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(Research Article)

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SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF SUBSTITUTED BENZOTHIAZOLES

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Keywords:
Anti-inflammatory Activity, Benzothiazole, NMR, Hetrocyclic compounds

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ABSTRACT

Benzothiozels are the common form of anti inflammatory chemical substances. The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities like anti-tumor, antimicrobial, anti-tubercular, anti-malarial, anti-convulsant, anti-helminthes, analgesic and anti-inflammatory activity. In view of the fact, imino thiazolidones were synthesized in our laboratory and were found to possess significant anti-inflammatory activity as compared to that of diclofenac sodium**.** Anti-inflammatory activity of all title compounds was carried out by Carrageenan-induced rat paw edema test as described by Winter *et al*. it is not needed. *Carrageenan-induced rat paw edema test*- Albino rats. Evidence show that the prepared benzothiazole derivatives i.e. 3-Methyl phenyl benzothiazole shows more potent anti-inflammatory activity followed by 4-Nitro phenyl benzothiazole, 2-Acetoxy phenyl benzothiazole, Meta-Hydroxy phenyl benzothiazole, Para-chloro phenyl benzothiazole.

INTRODUCTION

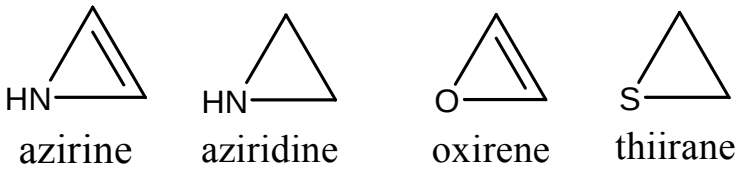
Heterocyclic compounds are organic compounds containing at least one atom of carbon and at least one element other than carbon such as sulfur, oxygen nitrogen within a ring structure. This structure may comprise either simple aromatic or non-aromatic rings.[1]

Compounds such as cyclopropane, an anesthetic with explosive properties and cyclohexane, a solvent, are not heterocyclic. They are merely cycloalkanes. In heterocyclic compounds, the term hetero represents an atom other than carbon and cyclic refers to the ring structure. Many hetero cyclic compounds including some amines are carcinogenic.

Heterocyclic compounds are classified based on the ring structure in the following classes.

3-Membered rings: Heterocyclic with three atoms in the ring is more active because of ring strain. Those containing one hetero atom are, in general, more stable.

Examples:

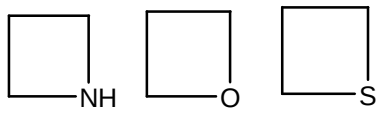


azirine aziridine oxirene thiirane

4-Membered rings: These compounds contain four membered rings with one or more hetero atoms.

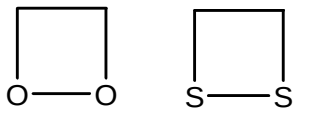
Examples:

Compounds with one heteroatom



azetidine oxetane thietane

Compounds with two heteroatoms

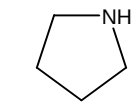


dioxetane dithietane

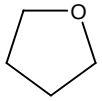
5-Membered rings: compounds contain five membered rings with one or more hetero atoms. Unsaturated compounds are frequently more stable because of aromaticity. The Five-membered rings with two heteroatoms at least one of which is nitrogen are collectively called as azoles. Thiazoles and Isothiazoles contain 1-sulfur and 1-nitrogen atom in the ring.

Examples:

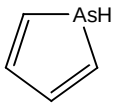
5-membered rings with a single heteroatom



tetrahydropyrrole

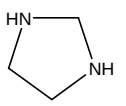


tetrahydrofuran

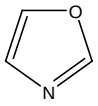


arsole

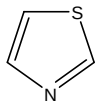
5-membered rings with two heteroatoms



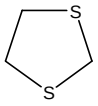
imidazolidine



oxazole

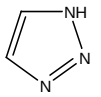


thiazole

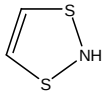


dithiolane

5-membered rings with three heteroatoms.

