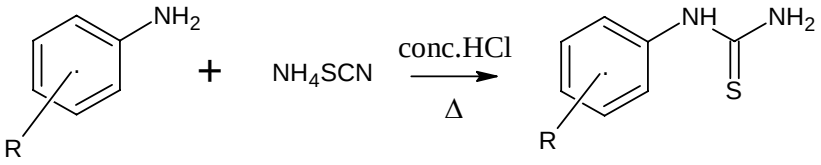
Yong-Qian Wu *et. al.* using Di(imidazole-1-yl)methanimine for formation of nitrogen containing heterocyclic nucleus. Di(imidazole-1-yl)methanimine was synthesized by treating cyanogens bromide with imdazole based on a previously reported procedure[32, 33]. As shown in the reaction of went smoothly with 2-substituted anilines, regardless of their nucleophilicity and this may suggest that second imidazole displacement is not the rate-limiting step due to the strong tendency towards cyclizations.



2.3 Synthetic Scheme Used For The Development of The Novel Substituted 1-(1, 3-benzothiazol-2-yl)-3-phenylthiourea.

Step 1

Ammoniumthiocyanate on reaction with different aniline derivatives gives phenylthiourea derivatives[12].

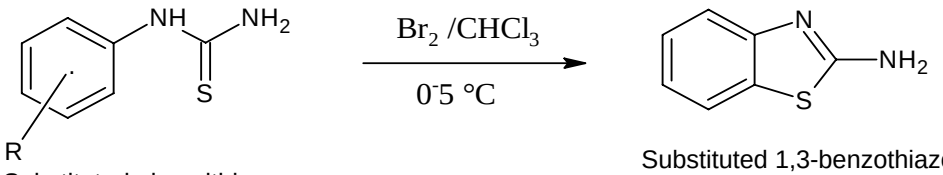


Substituted aniline

Substituted phenylthiourea

Step 2

When Substituted phenylthiourea is cyclised with bromine in presence of acidic media gives Substituted 1,3-benzothiazol-2 amine[13]

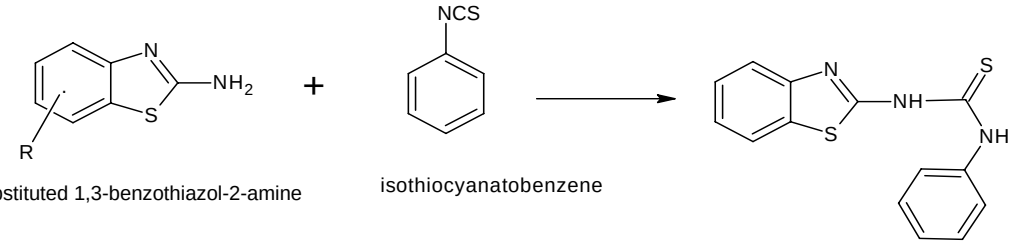


Substituted phenylthiourea

Substituted 1,3-benzothiazol-2-amine

Step 3

Substituted 1,3-benzothiazol-2 amine when reacted with phenylisothiocyanate produces substituted 1-(1, 3-benzothiazol-2-yl)-3-phenylthiourea.



Substituted 1,3-benzothiazol-2-amine

isothiocyanatobenzene

Substituted 1-(1,3-benzothiazol-2-yl)-3-phenylthiourea

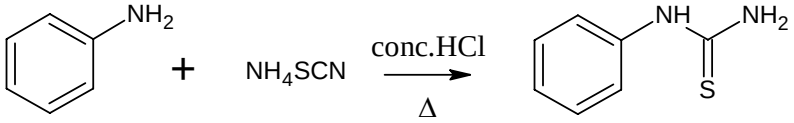
Where, R = CH_3 ,OCH_3,Cl

2.4 Synthesis of Intermediates

2.4.1 Synthesis of Substituted phenylthiourea derivatives

2.4.1 (A) Synthesis of 1-phenylthiourea

Reaction



aniline

1-phenylthiourea

Requirements

Reagents	Mol.Weight (g/mol)	Quantity (g)	Mol	Mol ratio
Aniline	93.12	5.00	0.053	1
Ammonium thiocyanate	76.12	4.08	0.053	1

Procedure

Aniline was dissolved in a mixture of concentrated hydrochloric acid(9 ml) and water(25 ml) by heating. The solution was cooled and ammonium thiocyanate was added. The reaction mixture was refluxed for 4 hr and then cooled. The separated product was filtered and washed with water. It was recrystallized from methanol. Reaction was monitored by Precoated TLC.

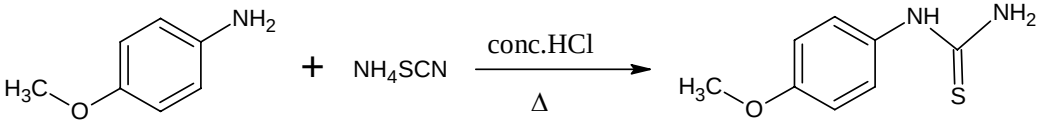
Solvent system used for TLC: Hexane:Ethylacetate(3:2)

Result

Product	Result
Percentage yield	80 %
R_f	0.45
Melting point	160 ^{o}C

2.4.1 B Synthesis of 1-(4-methoxyphenyl)thiourea

Reaction



4-methoxyaniline 1-(4-methoxyphenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
4-methoxyaniline	123.15	5.00	0.0406	1
Ammoniumthiocyanate	76.12	3.09	0.0406	1

Procedure

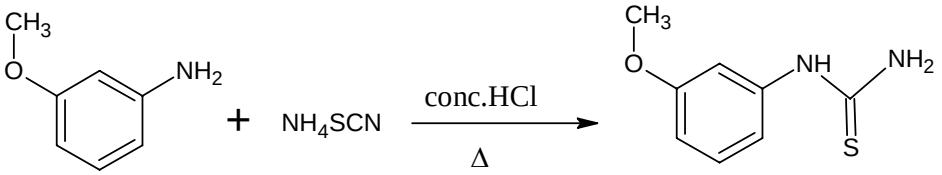
Same as 2.4.1 A

Result

Product	Result
Percentage yield	60 %
R_f	0.51
Melting point	170 ^oC

2.4.1 C Synthesis of 1-(3-methoxyphenyl)thiourea

Reaction



3-methoxyaniline

1-(3-methoxyphenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
3-methoxyaniline	123.15	5.00	0.0406	1
Ammonium thiocyanate	76.12	3.09	0.0406	1

Procedure

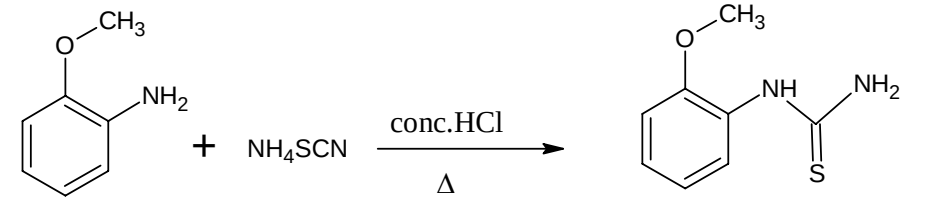
Same as 2.4.1 A

Result

Product	Result
Percentage yield	70.62 %
R_f	0.59
Melting point	150 ^oC

2.4.1 D Synthesis of 1-(2-methoxyphenyl)thiourea

Reaction



2-methoxyaniline

1-(2-methoxyphenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
2-methoxyaniline	123.15	5.00	0.0406	1
Ammonium thiocyanate	76.12	3.09	0.0406	1

Procedure

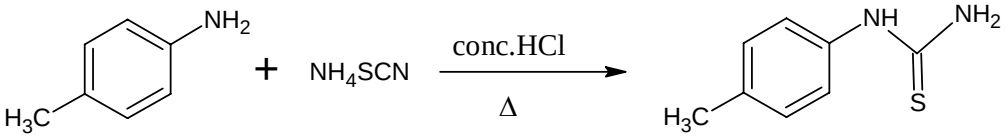
Same as 2.4.1 A

Result

Product	Result
Percentage yield	60%
R_f	0.67
Melting point	190^{o}C

2.4.1 E Synthesis of 1-(4-methylphenyl)thiourea(NP-smy$_4$)

Reaction



4-methylaniline

1-(4-methylphenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
4-methylaniline	107.15	5.00	0.046	1
Ammonium thiocyanate	76.12	3.54	0.046	1

