CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.3345871

Available online at: http://www.iajps.com *Research Article*

SYNTHESIS AND SCREENING OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF NOVEL BENZTRIAZOLE DERIVATIVES

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Article Received: May 2019 **Accepted:** June 2019 **Published:** July 2019

Abstract:
Benzotriazole is a lead molecule for designing potential bioactive molecule. Benzotriazole and its derivatives exhibits anti inflammatory, analgesic, antimicrobial and anticonvulsant activities. 1,2,4 triazole is one of the most biologically active classes of compounds possessing wide spectrum of activities including anti-inflammatory, analgesic, CNS stimulants, sedatives, antianxiety, antimicrobial agents and antimycotic agents. In this study, we planned to synthesize various Schiff's bases of 5 [1H,1,2,3 benzotriazol 1yl methyl] 4 [substituted amino]4H-1,2,4 triazole-3 – thiol. Finally characterization and pharmacological screening of synthesized derivatives were done for its antifungal activity.

Keywords: *Benzotriazole, antiinfammatoryl, Schiff's bases.*

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Please cite this article in press ***Padmaja Devi M.S*** *et al.,* ***Synthesis And Screening Of Analgesic And Anti-inflammatory Activity Of Novel Benztriazole Derivatives.,*** *Indo Am. J. P. Sci, 2019; 06(07).*

INTRODUCTION:

Azoles are five membered Heterocyclic compounds with two or more heteroatoms in which at least one is nitrogen. Azoles are found widely in natural sources and there are several drugs available which contain azole ring. Benzotriazole has various biological activities and Triazole is a polyfunctional heterocyclic compound, to exhibit biological activity in mammals.[1] Schiff bases and Mannich bases of Benzotriazole are known to possess a wide range of pharmacological properties including antibacterial, anticonvulsant, anti-HIV, antifungal and antiviral activity. It has been reported that Benzotriazole has anti-inflammatory l properties.

Inflammation:

Inflammation is the first response of the immune system to infection or irritation and may be referred to as the innate cascade. Inflammation is characterized by the following quintet : redness [rubor] , heat [calor] ,swelling [tumour] and pain [dolor] and the dysfunction of the organs involved. Inflammation is divided into acute and chronic patterns . Acute inflammation is rapid in onset , is of relatively short duration , lasting for minutes , several hours ,or a few days ; its main characteristics are the exudation of fluid and plasma proteins [edema] and the emigration of leukocytes , predominantly neutrophils . Chronic inflammation is of longer duration and is associated histologically with the presence of lymphocytes and macrophages, the proliferation of blood vessels , fibrosis and tissue necrosis . Many factors modify the course and morphologic appearance of both acute and chronic inflammation.

Pain and inflammation are essential life processes .Pain is a warning sign that something is wrong in the body ; inflammation is a protective mechanism that allows healing to occur . Histamine, eicosanoids, leukotrienes, platelet - activating factor, bradykinin, nitric oxide, neuropeptides and cytokinins are the mediators of inflammation.

The need for anti –inflammatory drugs rises when the inflammatory response is inappropriate ,aberrant or sustained and when it causes destruction to the tissue.

The analgesic effect has a ceiling effect , meaning that higher dose does not result in enhanced pain control .At lower doses NSAIDs are good for mild to moderate pain and higher doses have an anti – inflammatory effect .At lower doses NSAIDs are good for mild to moderate pain and higher doses have anti inflammatory effect.

Prostaglandins:

They are a group of naturally occurring 20 carbon fatty acid derivatives produced by the oxidative metabolism of arachidonic acid.Other so called eicosanoids , produced in the complex biological oxidation scheme called arachodonic acid cascade are thromboxane A2 (TXA2) ,the luekotrienes and the highly potent antithrombotic agent Prostacyclin.The related thromboxanes , were found in blood platlets and the leukotrienes ,whose biological effects include respiratory ,vascular and intestinal activities.

Eicosanoid biosynthesis:

The key precursor in eicosanoid biosynthetic pathways is arachidonic acid that is formed from linolenic acid through reactions catalyzed by a series of enzymes that dehydrate fatty acids. Cells store arachidonic acid as a component of membrane phospholipids such as phospoinositol. In response to appropriate stimuli , arachidonic acid is liberated from the storage lipid by an enzymatic reaction catalyzed by phospolipase A2. There are a number of drugs such as glucocorticoids that modulate phospolipase A2 . The conversion of free arachidonic acid to prostaglandins and other eicosanoids is intiated by oxidative enzymes of the cyclooxigenase and lipoxygenase families.

Cylooxygenases:

Cyclooxygenase (COX), also known as prostaglandin G\H synthase, is a membrane bound enzyme responsible for the oxidation of arachidonic acid to prostaglandins. Two cyclooxygenase isoforms have been identified and are referred to as cox-1 and cox - 2. Cox 1 and cox-2 are of similar molecular weight and having 65% aminoacid sequence homology and near identical catalytic sites , Cox -1 is normally expressed in the gastrointestinal tract ,Kidneys and platlets.It appears to be responsible for mediating the production of thromboxane A2 and prostaglandins . The isoenzyme cox2 is primarily associated with inflammation .Cytokines and growth factors increase the expression of COX -2 mainly at inflammatory sites , producing prostaglandins that mediate inflammation,pain and fever.