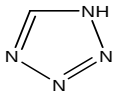


1,2,3-triazole



dithiazole

5-membered ring with four heteroatoms

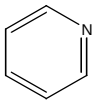


tetrazole

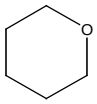
6-Membered rings:

Examples:

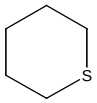
6-membered rings with a single heteroatom.



pyridine

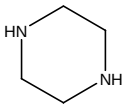


tetrahydropyran

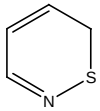


thiane

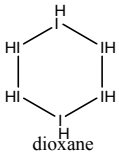
6-membered rings with two heteroatoms



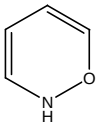
piperazine



thiazine

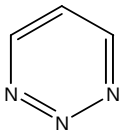


dioxane

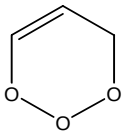


oxazine

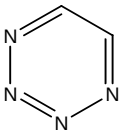
Six-membered rings with three and four heteroatoms.



triazine



trioxine

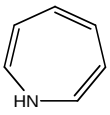


tetrazine

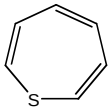
7-Membered rings: These compounds contain seven membered rings in their structures. With seven-membered rings, aromatic stabilization is not available.

Examples:

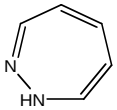
7-membered rings with one and two heteroatoms



azepine



thiepin



diazepine

Fused Rings

Heterocyclic ring systems that are formally derived from fusion with other rings, either carbocyclic or heterocyclic, have a variety of common and systematic names. For example with the benzo-fused unsaturated nitrogen heterocycles, pyrrole provides indole or isoindole depending on the orientation. The pyridine analog is quinoline or isoquinoline. For azepine, benzazipine is preferred name. Similarly, the compounds with two benzene rings fused to the central heterocycle are carbazole, acridine and dibezoazipine.

Type of hetero atom

The type of heteroatom is indicated by a prefix according to the following table. The sequence in this table also indicates the preferred order of prefixes (principle of decreasing priority).

Table1:

Element	Prefix	Element	Prefix
O	Oxa	Sb	Stilba
S	Thia	Bi	Bisma
Se	Selena	Si	sila

Ring size:

The ring size is indicated by a suffix according to the following table. Some of the syllables ae derived from latin numerals, namely ir from tri, et from tetra, ep from hepta, on from nona, ec from deca.

Table2:

Ring size	Unsaturated	Saturated
3	Irene[a]	Irane[b]
4	ete	etane
5	Ole	Olane
6	Ine	ane

The stem arine may be used for rings containing only N.

The traditional stems ‘irine’, ‘etidine’, and ‘olidine’ are preferred for N-containing rings and used for saturated hetero-monocycles having three, four, or five ring members respectively.

The stem for six-membered rings depends on the least preferred hetero atom in the ring, that immediately preceding the satem. To determine the correct stem for a structure, the set containing this least preferred heteroatom is selected.

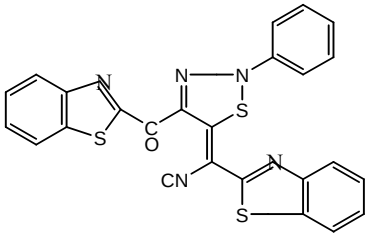
6A:O, S, Se, Bi,Hg; 6B:N,Si,Ge,N,Pb; 6C:B,P,As,Sb.

Chemical Properties

The metabolism of heterocyclic compounds very often involves ring hydroxylation, followed by ring cleavage. When attached to 6-membered rings, 5-membered rings are usually cleaved first, with or without initial hydroxylation. In addition, for sulphonated aromatic compounds, at least four desulfonation mechanisms have been an NADH-linked deoxygenation, a mono-oxygenation, a hydrolytic de sulfonation, and a Meta ring cleavage- associated desulfonation. All of these involve indirect oxygenation. [2]

Kamal dawood et al perform the synthesis of some 2, 3-dihydro-1, 3, 4,-thiadiazoles and unsymmetrical azines containing benzothiazole moiety and the chemical reaction with hydrazonoyl halides.