Procedure

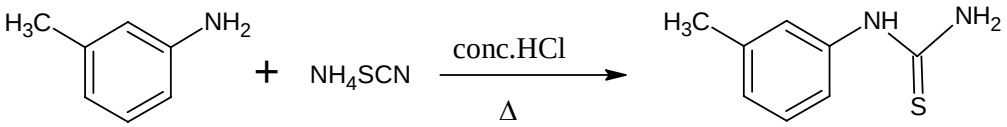
Same as 2.4.1 A

Result

Product	Result
Percentage yield	70.20%
R_f	0.508
Melting point	165 ^{o}C

2.4.1 F Synthesis of 1-(3-methylphenyl)thiourea

Reaction



3-methylaniline 1-(3-methylphenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
3-methylaniline	107.15	5.00	0.046	1
Ammonium thiocyanate	76.12	3.54	0.046	1

Procedure

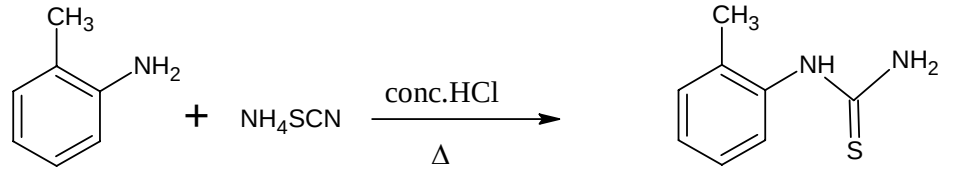
Same as 2.4.1 A

Result

Product	Result
Percentage yield	73.29 %
R_f	0.346
Melting point	177 ^{o}C

2.4.1 G Synthesis of 1-(2-methylphenyl)thiourea

Reaction



2-methylaniline 1-(2-methylphenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
2-methylaniline	107.15	5.00	0.046	1
Ammonium thiocyanate	76.12	3.54	0.046	1

Procedure

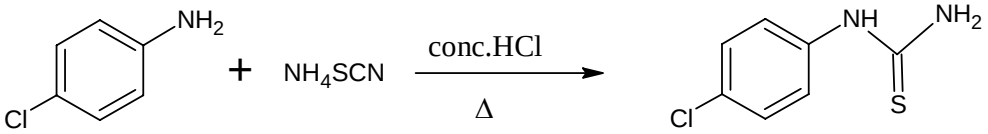
Same as 2.4.1 A

Result

Product	Result
Percentage yield	59.74 %
R_f	0.41
Melting point	200^{o}C

2.4.1 H Synthesis of 1-(4-chlorophenyl)thiourea

Reaction



4-chloroaniline 1-(4-chlorophenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
4-chloroaniline	127.57	5.00	0.039	1
Ammonium thiocyanate	76.12	2.98	0.039	1

Procedure

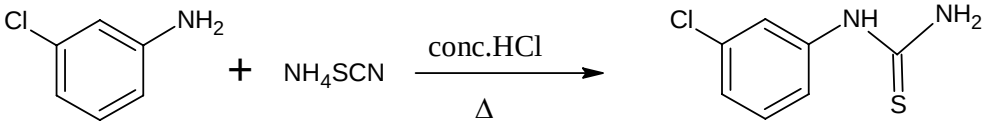
Same as 2.4.1A

Result

Product	Result
Percentage yield	71.86 %
R_f	0.163
Melting point	158 ^{o}C

2.4.1 I Synthesis of 1-(4-chlorophenyl)thiourea

Reaction



3-chloroaniline 1-(3-chlorophenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
3-chloroaniline	127.57	5.00	0.039	1
Ammonium thiocyanate	76.12	2.98	0.039	1

Procedure

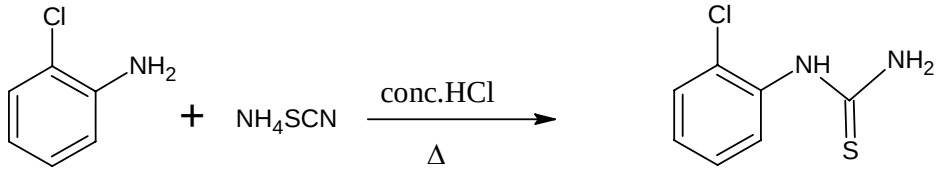
Same as 2.4.2 A

Result

Product	Result
Percentage yield	80.63 %
R_f	0.236
Melting point	190^{o}C

2.4.1. J Synthesis of 1-(4-chlorophenyl)thiourea

Reaction



2-chloroaniline 1-(2-chlorophenyl)thiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
2-chloroanilin	127.57	5.00	0.039	1
Ammonium thiocyanate	76.12	2.98	0.039	1

Procedure

Same as 2.4.1 A

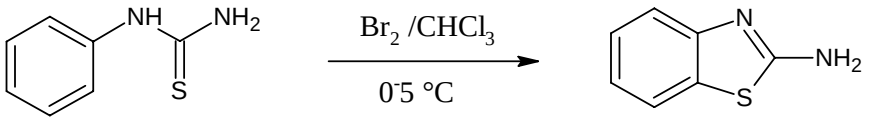
Result

Product	Result
Percentage yield	65.58 %
R_f	0.37
Melting point	180 ^oC

2.4.2 synthesis of substituted 1,3-benzothiazol-2-amine derivatives

2.4.2 A Synthesis of 1, 3-benzothiazol-2-amine

Reaction



1-phenylthiourea 1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1,3-benzothiazol-2-amine	152.21	4.00	0.026	1
Bromine	159.80	4.19	0.026	1

Procedure

1,3-benzothiazol-2-amine in chloroform was treated dropwise with a solution of bromine in chloroform while stirring the reaction mixture over period of 2 hr. the temperature of the reaction mixture over was maintained below 5 °c. stirring was continued for 1 hr more and the solution was refluxed on water bath till the evolution of hydrogen bromide vapours cease, then solution was neutralized with aqueous ammonia. the separated product was filtered and washed with water. It was crystallized from methanol. Reaction was monitored by Precoated TLC.

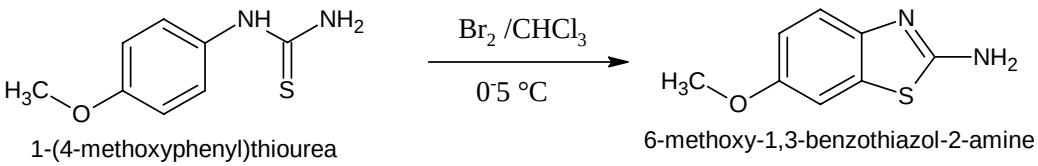
Solvent system used for TLC: Hexane:Ethylacetate(3:2)

Result

Product	Result
Percentage yield	55.58 %
R_f	0.47
Melting point	180 ^oC

2.4.2 B Synthesis of 6-methoxy-1,3-benzothiazol-2-amine

Reaction



1-(4-methoxyphenyl)thiourea

6-methoxy-1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(4-methoxyphenyl)thiourea	182.24	4.00	0.0219	1
Bromine	159.80	3.507	0.0219	1

Procedure

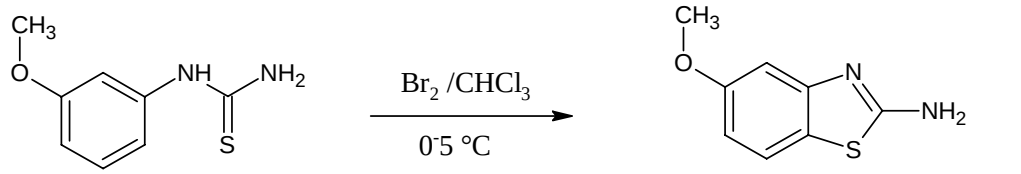
Same as 2.4.2 A

Result

Product	Result
Percentage yield	80.79 %
R_f	0.564
Melting point	198-204 ^oC

2.4.2 C Synthesis of 5-methoxy-1,3-benzothiazol-2-amine

Reaction



1-(3-methoxyphenyl)thiourea

5-methoxy-1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(3-methoxyphenyl)thiourea	182.24	4.00	0.0219	1
Bromine	159.80	3.507	0.0219	1