Cyclooxygenase stereospecifically add two molecules of oxygen to arachidonic acid to form the unique bicyclic endoperoxide PGG2 is then reduced by the cyclooxygenase to yield alcohol PGH2.PGH2 serves as a " branch point" for specific enzymes leading to

the formation of prostacyclin (PGI2) ,the various prostaglandins as well as the thromboxanes

Inhibition of Cyclooxygenase can occur by different mechanisms

1. Irreversible inhibition –e.g : aspirin causes acetylation of active site
2. Competitive inhibition -e.g: Ibuprofen acts a competitive substrate
3. Reversible, non competitive inhibition – e.g. Paracetamol has a free radical trapping action that interferes with the production of hydroperoxidase, which are believed to have as essential role in cyclooxygenase activity.

Cyclooxygenase -1:
Pico et al . reported the three dimensional structure of cox -1 providing new information for the action of cox inhibitors .Cox -1 is expressed in nearly all tissues including the colon ,kidney, spleen,stomach ,liver, lungs ,heart and brain. In the kidney and the stomach prostanoids synthesized by cox 1 act as vasodilators. In kdneys prostanoids help to maintain renal plasma flow and glomerular filtration during periods of systemic vasoconstriction. COX -1 in platelets,generate hromboxane which plays a key role in mediating platelet aggregation.

Cyclooxygenase-2:
The COX-2 enzyme is dimeric and each monomer consists of catalytic domain , and a membrane domain connected by the N-terminal EGF domain. The membrane binding domain forms a cannel which leads to the active site. The active site of COX -2 is slightly larger and can accommodate bigger structures than those which are able to reach the active site of COX -1 . Selectivity for COX -2 inhibitors can be conferred by replacing the His 513 and Ile 523 of COX-1 with Arg and Val respectively . This replacement removes the constriction at the mouth of the secondary side channel and allows the more bulky COX -2 selective inhibitors. The COX active site is a long ,hydrophobic channel . Aspirin like drugs inhibit Cox -1 by excluding arachidonate from the upper portion of the channel .Tyrosine 385 and serine 530 are at the apex of long active site. Aspirin irreversibly inhibits Cox -1 by acetylation of the Serine 530 ,thereby excluding the accesss of arachidonic acid.

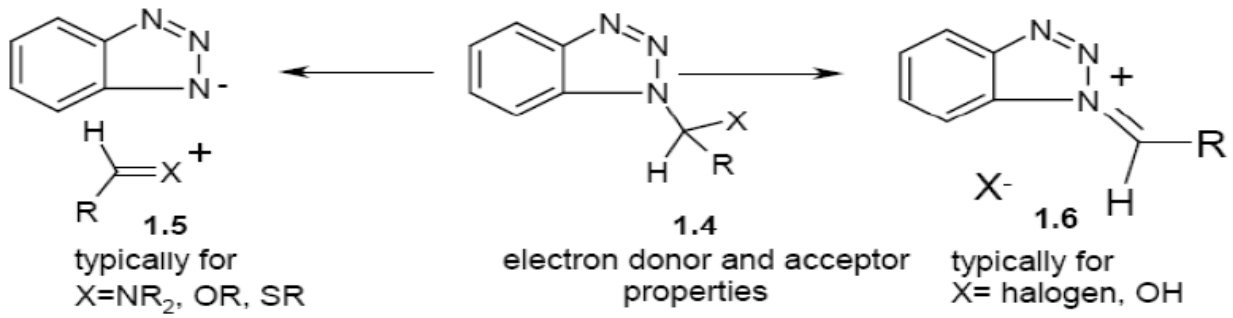
Like the other COX enzymes , COX -3 is involved in the synthesis of prostaglandins and plays a role in pain and fever . Unlike COX-1 and COX -2 , COX -3 appears to have no role in inflammation .The activity of Cox-3 is inhibited by the drug acetaminophen ,which has little effect on the other two COX enzymes.

Cyclooxygenase -3:
Binding site of CYCLOOXYGENASE
The cyclooxygenase active site is a channel that is lined with hydrophobic residues and protrudes towards the centre of the major globular domain of the enzyme . The channel contains areas with high electron density interacting with the aromatic system of the ligands. The phenyl ring is surrounded by hydrophobic residue Leu 352, Tyr355,Val 523 and backbone of serine 353.

The drug development process of anti-inflammatory and analgesic drugs of importance because inflammatory diseases and pain are the most common of diseases suffered by humanity. There is a regular search for novel anti inflammatory and analgesic drugs. As a contribution to the drug development process , design and synthesis of 4 substituted amino 3-mercapto triazole derivative of Benzotriazole as anti-inflammatory ,analgesic and antimicrobial agents.

N – substituted Benzotriazole exists as two isomers : 1- and 2- substituted. They typically exist in equilibrium and often show the same reactivity.. Benzotriazolyl moiety similarly provides activation to allow for deprotonation of an alpha C-H. Detachment of a proton in the 1- position of Benzotriazole most likely occurs due to the presence of two electron attracting pyridine like nitrogen atoms which increases the acidity of said proton. This type of activation to proton loss is an extremely important attribute of benzotriazole as it allows for subsequent reactions with electrophiles to form a variety of interesting compounds.[2]

Furthermore, benzotriazole possesses both electron – donor and electron -acceptor properties and because of this compounds with an alpha hetero atom (typically N,O, and S) attached to a Benzotriazole nitrogen can ionize in two ways. Ionization may form the benzotriazole anion and an immonium, oxonium, or thionium cation or result in the loss of the heteroatom substituent.



Electron donor and electron acceptor properties of benzotriazole

Review of literature:

Biological activity of benztriazole:

R Calvino *et al* [23](1980) synthesized 1-(beta-dimethylaminoethoxy) benzotriazole, 1-(gamma-dimethylaminopropoxy) benzotriazole and their N-oxide isomers 3-(beta-dimethylaminoethyl) benzotriazole1-oxide and 3-(gamma-dimethylaminopropyl) benzotriazole 1-oxide. The structure of these compounds has been confirmed by N.M.R. spectroscopy; the structures of the N-oxide derivatives were also elucidated by chemical reduction to the parent benzotriazoles. The two benzotriazoles were also tested for antiinflammatory activity in the rat, and for analgesic and depressant activity on the CNS of the mouse.