Benzothiazoloyl-N-aryl hydrazonoyl bromides were reacted with methyl2-thiazolylcyanomethinecarbodithioate and methyl thiocarbamates in the presence of tri ethyl amine to give 2, 3-dihydro-1, 3, 4-thiadiazoles in good yields.[3]

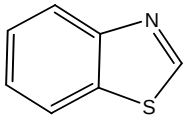


Physical Properties

Molecular formula: C_7H_5NS

Molecular weight: 135.9

Synonyms: 1-thia-3-azaindene, benzosulfonazole



Benzothiazole is a yellow liquid with unpleasant odour, which has a boiling point of 231^{o}C and a melting point of 2 ^{o}C. It has density of 1.2460gms/cm^{3} at 20 ^{o}C. Its flash point, which is defined the lowest, temperature at which it can form a ignitable mixture in air is greater than 110 ^{o}C. It is slightly soluble in water, very soluble in ethanol, diethyl ether and carbon disulfide; soluble in acetone. Its refractive index is 1.6379 at 20 ^{o}C. Its vapour pressure is 0.07mm Hg at 20 ^{o}C. It reacts with aldehyde or ketones to generate alpha- hydroxy carbonyl compounds

Stability: Benzothiazoles are stable compounds. They are regarded as highly persistent in the environment. They are incompatible strong oxidizing agents. The products formed on combustion are nitrogen oxides, carbon monoxide, carbon dioxide, sulphur oxide.

Toxicology: Benzothiazoles are harmful if swallowed or inhaled. They may cause skin or eye-irritation. [4]

Uses:

- It is used as a starting material for the synthesis of larger heterocyclic compounds.
- Its aromaticity makes it relatively stable, although as a heterocyclic, it has reactive sites which allow for cyclization.
- Mini dyes such as thioflavine and pharmaceutical drugs such as riluzole have benzothiazoles as a structural moiety.

Medicinal Importance of Benzothiazole Nucleus

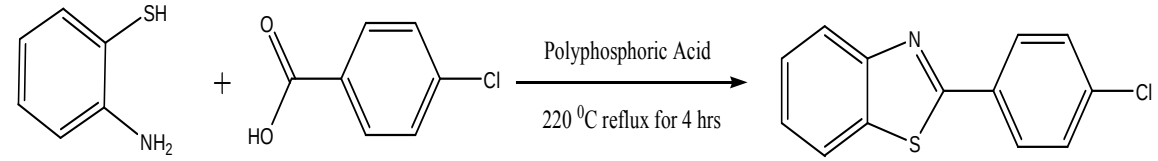
The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities like anti-tumor, 1-4 Thiazole antimicrobial, anti-tubercular, anti-malarial, anti-convulsant, anti-helminthes, analgesic and anti-inflammatory activity. The benzothiazole ring is present in various marine or terrestrial natural compounds, which have useful biological Coupling Reagent. Heterocycles containing the thiazole moiety are present in many natural products such as bleomycin, epothilone A, lyngbyabellin A & dolastatin. Benzothiazole is a privileged bicyclic ring system. Due to their important pharmaceutical utilities, the synthesis of these compounds is of considerable interests. [5]

METHODOLOGY

Synthesis of P-Chloro Phenyl Benzothiazole:

Equimolar quantities of ortho-amino thiophenol (2.5ml) &P-Chloro Benzoic acid (2.84gm) were added to 15 gm of polyphoshoric acid (PPA) (8.5ml) in a round bottom flask and condensed for four hours at 220^{o}C.

After four hours, the reaction mixture was cooled and poured into a beaker containing freshly prepared ice cold 10% sodium carbonate solution. The solution was then filtered. The residue thus obtained is purified by re-crystallization from hot methanol.