Procedure

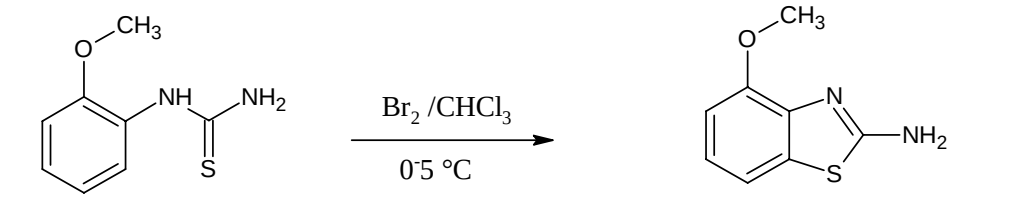
Same as 2.4.2 A

Result

Product	Result
Percentage yield	42.24 %
R_f	0.49
Melting point	202-205 ^{o}C

2.4.2 D Synthesis of 4-methoxy-1,3-benzothiazol-2-amine

Reaction



1-(2-methoxyphenyl)thiourea

4-methoxy-1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(2-methoxyphenyl)thiourea	182.24	4.00	0.0219	1
Bromine	159.80	3.507	0.0219	1

Procedure

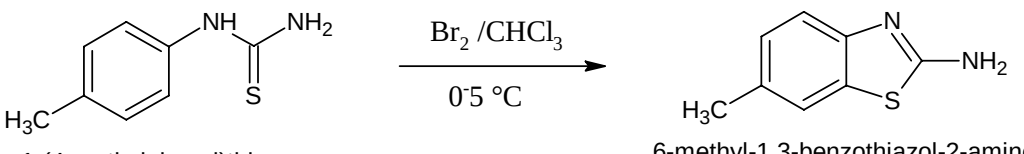
Same as 2.4.2 A

Result

Product	Result
Percentage yield	30.45 %
R_f	0.53
Melting point	220-228 ^{o}C

2.4.2 E Synthesis of 6-methyl-1,3-benzothiazol-2-amine

Reaction



1-(4-methylphenyl)thiourea

6-methyl-1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(4-methylphenyl)thiourea	166.24	4.00	0.024	1
Bromine	159.80	3.84	0.024	1

Procedure:

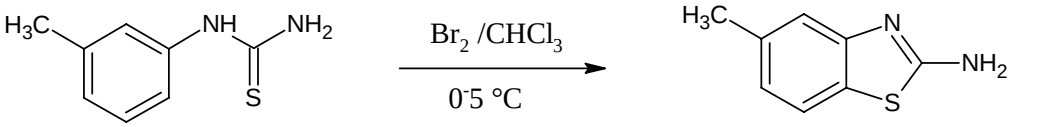
Same as 2.4.2

Result

Product	Result
Percentage yield	23.23 %
R_f	0.74
Melting point	220-224 ^{o}C

2.4.2 F Synthesis of 5-methyl-1,3-benzothiazol-2-amine

Reaction



1-(3-methylphenyl)thiourea

5-methyl-1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(3-methylphenyl)thiourea	166.24	4.00	0.024	1
Bromine	159.80	3.84	0.024	1

Procedure

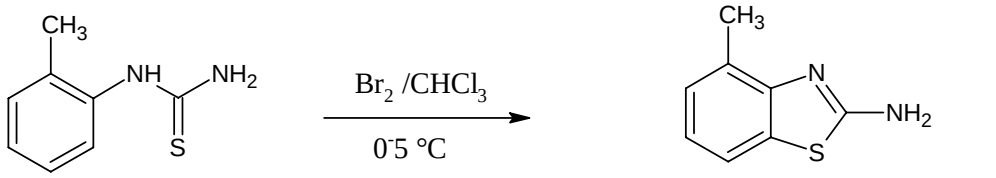
Same as 2.4.2 A

Result

Product	Result
Percentage yield	15.7 %
R_f	0.86
Melting point	72-80 ^oC

2.4.3 G Synthesis of 4-methyl-1,3-benzothiazol-2-amine

Reaction



1-(2-methylphenyl)thiourea

4-methyl-1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(2-methylphenyl)thiourea	166.24	4.00	0.024	1
Bromine	159.80	3.84	0.024	1

Procedure

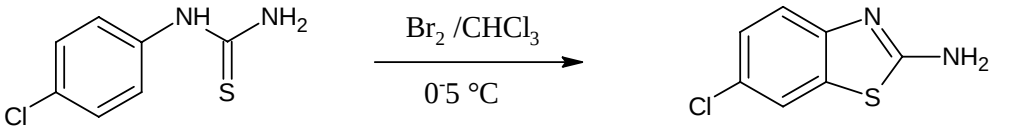
Same as 2.4.3 A

Result

Product	Result
Percentage yield	30.93 %
R_f	0.67
Melting point	130-132 ^oC

2.4.2 H Synthesis of 6-chloro-1,3-benzothiazol-2-amine

Reaction



1-(4-chlorophenyl)thiourea

6-chloro-1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(4-chlorophenyl)thiourea	174.65	4.00	0.0229	1
Bromine	159.80	3.65	0.0229	1

Procedure

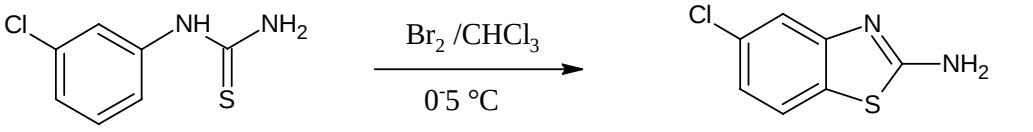
Same as 2.4.2 A

Result

Product	Result
Percentage yield	20.49 %
R_f	0.76
Melting point	176-180 ^{o}C

2.4.2 I Synthesis of 6-chloro-1,3-benzothiazol-2-amine

Reaction



1-(3-chlorophenyl)thiourea

5-chloro-1,3-benzothiazol-2-amine

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(3-chlorophenyl)thiourea	174.65	4.00	0.0229	1
Bromine	159.80	3.65	0.0229	1

Procedure

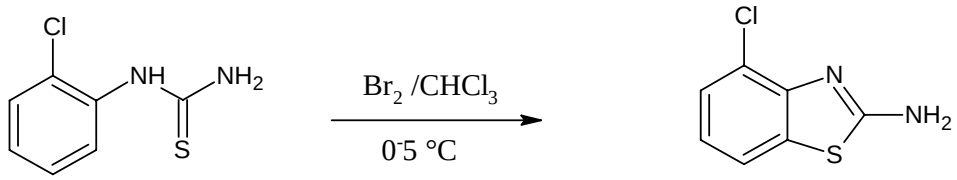
Same as 2.4.2 A

Result

Product	Result
Percentage yield	40.54 %
R_f	0.575
Melting point	135-140 ^oC

2.4.2 J Synthesis of 6-chloro-1,3-benzothiazol-2-amine

Reaction



Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1-(2-chlorophenyl)thiourea	174.65	4.00	0.0229	1
Bromine	159.80	3.65	0.0229	1

Procedure

Same as 2.4.2 A

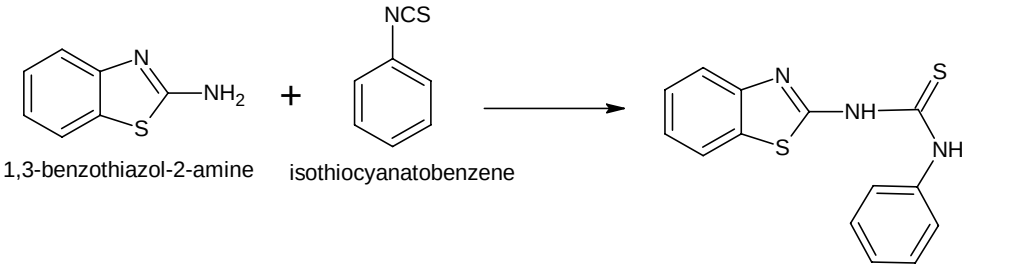
Result

Product	Result
Percentage yield	30.62 %
R_f	0.66
Melting point	150-155 ^oC

2.4.3 Synthesis of 1-(1,3-benzothiazol-2-yl)-3-phenylthiourea derivatives

2.4.3 A Synthesis of 1-(1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-a)

Reaction



1-(1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
1,3-benzothiazol-2-amine	150.20	3.00	0.0199	1
phenylisothiocyanate	135.18	2.69	0.0199	1