Nagarajan K *et al* [91](1986**)** synthesized several benzothiazole and benzotriazole carbamate analogues. All the products are found to be inactive in curing hamsters of N. americanus infection.

Boido A *et al* [18] (1989) prepared A number of N-oxides of 4'-(benzotriazol-2-yl)-phenylalkanoic and -phenoxyalkanoic acids bearing various substituents on position 6 of benzotriazole together with 4'-(benzotriazol-2-yl) phenylacetic acid and subjected to a wide pharmacological screening. Several compounds exhibited significant antiinflammatory and diuretic activities, while one was endowed with antihypertensive activity.

PurohitM, and Srivastava SK [110] (1992) synthesized some new chlorosubstituted phenoxy acetyl and propionyl benotriazoles and screened for their anti-inflammatory, analgesic, antibacterial and antifungal properties. Trichlorophenoxy acetyl benzotriazole exhibited better anti-inflammatory activity than its propionyl derivative. The 2,5-dichlorophenoxy-acetyl benzotriazole showed moderately better analgesic activity among the series. All the compounds showed mild to moderate antibacterial and antifungal activity.

Cappello B *et al* [24] (1993) synthesized a series of benzotriazole derivatives and tested in order to determine their activities for muscarinic receptor subtypes (M1, M2 and M3). The compounds showed antimuscarinic activity. Some of them displayed interesting selectivity profiles (M2/M1 and M2/M3).

G Caliendo *et al* [21] (1995) reported the synthesis and the pharmacological screening of a series of novel 1- and 2-[2-[4-(X)-1-piperazinyl] ethyl] benzotriazoles and 1-[3-[4-(X)-1-piperazinyl] propoxy] benzotriazoles, which are structurally related to trazodone. Antiserotonergic, antiadrenergic and antihistaminic in vitro activity and in vivo analgesic action were described. Some of the investigated compounds showed overall pharmacological profiles similar to that of the antidepressant trazodone.

G Caliendo *et al* [22] (1996) carried out the Structure-affinity relationship studies on benzotriazole derivatives binding to 5-HT receptor subtypes. Several of the compounds tested show interesting selectivity profiles. Structure—affinity relationship data suggest that not all the compounds exhibit the same binding mode at the 5-HT_{2A} and 5-HT_{1A} receptors.

Yoshimi Hirokawa *et al* [138] (1998) synthesized a novel series of 6-methoxy-lH-benzotriazole-5-carboxamide derivatives and their antiemetic and gastroprokinetic activities were evaluated. Among them, N-(l-ethylhexahydroazepin-3-yl)-, N-(1-ethyloctahydroazocin-3-yl)- and N-(1-ethyloctahydroazonin-3-yl)-6-methoxy-lH-benzotriazole-5-carboxamides showed a potent antiemetic activity along with gastroprokinetic activity.

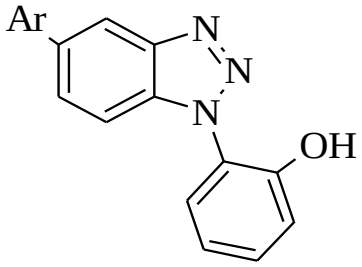
Janina Karolak *et al* [57] (1999) carried out the Structure-Activity Relationship studies of 4-[(1)-(Benzotriazolyl) alkyl]-1-(2-methoxyphenyl) piperazines with a different Pharmacological Activity. Crystallographic data, as well as the conformational analysis indicated that the investigated compounds could adopt a variety of conformations of small energy difference.

gatti *et al* [12] (2000) reported the synthesis and pharmacological evaluation of a series of 5-substituted-triazolyl benzotriazoles and the corresponding series of 5-substituted-triazolyl-benzimidazolones, as potential activators of the big-conductance calcium-activated potassium channels (BK (Ca)). Compounds with a benzotriazole ring showed full efficacy, with vasorelaxing properties and potency parameters a little lower than that of the reference compound.

Habib N.S *et al* [52] (2000) synthesized four novel series of 1H-benzotriazole derivatives; namely 1-[(3,4-disubstituted thiazolin-2-ylidene) hydrazinocarbonyl] methyl-1H-benzotriazoles; 1-[3-substituted 5-ethoxycarbonyl-4-methyl thiazolin-2-ylidene) hydrazinocarbonyl] methyl-1H-benzotriazoles; 1-[(3-substituted-4-oxo-thiazolidin-2-ylidene) hydrazinocarbonyl] methyl-1H-benzotriazoles; and 1-[(5-substituted aminothiadiazol-2-yl) methyl]-1H-benzotriazoles by cyclization of the key intermediates. Some compounds showed antiinflammatory activity comparable to indomethacin, and they also demonstrated minimum ulcerogenic activity.

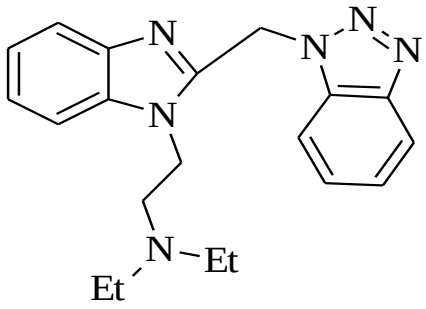
Romano Silvestri *et al* [114] (2000) designed ethyl 1-[(1*H*-benzotriazol-5(6)-yl) sulfonyl]-1*H*-pyrrole-2-carboxylate) as novel HIV-1 reverse transcriptase non-nucleoside inhibitors using structure-based computational methods. Although these compounds were inactive in the cell-based assay, they inhibited the target enzyme with micromolar potency (IC_{50}s=9 μM).