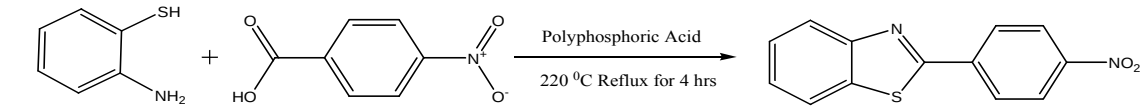


ORTHO AMINO THIO PHENOL PARA CHLORO BENZOIC ACID PARA CHLORO PHENYL BENZOTHIAZOLE

Synthesis of 4- Nitro Phenyl Benzothiazole:

Equimolar quantities of ortho amino thiophenol (2.5ml) & 4- Nitro Benzoic acid (2.84gm) were added to 15 gm of polyphoshoric acid (PPA) (8.5ml) in a round bottom flask and condensed for four hours at 220^{o}C.

After four hours, the reaction mixture was cooled and poured into a beaker containing freshly prepared ice cold 10% sodium carbonate solution. The solution was then filtered. The residue thus obtained is purified by re-crystallization from hot methanol.

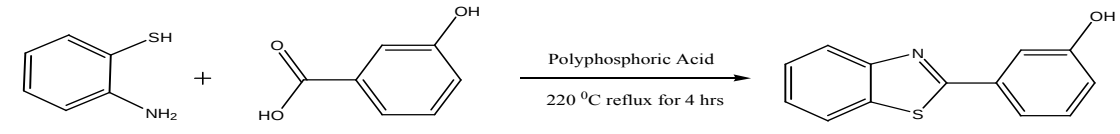


ORTHO AMINO THIO PHENOL 4-NITRO BENZOIC ACID 4-NITRO PHENYL BENZOTHIAZOLE

Synthesis of Meta Hydroxy Phenyl Benzothiazole:

Equimolar quantities of ortho amino thiophenol (2.5ml) & Meta hydroxy Benzoic acid (2.84gm) were added to 15 gm of polyphoshoric acid (PPA) (8.5ml) in a round bottom flask and condensed for four hours at 220^{o}C.

After four hours, the reaction mixture was cooled and poured into a beaker containing freshly prepared ice cold 10% sodium carbonate solution. The solution was then filtered. The residue thus obtained is purified by re-crystallization from hot methanol.

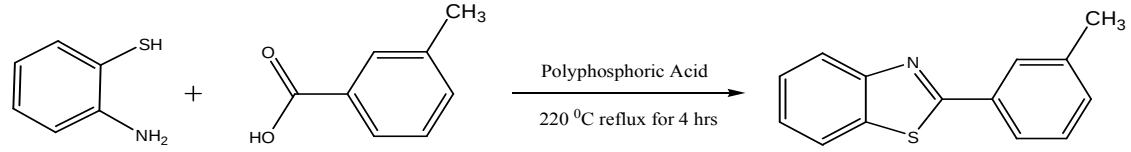


ORTHO AMINO THIO PHENOL META HYDROXY BENZOIC ACID META HYDROXY PHENYL BENZOTHIAZOLE

Synthesis of 3-Methyl Phenyl Benzothiazole:

Equimolar quantities of ortho amino thiophenol (2.5ml) & 3-methyl Benzoic acid (2.84gm) were added to 15 gm of polyphoshoric acid (PPA) (8.5ml) in a round bottom flask and condensed for four hours at 220^{o}C.

After four hours, the reaction mixture was cooled and poured into a beaker containing freshly prepared ice cold 10% sodium carbonate solution. The solution was then filtered. The residue thus obtained is purified by re-crystallization from hot methanol.

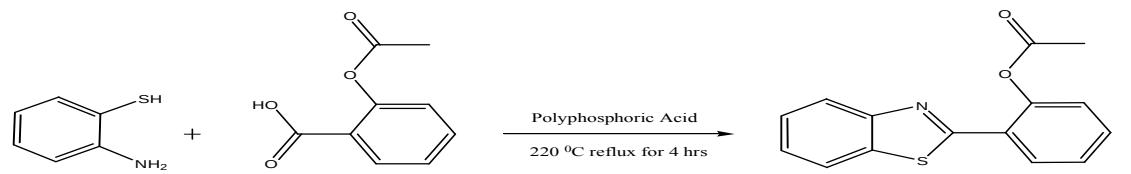


ORTHO AMINO THIO PHENOL 3 METHYL BENZOIC ACID 3-METHYL PHENYL BENZOTHIAZOLE

Synthesis of 2-Acetoxy Phenyl Benzothiazole:

Equimolar quantities of ortho amino thiophenol (2.5ml) & 2-Acetoxy Benzoic acid (2.84gm) were added to 15 gm of polyphoshoric acid (PPA) (8.5ml) in a round bottom flask and condensed for four hours at 220^{o}C.