Procedure

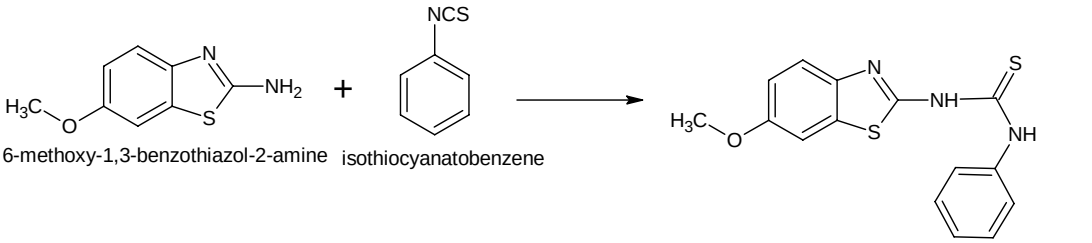
To solution of 1,3-benzothiazol-2-amine in acetonitrile, phenylisoithiocyanate in acetonitrile was added drop wise at room temperature. The mixture was refluxed with stirring for 4hr until the completion of the reaction and the resultant precipitate obtained after cooling of the reaction mixture was filtered , washed with acetonitrile , and dried. The product was recrystalised from ethanol.

Result

Product	Result
Percentage yield	60 %
R_f	0.45
Melting point	240-242 ^oC

2.4.3 B Synthesis of 1-(6-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-x_4)

Reaction



1-(6-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
6-methoxy-1,3-bezothiazol-2-amine	180.22	3.00	0.0166	1
phenylisothiocyanate	135.18	2.24	0.0166	1

Procedure

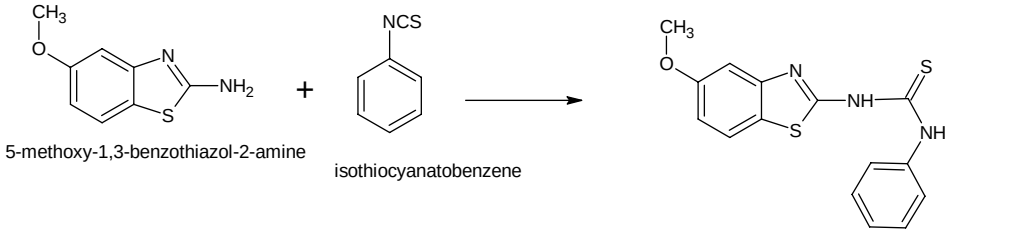
Same as 2.4.3 A

Result

Product	Result
Percentage yield	40 %
R_f	0.51
Melting point	200-205 ^oC

2.4.3 C Synthesis of 1-(5-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-x_3)

Reaction



5-methoxy-1,3-benzothiazol-2-amine + isothiocyanatobenzene → 1-(5-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements:

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
5-methoxy-1,3-bezothiazol-2-amine	180.22	3.00	0.0166	1
phenylisothiocyanate	135.18	2.24	0.0166	1

Procedure

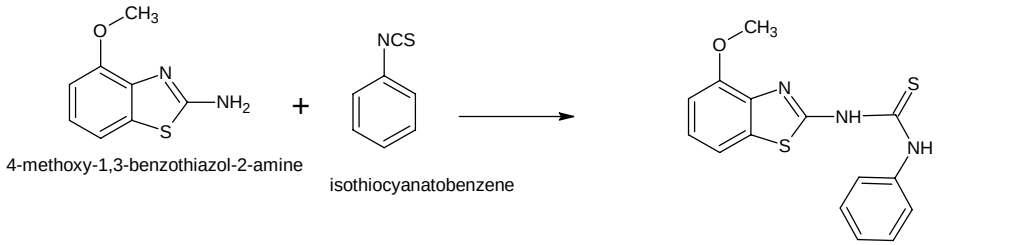
Same as 2.4.3 A

Result

Product	Result
Percentage yield	30.32 %
R_f	0.53
Melting point	232-234 ^{o}C

2.4.3 D Synthesis of 1-(4-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-x_4)

Reaction



4-methoxy-1,3-benzothiazol-2-amine + isothiocyanatobenzene → 1-(4-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
4-methoxy-1,3-bezothiazol-2-amine	180.22	3.00	0.0166	1
phenylisothiocyanate	135.18	2.24	0.0166	1

Procedure

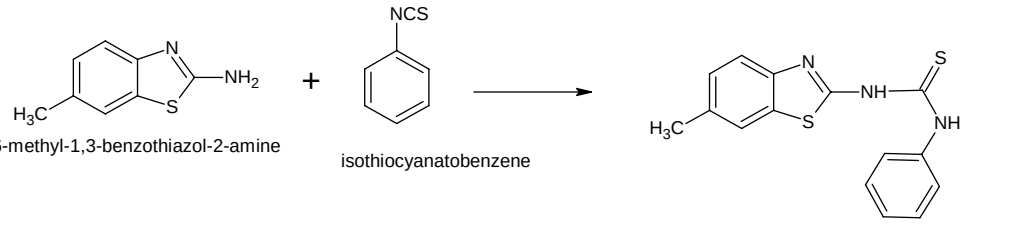
Same as 2.4.3 A

Result

Product	Result
Percentage yield	33.33 %
R_f	0.57
Melting point	162-164 ^oC

2.4.3 E Synthesis of 1-(6-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-y_4)

Reaction



1-(6-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
6-methyl-1,3-bezothiazol-2-amine	164.22	3.00	0.0182	1
phenylisothiocyanate	135.18	2.46	0.0182	1

Procedure

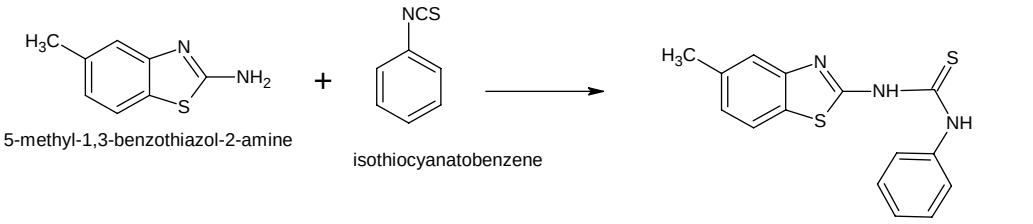
Same as 2.4.3 A

Result

Product	Result
Percentage yield	12.26 %
R_f	0.603
Melting point	166-170 ^oC

2.4.3 F Synthesis of 1-(5-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-y_3)

Reaction



5-methyl-1,3-benzothiazol-2-amine + isothiocyanatobenzene → 1-(5-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
5-methyl-1,3-bezothiazol-2-amine	164.22	3.00	0.0182	1
phenylisothiocyanate	135.18	2.46	0.0182	1

Procedure

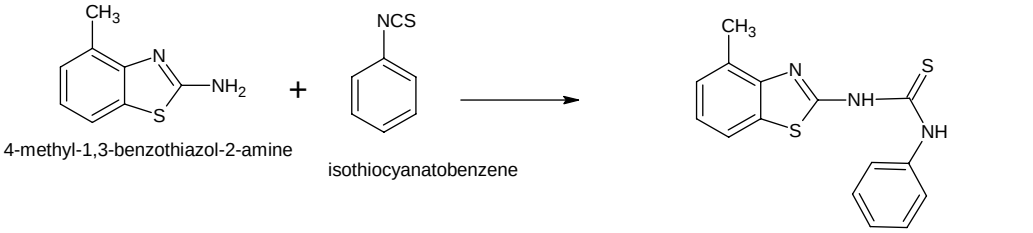
Same as 2.4.3 A

Result

Product	Result
Percentage yield	15.75 %
R_f	0.55
Melting point	220-222 ^oC

2.4.3 G Synthesis of 1-(4-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-y_2)

Reaction



4-methyl-1,3-benzothiazol-2-amine

isothiocyanatobenzene

1-(4-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
4-methyl-1,3-bezothiazol-2-amine	164.22	3.00	0.0182	1
phenylisothiocyanate	135.18	2.46	0.0182	1