G Biagi, *et al* [16] (2001) synthesised a series of new 5-substituted-1-(2-hydroxybenzoyl)-benzotriazoles, which have been tested for their activity as possible activators of potassium channels. Most remarkable effects were recorded for 2-hydroxy benzoyl Benzotriazole .They have cardioprotective activity.

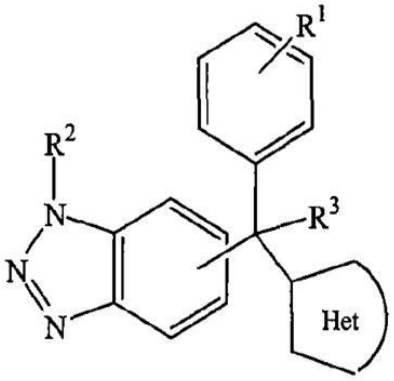


Boido A *et al* [17] (2001) prepared thirteen [(aryl/heteroaryl-piperazinyl) alkyl]benzotriazoles as potential trazodone- and buspirone-like drugs. The synthesized compounds displayed from moderate to good affinity to theserotonin 5-HT1A receptor and only modest or poor affinity to the dopamine D2 receptor, similar to buspirone. In a general pharmacological screening. Some compounds strongly inhibited the guinea pig ileum contractions, induced either electrically or by several agonists. At higher concentrations also the spontaneous tone of the guinea pig trachea was reduced and showed good analgesic activity. The same at 30 mg ⁄ kg p.o. also displayed antihypertensive activity probably related to calcium channel blockade and adrenergic $\alpha_1$antagonism.

Nicholas A. Meanwell *et al* [94] (2003) reported the Structure–activity relationships surrounding the dialkylamino side chain of a series of benzotriazole-derived inhibitors of respiratory syncytial virus. The results indicate that the topology of the side chain is important but the terminus element offers considerable latitude to modulate physical properties.



BERWAER *et al* (2004) synthesized a group of benzotriazole derivatives, that are potent cannabinoid-CB_i modulators (known as antagonists or inverse agonists), useful in the treatment of obesity, psychiatric and neurological disorders, as well as other diseases involving cannabinoid-CB_1 neurotransmission.



R^I is hydrogen, halo, trifluoromethyl, C_{1-4} alkyl, C_{1-4} alkyloxy- or C_{1-4} alkyloxycarbonyl**;**

R^2 is hydrogen, phenyl, C_{3-7}cycloalkyl or C_{1-6}alkyl optionally substituted with Ar^1**;**

R^3 is hydrogen, hydroxyl or C_{1-6}alkyl;

Ar^1 is phenyl or phenyl substituted with up to three halo substituents; and

Het represents a monocyclic 5 or 6 membered partially saturated or aromatic heterocycle selected from furanyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyrimidinyl, pyridinyl, pyrazinyl, triazinyl, pyridazinyl, 2H-pyranyl or 4H-pyranyl wherein said heterocycle is optionally substituted with C_{1-6}alkyl.

and

Carta A *et al* [25] (2004) Synthesized a new series of variously substituted 3-aryl-2-[1H(2H)-benzotriazol-1(2)-yl]acrylonitriles and tested for antiproliferative antitubercular activity. The compound E-2-(5,6-dimethyl-1H-benzotriazol-1-yl)-3-(3-nitrophenyl)acrylonitrile found to be more potent than 6-MP on all cell lines.

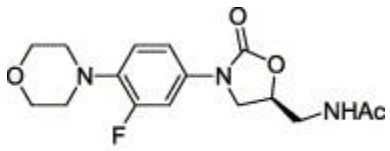
Katarzyna Kopańska *et al* [64] (2004) synthesized Chloro-, bromo- and methyl- analogues of 1H-benzimidazole and 1H-benzotriazole and their N-alkyl derivatives and tested in vitro against the protozoa Acanthamoeba castellanii. The results indicate that 5,6-dimethyl-1H-benzotriazole and 5,6-dibromo-1H-benzotriazole have higher efficacy than the antiprotozoal agent chlorohexidine

Alessandro Boido *et al* [6] (2005) synthesized series of pharmacologically interesting 1- and 2-[(4-arylpiperazin-1-yl) alkyl]-1,2,3-benzotriazoles, and subjected to various biological studies to identify structure-activity relationships (SAR). The new compounds were found to exhibit good non-selective binding affinity towards the α_1 adrenoreceptor comparable to that of prazosin.

Maria Bretner *et al* [82] (2005) synthesized N-alkyl derivatives of 1H-benzotriazole and 1H-

benzimidazole and tested for antihelicase activity against enzymes of selected Flaviviridae. N-alkylation of this benzotriazole compound enhanced inhibitory activity and selectivity towards the helicase activity of HCV NTPase/helicase. The most active were the 2-methyl, 2-ethyl and 2-propyl derivatives. Derivatives of the benzotriazole in which hydroxyethyl or chloroethyl replaced the alkyl substituents lost their inhibitory activity.