After four hours, the reaction mixture was cooled and poured into a beaker containing freshly prepared ice cold 10% sodium carbonate solution. The solution was then filtered. The residue thus obtained is purified by re-crystallization from hot methanol.



ORTHO AMINO THIO PHENOL 2-ACETOXY BENZOIC ACID 2-ACETOXY PHENYL BENZOTHIAZOLE

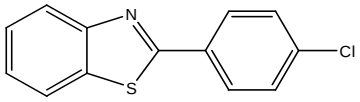
Para-Chloro Phenyl Benzothiazole:

Molecular formula: $C_{13}H_8SNCl$

Molecular weight: 245.5gms

Melting point: 110^oC

Solubility: methanol



PARA CHLORO PHENYL BENZOTHIAZOLE

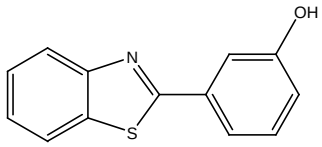
Meta-Hydroxy Phenyl Benzothiazole:

Molecular formula: $C_{12}H_9NOS$

Molecular weight: 227gms

Melting point: 130^oC

Solubility: methanol



META HYDROXY PHENYL BENZOTHIAZOLE

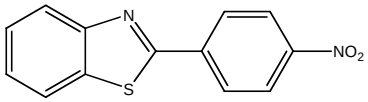
4-Nitro Phenyl Benzothiazole:

Molecular formula: $C_{13}H_8NSNO_2$

Molecular weight: 224gms

Melting point: 105^oC

Solubility: methanol



4-NITRO PHENYL BENZOTHIAZOLE

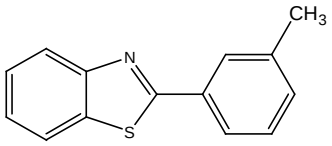
3-Methyl Phenyl Benzothiazole:

Molecular formula: $C_{13}H_{11}NS$

Molecular weight: 213gms

Melting point: 115^oC

Solubility: methanol



3-METHYL PHENYL BENZOTHIAZOLE

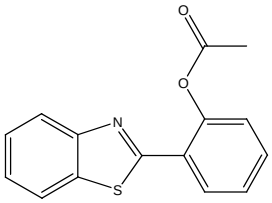
2-Acetoxy Phenyl Benzothiazole:

Molecular formula: $C_{15}H_{13}NO_2S$

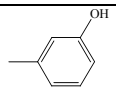
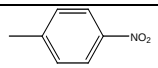
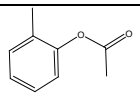
Molecular weight: 271gms

Melting point: 108°C

Solubility: methanol



2-ACETOXY PHENYL BENZOTHIAZOLE

Compd No.	R	Mol. Formula	Melt. Pt.	Mol. Wt.	Percentage Yield.
1		$C_{13}H_8SNCl$	110°C	245.5 gms	82%
2		$C_{12}H_9NOS$	130°C	227 gms	75%
3		$C_{13}H_8NSNO_2$	105°C	224gms	65%
4		$C_{13}H_{11}NS$	115°C	213gms	78%
5		$C_{15}H_{13}NO_2S$	108°C	271gms	62%

CHARACTERISATION

NMR SPECTROSOPY:

Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy, is the name to a technique which explodes the magnetic properties of certain nuclei. The most important application for the organic chemist is proton NMR and ^{13}C NMR spectroscopy.[6]

Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected. Although larger amount of samples are needed than for a mass spectroscopy, NMR non destructive, and with modern instrument good data may be obtained from samples weighing less than a milligram. To be successful in using NMR an analytical tool, it is necessary to understand the physical principles on which the methods are based. [7]