Procedure

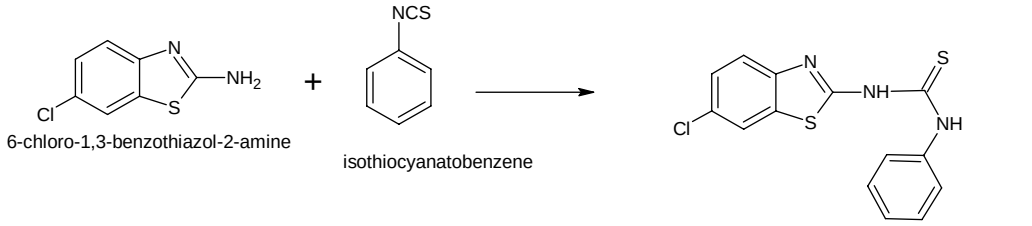
Same as 2.4.3 A

Result

Product	Result
Percentage yield	18.05 %
R_f	0.53
Melting point	270-274 ^oC

2.4.3 H Synthesis of 1-(6-chloro-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-z_4)

Reaction



6-chloro-1,3-benzothiazol-2-amine

isothiocyanatobenzene

1-(6-chloro-1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
6-chloro-1,3-bezothiazol-2amine	184.22	3.00	0.0162	1
phenylisothiocyanate	135.18	2.19	0.0162	1

Procedure

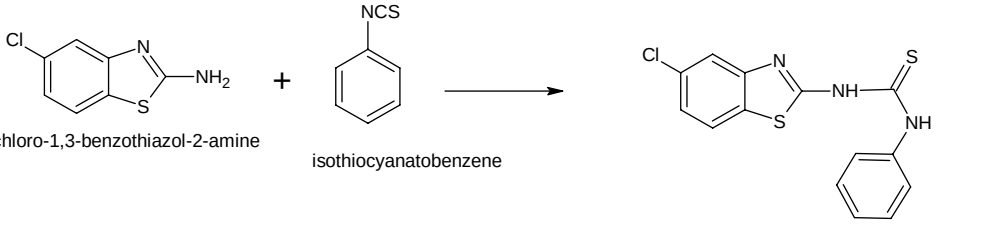
Same as 2.4.3 A

Result

Product	Result
Percentage yield	15.75 %
R_f	0.55
Melting point	220-222 ^{o}C

2.4.3 I Synthesis of 1-(6-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-z_3)

Reaction



5-chloro-1,3-benzothiazol-2-amine

isothiocyanatobenzene

1-(5-chloro-1,3-benzothiazol-2-yl)-3-phenylthiourea

Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
5-chloro-1,3-bezothiazol-2amine	184.22	3.00	0.0162	1
phenylisothiocyanate	135.18	2.19	0.0162	1

Procedure

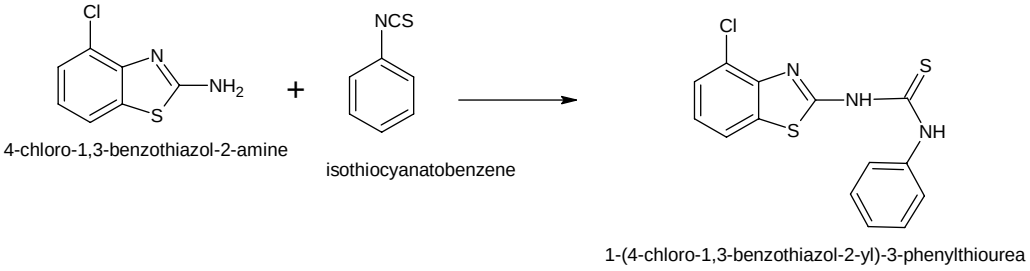
Same as 2.4.3 A

Result

Product	Result
Percentage yield	15.75 %
R_f	0.55
Melting point	220-222 ^{o}C

2.4.3 J Synthesis of 1-(6-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea(NP-z_2)

Reaction



Requirements

Reagents	Mol. Weight (g/mol)	Quantity(g)	Mol	Mol ratio
4-chloro-1,3-bezothiazol-2-amine	184.22	3.00	0.0162	1
phenylisothiocyanate	135.18	2.19	0.0162	1

Procedure

Same as 2.4.3 A

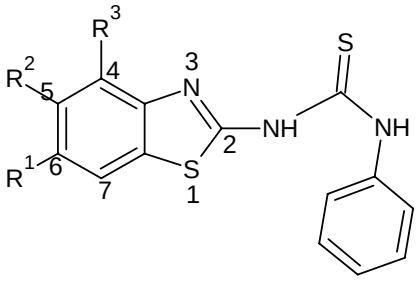
Result

Product	Result
Percentage yield	15.75 %
R_f	0.55
Melting point	220-222 ^oC

2.5 Result and Discussion

In order to obtain effective anti-inflammatory agents, new substituted 1-(Benzothiazol-2-yl)-3-Phenylthiourea compounds were successfully synthesized.. A series of total 15 compounds was prepared having new substituted 1-(Benzothiazol-2-yl)-3-Phenylthiourea moiety which are mentioned in Table 2.1.

Table 2.1 List of Target Compounds with IUPAC Name



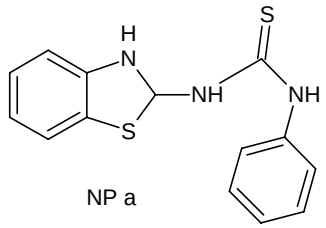
No. of Compound	Compound Code	IUPAC Name	Substitutions		
			R^1	R^2	R^3
1	NP-a	1-(1,3-benzothiazol-2-yl)-3-phenylthiourea	H	H	H
2	NP-x_4	1-(6-methoxy-1,3benzothiazol-2-yl)-3-phenylthiourea	-OCH_3	H	H
3	NP-x_3	1-(5-methoxy-1,3benzothiazol-2-yl)-3-phenylthiourea	H	-OCH_3	H
4	NP-x_2	1-(4-methoxy-1,3benzothiazol-2-yl)-3-phenylthiourea	H	H	-OCH_3
5	NP-y_4	1-(6-methoxy-1,3benzothiazol-2-yl)-3-phenylthiourea	CH_3	H	H
6	NP-y_3	1-(5-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea	H	CH_3	H
7	NP-y_2	1-(4-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea	H	H	CH_3
8	NP-z_4	1-(6-chloro-1,3-benzothiazol-2-yl)-3-phenylthiourea	Cl	H	H
9	NP-z_3	1-(5-chloro-1,3-benzothiazol-2-yl)-3-phenylthiourea	H	Cl	H
10	NP-z_2	1-(4-chloro-1,3-benzothiazol-2-yl)-3-phenylthiourea	H	H	Cl

The structures of the novel synthesized compounds of substituted 1-(Benzothiazol-2-yl)-3-Phenylthiourea were elucidated by IR, ^{1}H NMR, and MASS spectroscopic tools. The summery of physical data and spectral analysis data of the synthesized compounds shown in Table 2.2

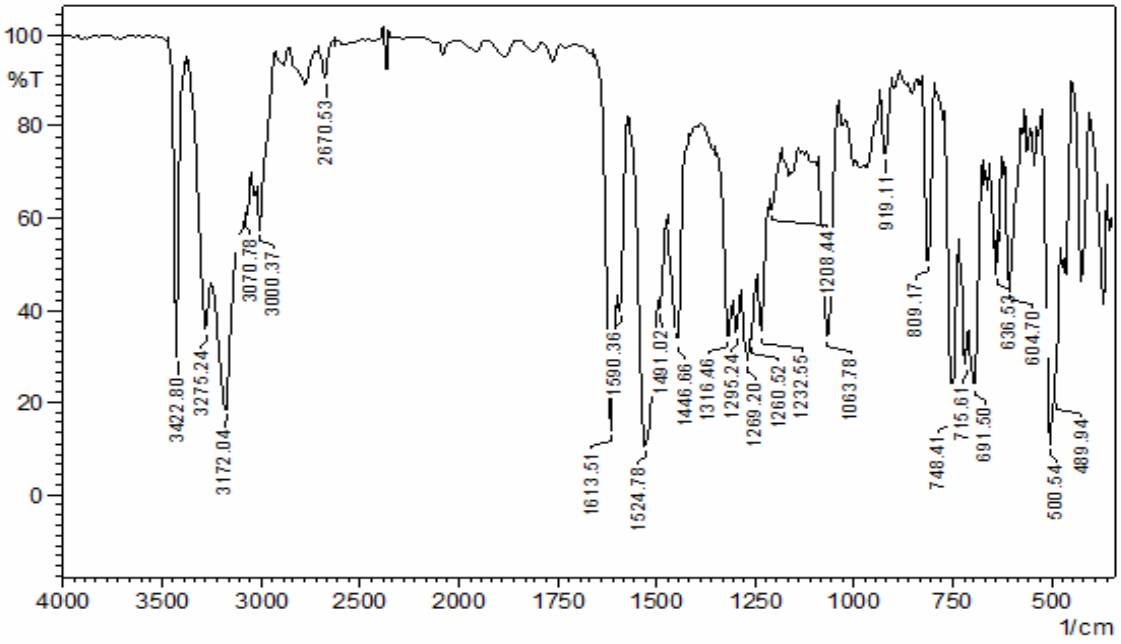
Table 2.2 Summary of The Physical Data of the Novel 2,5-disubstituted-1,3,4-thiadiazole compounds