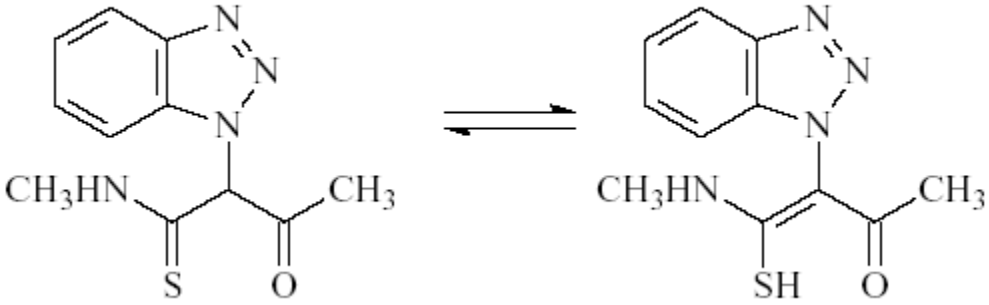
Prasad P. Dixit *et al* [107] (2005) have designed and synthesized various oxazolidinone derivatives containing (un) substituted-benzotriazoles, which have shown enhanced antibacterial activities against many antibiotic-resistant strains compared to linezolid.



Linezolid

Anna Sparatore *et al* [10] (2006) synthesized a series of [4-(2*H*-1, 2,3-benzotriazol-2-yl) phenoxy] alkanoic acids and tested as agonists of Peroxisome Proliferator-Activated Receptor (PPAR) α, ϒ, and δ . Three compounds displayed 56 to 96% of maximal activity of the reference drug rosiglitazone.

Dawood. K.M *et al* [34] (2006) reported the synthesis of some new heteroaromatic derivatives having benzofuran and benzotriazole moieties such as 2-(5-acetyl-4-methyl-3-phenyl-3h-thiazol-2-ylidene)-1-(2-benzofuryl)-2-(1-benzotriazolyl) ethanone. The biological activity and structure–activity relationship (SAR) of the newly synthesized compounds were evaluated using Wistar albino mice and some of the obtained products were found to possess anticonvulsant, antinociceptive, and anti-inflammatory activities.



N1-methyl-2- (*1H*-1, 2,3-benzotriazol-1-yl)-3-oxobutanethioamide (MBOBT)

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Of the key intermediates.Some compounds showed anti inflammatory activity comparable to indomethacin and they also demonstrated minimum ulcerogenic activity.

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NovellinoE et al reported the synthesis and the pharmacological screening of a series of novel 1and 2-[2-[4-(X) 1-piperazinyl]ethyl benzotriazole and 1-[-[4-(X) – 1-piperazinyl]ethyl benzotriazoles and 1-[3-[4-(X)-1-pipearazinyl] propoxyl] benzotriazole, which are structurally related to trazodone. Antiserotoninergic ,antiadrenergic and antihistaminic in vitro activity and in vivo analgesic action were described. Some of the investigated compounds showed overall pharmacological profiles similar to that of the antidepressant trazodone

MATERIALS AND METHODS

Synthetic Procedure:

STEP 1: Synthesis of ethyl 1H-1,2,3-benzotriazol-1-ylacetate[I][5]

To a mixture of benzotriazole (0.1 mole, 11.91g) in acetone, ethylchloroacetate (0.1 mole, 10.7 ml) was added dropwise and potassium carbonate (0.2 mole, 27.64g) in 60 ml acetone was added as base. The reaction mixture was refluxed for 7 hrs on a water bath and filtered hot. Solvent was evaporated from the filtrate to yield the product as white shining crystals. The product thus obtained is recrystallized from ethanol to yield white shining crystals. Yield: 62.21%w/w, 12.75g.

STEP 2- Synthesis of 2-(1*H*-benzotriazol-1-yl) acetohydrazide [II][7]

A mixture of ethyl 1H- benzotriazolyl acetate (0.1mole, 20.5g) and hydrazine hydrate 99% (0.15 mole, 7.5ml) in ethanol (30ml) was refluxed for about 8 hrs. The reaction mixture was cooled to room temperature. The separated solid was filtered, washed with ethanol and dried. The dried product obtained was recrystallized from ethanol to white needle shaped crystals. Yield: 85.52% w/w, 12.25g.

STEP 3- Synthesis of potassium 2-(1*H*-benzotriazol-1-yl acetyl) hydrazine carbodithioate [III]

Potassium hydroxide (0.15 mole, 5.39g) was dissolved in absolute ethanol (200ml). To the above solution 2-(1*H*-benzotriazol-1-yl) acetohydrazide [II] (0.1mole, 12.25g) was added and the solution was cooled in ice. To this carbon disulphide (0.15 mole, 7.31ml)) was added in small portion with constant stirring. The reaction mixture was agitated continuously for a period of 16 hrs. It was then diluted with anhydrous ether. The precipitated compound was washed with anhydrous ether (100ml) and dried under[8]

STEP 4 – Synthesis of 4-amino-5-(1*H*-benzotriazol-1-ylmethyl)-4*H*-1,2,4-triazole-3-thiol[IV]

A suspension of potassium salt (0.05 mole,15.2710g) in water(50ml) and hydrazine hydrate 99%(0.15mole,7.5ml) was refluxed for 10 hrs with occasional shaking. The colour of the reaction mixture changed to green with the evolution of hydrogen sulphide gas. The reaction mixture was cooled to room temperature and then diluted with water (100ml). On acidification with concentrated hydrochloric acid, the required triazole was precipitated. It was then filtered,[9] washed thoroughly with cold water and dried. The dried product obtained is recrystallized from ethanol. Yield: 75.9%.