RESULTS

Mass Spectral Data of Benzothiazole

In The Below Table

A: 4-Nitrophenyl benzothiazole

B: Para-chloro phenyl benzothiazole

C: 3-Metyl phenyl benzothiazole

Indomethacin (standard)

Nuclear Magnetic Resonance Spectral Data of Novel Substituted Benzothiazole Derivatives

Compd. No.	Chemical shift in (δ) ppm	
	^{1}H-NMR	^{13}C-NMR
A.	δ 3.8463 (S, 1H, OH), δ 7.2548 – 7.9672(m, Ar-H,8H,H_1,H_2,H_3-H_8)	δ40.5(C_6H_6),80.5(OH),118(C_1),118.5(C_2), 121(C_3),120.5(C_4),122(C_5),125(C_6), 161(NH)
B.	δ7.0206-7.8160 (m, Ar-H ,7H , H_1-H_7)	δ40.5(C_6H_6),80.5(Cl),114(C_1),116(C_2), 122(C_3),129(C_4),133(C_5),136(C_6), 152(NH)
C.	δ7.0255-7.3866 (m, Ar-H ,8H , H_1-H_8) δ 0.7845-1.1846 (m, Ar-H ,3H , CH_3)	δ 21(CH_3),40.5(C6H5),115(C_1),121(C_2), 124(C_3),126(C_4),132(C_5),137(C_6), 154(NH)

Compound number	Molecular weight	Fragment ions (m/z)
A	245.5	228,209,185,161,145,133,119,105,73
B	224	281,252,235,209,196,180,134,120,94
C	227	226,213,182,154,142,128,112,101,73

Anti-Inflammatory Activity:

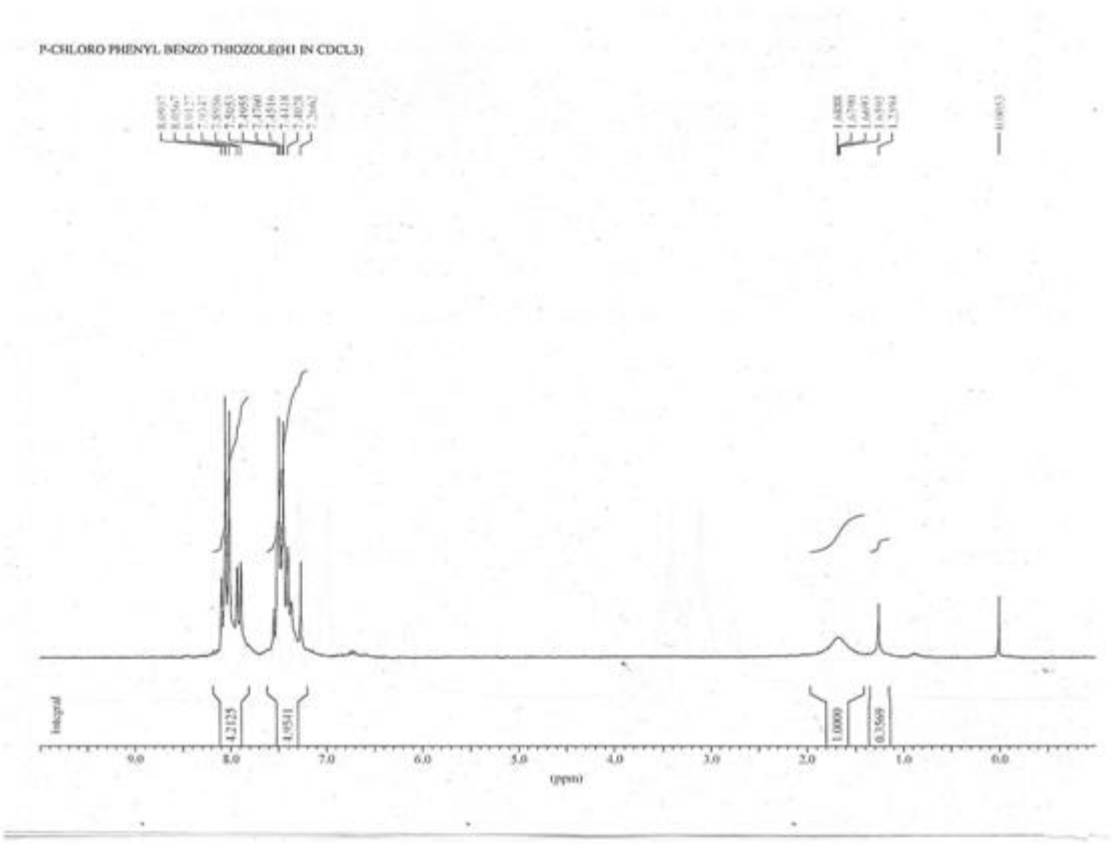
Following the discovery of indomethacin, a large number of aryl and heterocyclic acetic acids have been synthesized and many of them have shown significant anti-inflammatory activity. 1, 2, 3 Potent anti-inflammatory activities have been reported in number of aryl thiazole 4, 5 and benzothiazole derivatives. Thiazolidones also shows antimicrobial, anticonvulsant, antibacterial, fungicidal and congeners as central nervous system active agents. In view of the fact, imino thiazolidones were synthesized in our laboratory and were found to possess significant anti-inflammatory activity as compared to that of diclofenac sodium**.**