Code of the compound	Chemical Formula	% Yield	R_f Value	Melting Point (^{o}C)	Molecular weight
NP-a	$C_{14}H_{11}N_3S_2$	49	0.64	150-154	285.387
NP-x_4	$C_{15}H_{13}N_3OS_2$	15.12	0.45	185-190	315.413
NP-x_3	$C_{15}H_{13}N_3\ OS_2$	20.45	0.51	165-170	315.413
NP-x_2	$C_{15}H_{13}N_3\,OS_2$	18.05	0.63	270-274	315.413
NP-y_4	$C_{15}H_{13}N_3S_2$	17.26	0.503	220-225	299.413
NP-y_3	$C_{15}H_{13}N_3S_2$	25.75	0.45	220-222	299.413
NP-y_2	$C_{15}H_{13}N_3S_2$	21.93	0.61	180-185	299.413
NP-z_4	$C_{14}H_{10}N_3S_2\ Cl$	23.25	0.74	220-224	319.823
NP-z_3	$C_{14}H_{10}N_3S_2Cl$	25.7	0.56	140-145	319.823
NP-z_2	$C_{14}H_{10}N_3S_2Cl$	30.62	0.66	240-245	319.823

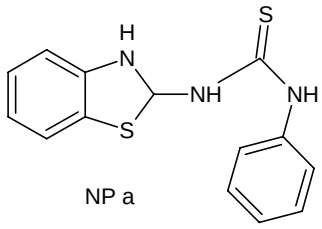
- **Summary of IR Spectral Data of novel NP-a compounds**



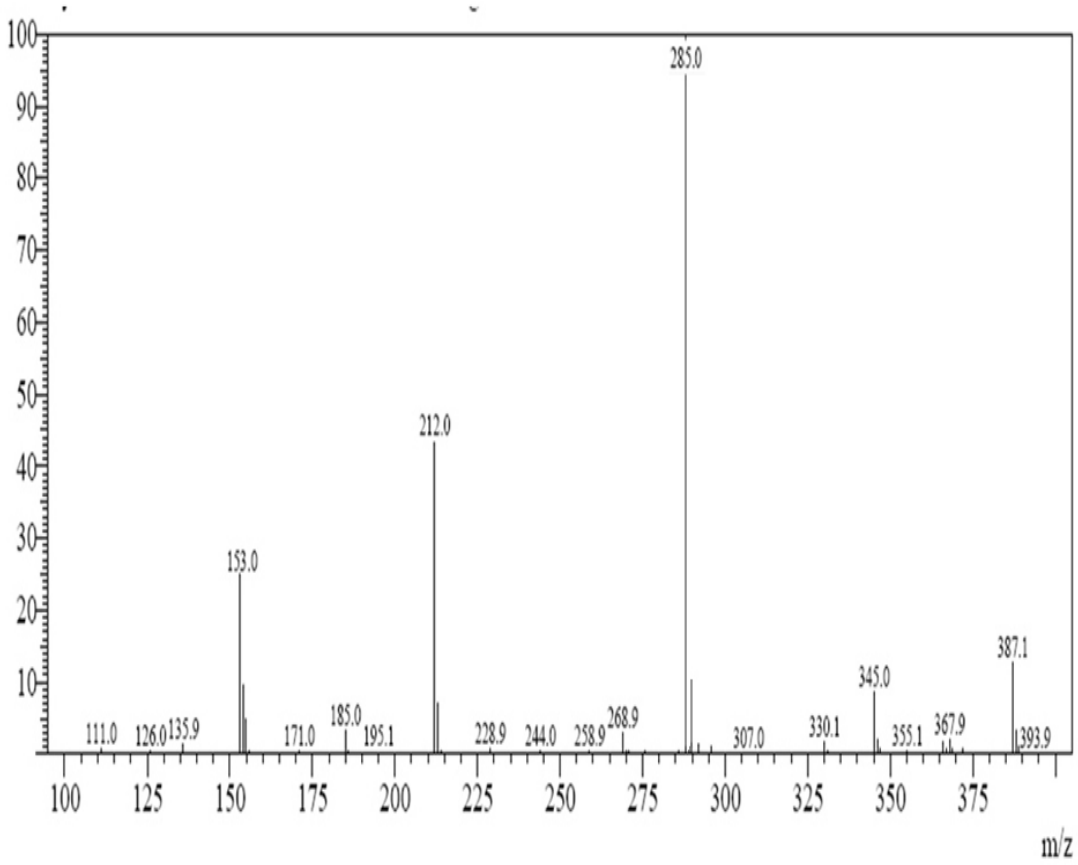
NP a



- **Summary of MASS Spectral Data of novel NP-a compounds**



NP a



- **Spectral analysis of synthesized compounds by means of IR Spectoscopy**

Functional group	Spectral data (KBr, Cm^{-1})				
	Compound Code				
	NP-a	**NP-y_3**	**NP-X_2**	**NP-Z_2**	**NP-X_3**
-N-H(stretch)	3422.80	3458.13	3381.33	3286.48	3423.41
Ar-C-H(sp^2 stretch)	3112.04	3186.18	3078.49	3184.26	3172.68
-C-H(sp^3 stretch)	-	2970.17	2901.64	-	2968.24
-C-N (stretch)	1316.46.	1305.72	1352.14	1321.28`	1317.29
-N-H (bending)	1524.78	1504.37	1527.67	1535.69	1519.80
-C=S(stretch)	715.41	765.69	712.72	727.11	748.33
-C=N(stretch)	1590.36	1606.59	1627.01	1591.16	1591.16
-C-S(stretch)	1260.52	1228.57	1269.20	1227.79	1230.50
-C-O(stretch)	-	-	1201.20	-	1230.50
-C-Cl(stretch)	-	-	-	686.61	-

- **Spectral analysis of synthesized compounds by Mass Spectoscopy**

Compound Code	Molecular Weight	ESI-MS(m/z)
NP-a	285.38	M^+(m/z)= 285.0
NP-x_2	315.41	M^+(m/z)=315.0

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Experimental Work-II

3.1 Introduction

Inflammation is protective and defense mechanism of the body. During inflammatory conditions various pathological changes are take place. The production of active inflammatory mediators is triggered by microbial products or by host proteins, such as proteins of the complement, kinins and coagulation systems that are themselves activated by microbes and damaged tissues**.**

In preclinical studies, these changes can be induced by administration of the agents causing inflammation. For purpose of evaluation of anti-inflammatory activity we have focused on some in vivo animal models which are commonly used in laboratory practice.

Numerous reports have been demonstrated in increase incidence of inflammatory condition. It is one of the most important natural defence mechanisms. Its main purpose is to destroy the injurious agent and/or to minimize its ill effects by limiting its spread. Though inflammation is protective in some situations if untreated can lead to serious complications. Inflammation is the dynamic pathological process consisting of a series of interdependent changes

3.2 Signs of inflammation:

The 4 cardinal signs of inflammation are given by roman writer Celsus in 1st century A.D. as: rubor (redness), tumor (swelling), calor (heat), dolor (pain), fifth sign of inflammation function laesa (loss of function) was later added by Virchow. The word inflammation means burning but burning is not only one of the signs of inflammation (Harsh, 2002).