STEP 5 – General procedure for the synthesis of 5-[1H, 1,2,3 benzotriazolyl methyl] 4{substituted} amino 4H 1,2,4 triazole 3-thiol.[10]

An equimolar mixture of 4-amino-5-(1*H*-benzotriazol-1-ylmethyl)-4H-1,2,4-triazole-3-thiol (0.01mole), respective aromatic aldehydes (0.01mole) in alcohol (15ml) and few drops of glacial acetic acid was refluxed for 5 hours After refluxing, the reaction mixture was cooled to room temperature. Then it is filtered into petridish and solvent was allowed to evaporate. The dreied product is then recrystallized from ethanol to yield desired compounds [BTT1-BTT7] respectively. Yield 75 percent. Rf 0.74(petroleum ether: Ethyl acetate 1:1).

Step 5.1: Synthesis of 5-[1H,1,2,3 benzotriazolyl methyl] 4- {phenyl methylene amino} 1,2,4 triazolo 3-thiol.[BTT1]

Compound BTT1 was synthesized by reacting compound 5 (0.01mole) and benzaldehyde (0.01 mole), 15 ml ethyl alcohol and few drops of glacial acetic acid was refluxed for 5 hours. Yield:60%.

Step 5.2: Synthesis of 5[1H - 2 hydroxy phenyl methylene amino] 1,2,4 triazolo 3-thiol.[BTT2]

Compound BTT2 was synthesized by reacting compound 5(0.01 mole) and (0.01 mole) salicylaldehyde, 15 ml alcohol and few drops of

glacial acetic acid and was refluxed for 5 hours. Yield 64%.

STEP 5.3: Synthesis of 5 [1H- Benzotrizolyl methyl-] 4{3-nitro phenyl methylene amino} 1,2,4 triazolo 3-thiol.[BTT3]

Compound BTT3 was synthesized by refluxing compound (0.01mole) and 3 nitro benzaldehyde (0.01 mole) in 15 ml alcohol and few drops of glacial acetic acid. Yield:65%.

Step 5.4: Synthesis of 5-[1H- benzotriaolyl methyl] 4- {3-hydroxy, 4-methoxy phenyl methylene} amino 1,2,4,triazolo 3 –thiol. [BTT4]

Compound BTT4 was synthesized by refluxing compound 5(0.01 mole) with 0.01 mole vanillin in presence of 15 ml alcohol and few drops of glacial acetic acid. Yield 70%.

Step 5:5 Synthesis of 5-[1H benzotriazolyl methyl 4- {4-methoxy phenyl methylene} amino 1,2,4 triazolo 3-thiol.[BTT5]

Compound BTT5 was synthesized by refluxing compound 5 (0.01 mole) and anisaldehyde (0.01 mole) in presence of 15 ml alcohol and few drops of glacial acetic acid. Yield63%.

Step 5.6: Synthesis of 1H [benzotriazolo methyl]4-{4-chloro phenyl methylene}amino 1,2,4 triazolo 3-thiol.[BTT6]

Compound BTT6 was synthesized by refluxing compound 5 (0.01 mole) and 4-chloro benzaldehyde (0.01 mole) in presence of 15 ml alcohol and few drops of glacial acetic acid. Yield: 62%Step 5.7:

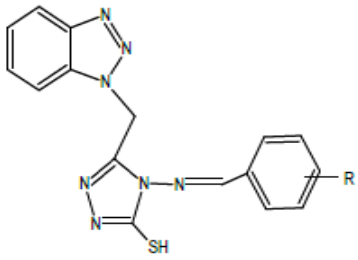
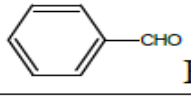
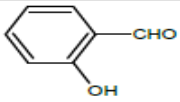
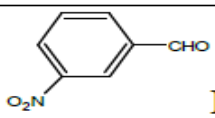
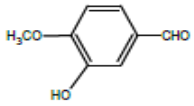
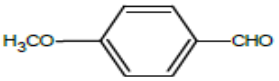
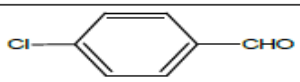
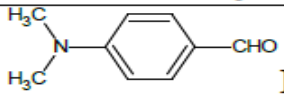
Step 5.7: Synthesis of 5- [1H benzotriazolyl methyl] 4-{4- Dimethyl amino phenyl methylene} amino, 1,2,4 triazolo 3-thiol.[BTT7]

Compound BTT7 was synthesized by refluxing (0.01 mole) compound 5 and 0.01 mole dimethyl amino benzaldehyde in presence of glacial acetic acid. Yield:60%.