Procedure:

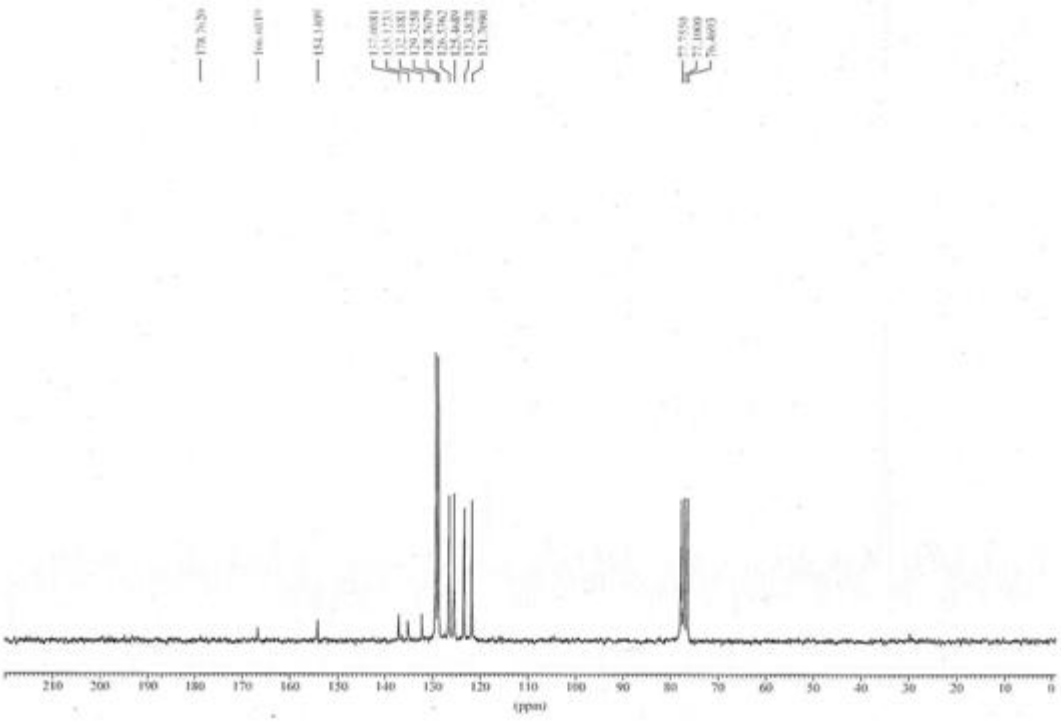
Anti-inflammatory activity of all title compounds was carried out by Carrageenan-induced rat paw edema test as described by Winter *et al.* it is not needed. *Carrageenan-induced rat paw edema test*-Albino rats of either sex (150-200 g) were divided into different groups, each containing six individuals. Animals were fasted for 12 h before experiment and only water was allowed. While the first group was a control one and received vehicle (Tween 80 in propylene glycol (10%, *v/v*), 0.5 mL per rat), the second group received indomethacin 10 mg kg^{-1} body mass. All the remaining groups received orally the test compounds at the same dose. All the suspensions for oral dose were prepared in the vehicle mentioned above and administered in a constant volume of 0.5 mL per rat.