- **Redness (Rubor):** Due to gross and persistent dilatation of arterioles, capillaries and venules in the injured area.
- **Swelling (Tumour):** Due to increased permeability of small blood vessels which allows the exudates to escape into the tissues of the damaged area.
- **Heat (Calor):** Due to considerable increase in blood flow.
- **Pain (Dolar):** Due to the release of certain endogenous chemical substances such as bradykinin, 5HT and certain prostaglandins.

3.3 Acute Inflammation models

3.3.1 Carrageenan-induced Paw Edema in Rats

This model is based on the principle of release of various inflammatory mediators by carragenan. Edema formation due to carrageenan in the rat paw is biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasation, increased tissue water and plasma protein exudation along with neutrophil extravasation, all due to the metabolism of arachidonic acid. The first phase begins immediately after injection of carrageenan and diminishes in two hours. The second phase begins at the end of first phase and remains through third hour up to five hours.[4,5,6]

3.3.2 Histamine Induced Paw Edema in Rats

Histamine induced paw edema is said to occur in earlier stage in mounting of vascular of the vascular reaction in the chemically induced inflammation. In this, swelling occurs primarily due to action of histamine. Generally histamine is released following the mast cell degranulation by number of inflammatory mediators including substances P interleukin-1 (IL-1). This is likely to evoke the release of neuropeptide as well as release of prostaglandins and monohydroxy eicosatetranoic-acid from endothelial cell leading to hyperalgesia and other pro-inflammatory effects.[8]

3.3.3 Acetic Acid-Induced Vascular Permeability

The test is used to evaluate the inhibitory activity of drugs against increased vascular permeability which is induced by acetic acid by releasing inflammatory mediators.[10] Mediators of inflammation, such as histamine, prostaglandins and leukotrienes are released following stimulation of mast cells. This leads to a dilation of arterioles and venules and to

an increased vascular permeability. As a consequence, fluid and plasma protein are extravaseted and edemas are formed.

3.3.4 Xylene Induced Ear Edema (Thickness and weight parameter)

In xylene induced ear edema model, the application of xylene induces neurogenous edema. It is partially associated with the substance P. Substance P is an undecapeptide, which is widely distributed in the central and peripheral nervous system and it functions as a neuro-transmitter or neuro-modulator in variety of physiological processes. Substance P is released from the neurons in the midbrain in response to stress, where it facilitates dopaminergic neurotransmission from sensory neurons in the spinal cord in response to noxious stimuli where it excites dorsal neurons. In the periphery, release of substance P from sensory neurons causes vasodilatation and plasma extravasations suggesting its role in neurogenous inflammation. Thus it can cause the swelling of ear in the mice.[11]

3.3.5 Arachidonic Acid-Induced Ear Edema

This model is based on the principle of metabolism of arachidonic acid by COX (Cyclooxigenase) leads to the generation of PGs and thromboxanes that mediate pain and edema associated with inflammation and inhibition of these mediators by test drug is evaluated.[12]

3.3.6. Phorbol Myristate Acetate-Induced Ear Edema in Mice

Phorbol myristate acetate (PMA) is a protein kinase C (PKC) promoter, which induces the formation of free radicals in vivo. It has been also demonstrated that pre-treatment of mouse skin by antagonists of PKC suppresses inflammation and ROS (reactive oxygen species). This species involved in the synthesis of mediators and regulate the production of TNFα this in turn stimulate PLA_2 activity, which releases arachidonic acid from phospholipids and stimulate the activity of COX and LOX (Lipoxygenase) these enzyme involved in release different inflammatory mediators.[13]

3.3.7 Oxazolone-induced Ear Edema in Mice

The oxazolone-induced ear edema model in mice is a model of delayed contact hypersensitivity that permits the quantitative evaluation of the topical and systemic anti-inflammatory activity of a compound following topical administration[15]

The above mentioned models have given broad spectrum for the evaluation of the anti-inflammatory activity. In different models, the inflammation has produced by different inducers by releasing inflammatory mediators. Each is having different mechanism of action for producing inflammation either by increased in vascular permeability, the infiltrations of leukocytes from the blood into the tissue or granuloma formation and tissue repair.

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, Aerosil®, sulfated polysaccharides like carrageenan or naphthoylheparamine. For producing edema, histamine, xylene, arachidonic acid, phorbol myristate acetate, oxozolone, croton oil and formalin are also used.

For evaluating the most effective and widely used model for inflammation is carrageenan-induced paw edema, Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea moss, Chondrous crispus. Its use as an endemogen was introduced by Winter et.al. Carrageenan initially releases histamine and serotonin followed by release of prostaglandins, protease and lysosomes producing edema.

3.4 Experimental protocol

3.4.1 Animals

Wistar rat of either sex were procured from Zydus Research Center, ahmedabad which were used in the present study and were maintained in colony cages at 25±2°C and relative humidity of 45-55% under a 12 hrs light and dark cycle. They were fed

standard animal food. The institutional animal ethics committee approved the protocol adopted for the experimentation of animals**. (Project Number: IPS/PCHEM/MPH10/003)**

3.4.2 Test compounds

The test compound and standard drugs were administered in the form of suspension 0.5%W/V sodium carboxy methyl cellulose (CMC) as vehicle by the same route of administration. Diclofenac sodium as dose of 13.5mg/kg of body weight and nimesulide as dose 20mg/kg of body weight were administered orally as reference of drug comparison. The test compounds as dose of 20mg/kg of body weight were also administered orally.

3.4.3 SCREENING METHOD

For this activity overnight starved wistar rats of 250-350 g were divided into 8 groups of six animals in each group:

Group I : Normal control
Group II : Treated with standard Diclofenac (13.5 mg/kg orally).
Group III : Treated with NP-a (20 mg/kg orally).
Group IV : Treated with NP-x_2 (20 mg/kg orally)
Group V : Treated with NP-x_3 (20 mg/kg orally).
Group VI : Treated with NP-x_4 (20 mg/kg orally).
Group VII : Treated with NP-y_2 (20 mg/kg orally).
Group VIII: Treated with NP-y_3 (20 mg/kg orally).
Group IX : Treated with NP-y_4 (20 mg/kg orally).
Group X : Treated with NP-Z_2 (20 mg/kg orally).
Group XI : Treated with NP-Z_3 (20 mg/kg orally).
Group XII: Treated with NP-Z_4(20 mg/kg orally).

All the animals were injected subcutaneous with 0.1 ml. of 1% freshly prepared Carrageenan suspension in normal saline, into subplantar region of right hind paw to induce inflammation.

3.4.4 Procedure:

The acute inflammation was produced according to the method of Winter *et al.* (1962) in all the test animals. The animals were received vehicle/test drug, Diclofenac sodium and sixty minute later all the animals were challenged by injecting of 0.1 ml of 1% freshly prepared carrageenan suspension into the sub plantar region of the right hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in water up to this mark. The paw volume was measured plethysmographically before injection, immediately after injection and again at 1, 2, and 4 hours after challenge with carrageenan. The percentage protection of oedema was calculated for each group on each hour recording as follows:

Percentage protection = $[(Vo - Vt) / Vo] \times 100$

Where, Vo = Volume of the paw of control at time‘t’.