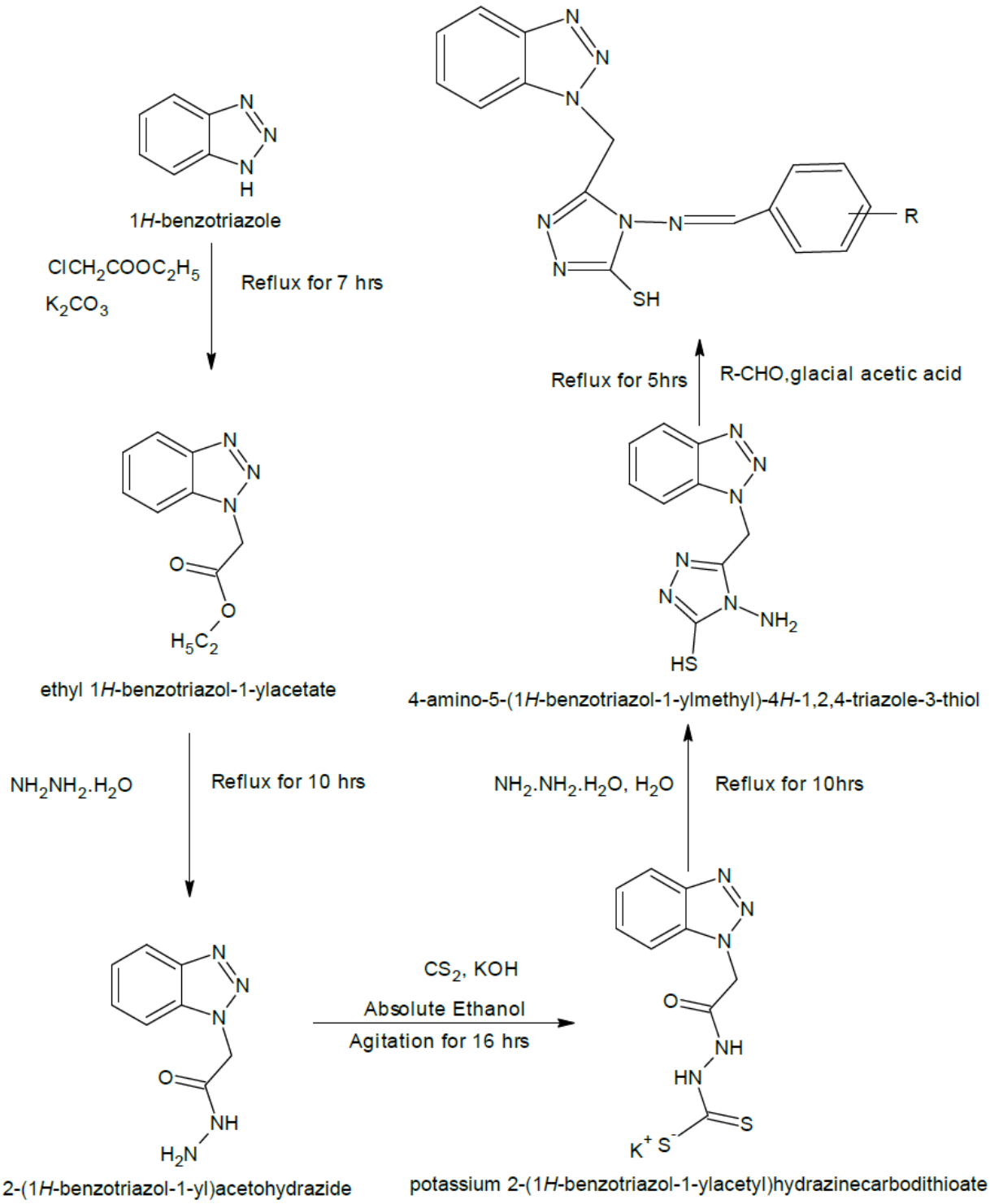
Characterization of synthesized compounds

Compound	Mol .formula	Mol.wt	Yield	m.p(^{0}c)	Rf
BTT_1	$C_{16}H_{13}N_7S$	335.388	60%	204 c	0.85
BTT_2	$C_{16}H_{13}N_7OS$	351.387	64%	208 c	0.69
BTT_3	$C_{16}H_{12}N_8O_2S$	380.385	65%	199 c	0.72
BTT_4	$C_{17}H_{15}N_7O_2S$	381.413	70%	224 c	0.78
BTT_5	$C_{17}H_{14}N_7OS$	364.413	63%	236 c	0.71
BTT_6	$C_{16}H_{12}clN_7S$	369.832	62%	197 c	0.69
BTT_7	$C_{18}H_{19}N_8S$	379.388	60%	189 c	0.71

Synthesized derivatives

GENERAL STRUCTURE	PROPOSED DERIVATIVES
	 BTT-1
	 BTT-2
	 BTT-3
	 BTT-4
	 **BTT-5**
	 BTT-6
	 BTT- 7

Scheme of Synthesis[11]



Characterization of Synthesized Derivatives[12,13,14,15]

Characterization of the synthesized compounds was carried out by UV and IR spectra, and mass spectra. Thin layer chromatography was used to check purity of the synthesized compounds The purity of the analogues was further ascertained by consistency in melting point.

Characteristic IR absorption peaks for basic nucleus and its modified compounds

Derivatives	IR absorption peak value
BTT_1	C-N stretching at 1700 cm-1,Aromatic C-H stretching at 3000cm-1,S-H group at 2580 cm-1,Aromatic C-H bending at 707.747 cm-1
BTT_4	C-N stretching at 1700 cm-1,Aromatic C-H stretching at 3000cm-1, Aromatic C-H bending at 741.496 cm-1
BTT_3	C=N stretching at 1700.91 cm-1, S-H group at 2360 cm-1 ,Aromatic C-H stretching at 3050 cm-1,Aromatic C-H bending at 744.388cm-1,C-N stretching at 1264cm-1
BTT_7	Aromatic C-H sretching at 3050 cm-1,C=N stretching at 1598.7cm-1,aromatic C-H bending at 727 cm-1,C-N sretching at 1232.29cm-1
BTT_2	O-H stretching at 3500cm-1,Aromatic C-H stretching at 3050 cm-1, C=N stretching at 1682.59 cm-1,Aromatic C-H bending at 745.352cm-1
BTT_6	Aromatic C-H stretching at 3049.87 cm-1,S-H group at 2389 cm-1 ,C=N stretching at 1689.34 cm-1 ,C-N sretching at 1267.99cm-1,C-Cl stretching at 740 cm-1

Pharmacological studies:
Clearance from the Institutional Animal Ethics Committee was obtained for carrying out the animal experiments. Inbred, Male, healthy Wilstar albino rats (150-200g) and Swiss albino mice (20-25g) maintained at a room temperature of 25±50C with relative humidity of 60±5% were used. The animals were housed in polypropylene cages with access to standard rodent pellet diet and water *ad libitum*. A twelve hour, light dark cycle was maintained for experiments. Solid food and water were withdrawn 18 hours before the administration of the test compound.

Preparation of solutions of test compounds:
The synthesized compounds are insoluble in water and soluble only in organic solvents. Therefore a homogenous suspension of the individual compounds were prepared using 0.1% Carboxy Methyl Cellulose Sodium (CMC Sodium). The respective suspensions were used as the test material.

Acute Toxicity Study:
To study the acute toxicity and to find out the LD50 of given product

Animals used –albino mice (20-25g)

Number of animals in each group-2

Number of groups-5

Total number of animals-1o

Stair case method:
Healthyadult albino mice of either sex weighing 20-25 g were randomly selected for study. All the animals were fasted overnight. The first group serves as the control and was given 0.1%CMC orally at a dose of 10ml/kg body weight.

Test compounds as a suspension in 0.1 % CMC is given orally in a dose of 500mg/kg body weight. If the initial dose was tolerated, then doses are increased by a factor of 1.5 and decreased by factor of 0.7 if. The initial dose was lethal. After dosing the animals were closely observed for first two hours and intermittently for another four hours and also at the end of 24 hours to assess the morbidity and mortality. Any change in skin, eyes, mucous membrane and behavioral pattern were recorded.

Anti-inflammatory studies [in vitro method]:
The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer. The collected blood was mixed with equal volume of sterilized Alsever solution(2% dextrose,0.8% sodium chloride,

0.5%citric acid and 0.42%sodium chloride in water).The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%)PH 7.2 and a 10% (v|v) suspension was made with isosaline. The assay mixture contained the drug,1ml phosphate buffer(0.15M,PH 7.4), 2ml of hyposaline(0.36%) and 0.5ml of HRBC suspension . Diclofenac was used as reference drug .Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixture were incubated at 37 c for 30 minutes and centrifuged .The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm.The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100%. The percentage of haemolysis was calculated using the formula

% Hemolysis =(O.D 1-OD2)* 100\O.D1

OD1 –absorbance of control

OD2-absorbance of drug

Analgesic activity:

The hot plate was maintained at 56±1°C.Animals were placed into a glass cylinder of 24cm diameter on the heated surface and the time between placement and licking the paws or jumping was recorded as response latency. The reaction time was recorded for control mice or animals treated with morphine (5mg/kg s.c) and test compounds at 0,15,30,60,90 minutes. The test was terminated at 15s to prevent tissue damage.

Pharmacological Screening:

Acute toxicity study:

The objective of acute toxicity study was to find out the LD50 of the synthesized compounds and to establish safer doses for the further studies. Due to difficulty in administering a dose higher than 1600 mg/Kg to mice, the maximum dose was the same in which no mortality was found. One molecule was randomly selected for the study of safety dose range of the analogues. In the study it was found that dose of up to 1600 mg/Kg the compound was safe, i.e., there was no mortality or gross behavioral change in the animals used. Hence LD50 of the all synthesized 5-[1H benzotriazolo methyl 4- (substituted amino]-12,4triazolo 3-thiol derivatives was found to be more than 1600 mg/kg.

Anti-Inflammatory Activity:

In-vitro method was used in albino rats for screening anti-inflammatory potential of the compound. The compound BTT1 was selected and were evaluated for anti-inflammatory activity at a dose of 50,100 and 250 miro gram\ml. Diclofenac was used as standard drug at the dose of 50,100 and 250 micro gram \ml .Dimethyl sulfoxide was used as control.

% inhibition of Haemolysis = 100 (0D1-OD2)\OD1

Anti –inflammatory activity screening

Concentration(micro gram\Ml	Drug	Diclofenac	control
50	0.1260	0.1002	
100	0.1266	0.1007	0.4195
250	0.1278	0.1012	

% inhibition of hemolysis at 50 microgram \ml =69.96%

% inhibition of hemolysis at 100 micro gram \ml = 69.821%

% inhibition of hemolysis at 250 micro gram \ml = 69.53 %

The test compound BTT1showed significant anti inflammatory activity at 50 micro gram \ml.

Analgesic Activity:

Analgesic activity was determined by hot plate method .Morphine is used as standard. It is given by subcutaneous route. 1% carboxy methyl cellulose is used as control.Drug solution is injected by subcutaneous Route.

Screening of analgesic activity:

Treatment(subcutaneous)	Jumping response	P value
Morphine	5.257	<0.001
Control	5.12	
Drug solution(500 mg/Kg body weight)	5.1245	<0.001

The drug substance BTT1 show significant analgesic activity

SUMMARY AND CONCLUSION:

This work was focused at the development of novel Benzotriazole

Derivatives Benzotriazole moiety is very active pharmacophore reported to possess considerable anti-inflammatory, analgesic , antibacterial and antifungal activity. The research work involved preliminary *in silico* screening of various 5[1H benzotriazolo methyl 4-(substituted amino) 1,2,4, triazolo 3-thiol anlogues for quantifying their drug likeness using molinspiration software. Among the proposed analogues, only those candidates which obeyed Lipinski rule of five were taken for wet lab synthesis. Five different analogues were synthesized by both conventional and microwave methods and a comparative study of the yield and reaction time was carried out. Purity of the compounds thus synthesized was ascertained by consistency in melting point and Rf value and characterized by UV, IR, Mass and 1H NMR spectral studies.

Out of seven synthesized analogues,BTT1 have screened for their analgesic and antinflammatory activity and it showed moderate to good activity. Since the compounds showed good results, the importance of *in silico* screening in biological activity is emphasized.

The proposed derivatives were synthesized by both conventional and microwave methods. This work clearly reveals the advantages of microwave assisted organic synthesis over conventional method. Microwave method can be considered as an efficient method for Schiff base synthesis.

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