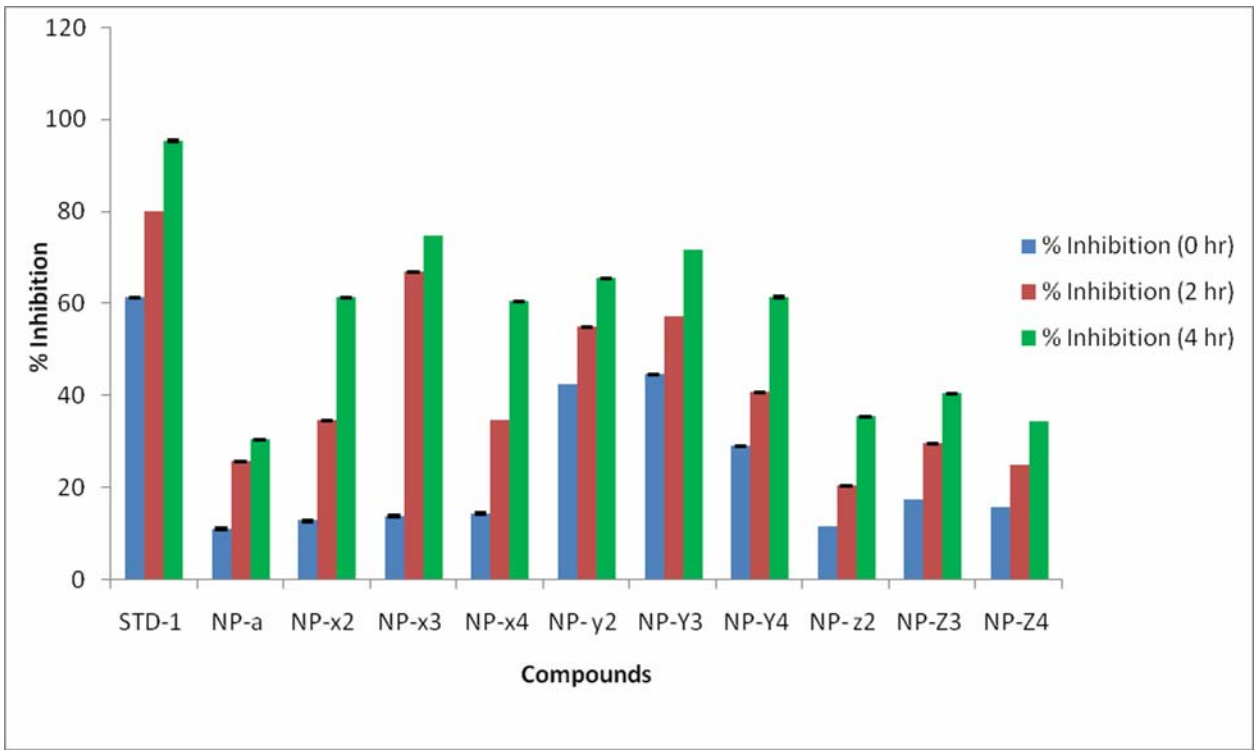
Vt = Volume of the paw of drug treated at time‘t’.

3.4.5 Results and Discussion

Anti-inflammatory activity of compounds

Drugs	%Inhibition (0 hrs)	% Inhibition (2hrs)	% Inhibition (4hrs)
Control	^{00}±0.06	00±0.09	00±0.09
STD-1	61.27±0.01	100±0.06	29.55±0.09*
NP-a	10.97±0.05	1.96±0.01	70.35±0.02
NP-x_2	12.71±0.01	13.41±0.07	61.23±0.03*
NP-x_3	**13.72±0.02**	**66.79±0.01**	**64.74±0.02***
NP-x_4	62.28±0.02	14.56±0.04	60.48±0.04*
NP- y_2	52.40±0.06	24.81±0.03	65.53±0.02*
NP-Y_3	73.62±0.09	37.18±0.07	71.64±0.03*
NP-Y_4	28.95±0.02	40.68±0.09	61.36±0.06
NP- z_2	41.54±0.04	59.06±0.01	35.45±0.05
NP-Z_3	57.37±0.05	89.52±0.02	40.36±0.02*
NP-Z_4	75.46±0.07	15.65±0.03	34.36±0.07

Values are mean ±SEM,(n=6), *p<0.05,students t-test as compared to control



The synthesized compounds show anti-inflammatory activity. Substitude benzothiazole derivatives have shown good anti-inflammatory activity than unsustituted benzothiazole derivative.

Amongst all NP-X_3 (1-(5-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea) has good anti-inflammatory activity other than other derivative. NP-a (1-(1,3-benzothiazol-2-yl)-3-phenylthiourea), NP-x_2 1-(4-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea, NP-x_4(1-(6-methoxy-1,3-benzothiazol-2-yl)-3-phenylthiourea),NP-y_4(1-(6-methyl-1,3-benzothiazol-2-yl)-3-phenylthiourea) also found to be good anti-inflammatory activity.Substitution at 3 position of 1-(benzthiazol-2-yl)-3-phenylthiourea more active than 2 and 4 position of 1-(benzthiazol-2-yl)-3-phenylthiourea.

Methyl and Methoxy groups substituted 1-(benzthiazol-2-yl)-3-phenylthiourea more anti-inflammatory activity than halogen substituted 1-(benzthiazol-2-yl)-3-phenylthiourea derivative.

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Summary

Inflammation involving the innate and adaptive immune systems is a normal response to infection. However, when allowed to continue unchecked, inflammation may result in autoimmune or auto inflammatory disorders, neurodegenerative disease, or cancer. A variety of safe and effective anti-inflammatory agents are available, including aspirin and other nonsteroidal anti-inflammatories, with many more drugs under development

The NSAIDs are among the most widely used of all therapeutic classes of drugs. These agents have been understood for many years to act peripherally to reduce the production of prostaglandins that sensitize nerve endings at the site of injury. This effect occurs due to inhibition of the cyclooxygenase (COX) enzyme that converts arachidonic acid liberated from the phospholipid membrane by phospholipases to prostanoids such as prostaglandins Certain adverse effects with NSAIDs has been the development of selective COX2 inhibitors. This strategy targets the production of prostaglandins specifically involved in pain and inflammation while sparing constitutive prostaglandins that exert important physiological roles such as maintaining the integrity of the gastric lining and normal renal function. A further enzyme, COX-3, has recently been described; this has a prominent central distribution, is selectively inhibited by acetaminophen, and is potently inhibited by NSAIDs. NSAIDs are cleared from the blood stream by the kidney NSAIDs may also cause stomach upset or possibly ulcers.

Growing evidence suggests several members of the TRP superfamily are involved in the detection of acute noxious, mechanical and chemical as well as in inflammatory pain. Recently, it was reported selective TRPV1 antagonist with good oral bioavailability and effectiveness in reducing hyperalgesia and allodynia in animal models. Furthermore, encouraging pharmacodynamic effects, including an effect on heat pain threshold and a reduction in UV burn-induced flare in the skin, indicating on target activity versus inflammatory hyperalgesia, have been reported in Phase 1 healthy volunteer studies. This demonstrates that this compound is pharmacologically active in humans at the dose tested and provides further confidence in the progression and design of clinical trials to assess the efficacy of TRPV1 antagonists in patients. Intense investigation is being carried out on thiourea compounds owing to their anti-

inflammatory activity. In the recent years many article shows that thiourea containing compounds give good anti-inflammatory activity.

Prompted by these observations, as a part of present study aimed at developing new biologically active (Benzothiazole-2-yl)-3-phenylthiourea Derivatives were synthesized. The target molecules were synthesized according to the steps reported in literature and all the reaction steps were optimized in context of present study.

(Benzothiazole-2-yl)-3-phenylthiourea Derivatives were synthesized from the substituted aromatic amines through the intermediate substituted 1-phenylthiourea oxidation by bromine water in acidic medium then reacted with Phenylisothiocyanate.

The structures of the synthesized compounds were established by ^{1}H NMR, MASS and IR spectroscopic techniques. The data of the physical characterization of the compounds are given below in table.

Table 4.1 Physical characterization of synthesized compounds

Code of the compound	**Chemical Formula**	**% Yield**	**R_f Value**	**Melting Point (^{o}C)**	**Molecular weight**
NP-a	$C_{14}H_{11}N_3S_2$	49	0.64	150-154	285.387
NP-x_4	$C_{15}H_{13}N_3OS_2$	15.12	0.45	185-190	315.413
NP-x_3	$C_{15}H_{13}N_3\ OS_2$	20.45	0.51	165-170	315.413
NP-x_2	$C_{15}H_{13}N_3\,OS_2$	18.05	0.63	270-274	315.413
NP-y_4	$C_{15}H_{13}N_3S_2$	17.26	0.503	220-225	299.413
NP-y_3	$C_{15}H_{13}N_3S_2$	25.75	0.45	220-222	299.413
NP-y_2	$C_{15}H_{13}N_3S_2$	21.93	0.61	180-185	299.413
NP-z_4	$C_{14}H_{10}N_3S_2\ Cl$	23.25	0.74	220-224	319.823
NP-z_3	$C_{14}H_{10}N_3S_2Cl$	25.7	0.56	140-145	319.823
NP-z_2	$C_{14}H_{10}N_3S_2Cl$	30.62	0.66	240-245	319.823