After one hour of administration of test compound and Indomethacin 0.1 mL of 1% w/v suspension of carangeenan was injected in to the sub plantar of left paw of control and test animals. Immediately, the paw volume was measured using plethsmometer (initial paw volume), there after the paw volume was measured every half an hour till three hours. The difference between initial and subsequent readings gave the edema volume for the corresponding time.[8]

P-CHLORO PHENYL BENZO THIOZOLE(H1 IN CDCL3)



P-CHLORO PHIENYL BENZO THIOZOL3(13C IN CDCL3)



LCMS-2010A DATA REPORT
SHIMADZU

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Inj. Volume	: 5.000
Data Name	: C:\LCMSsolution\User\Data\P-CHLORO PHENYUL BENZOTHIOZOLE-APCI-P
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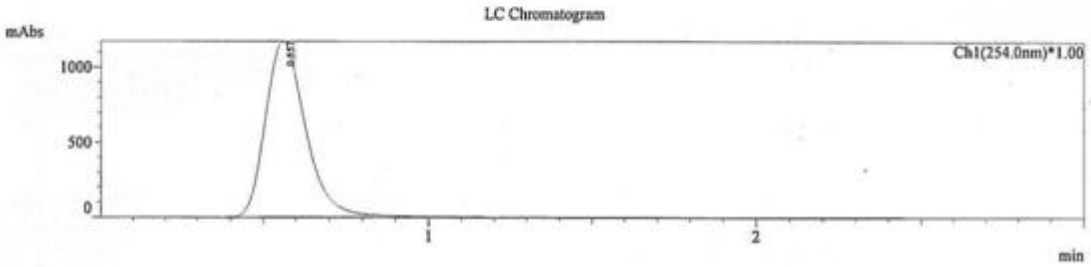

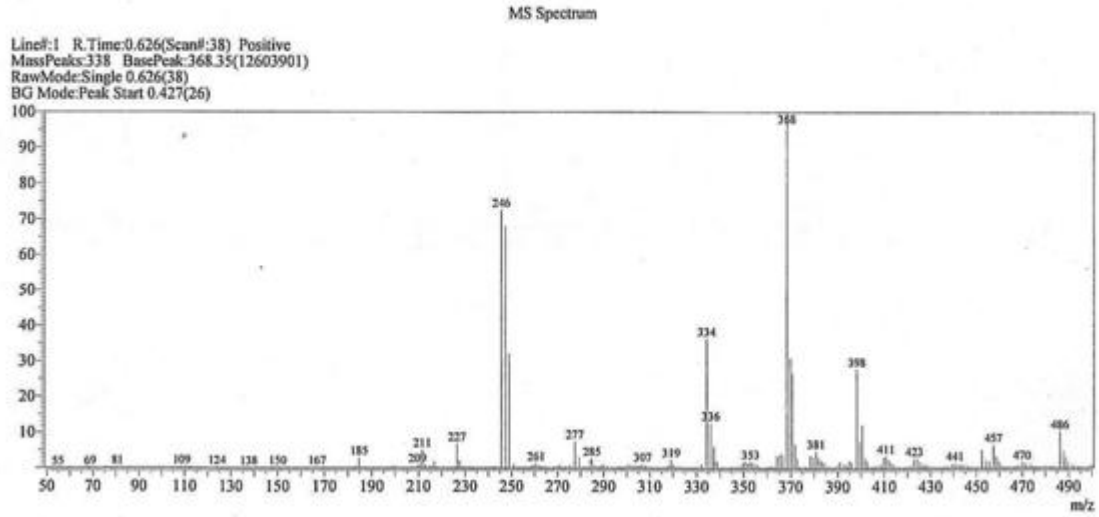

LCMS-2010A DATA REPORT
SHIMADZU

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Inj. Volume : 5.000
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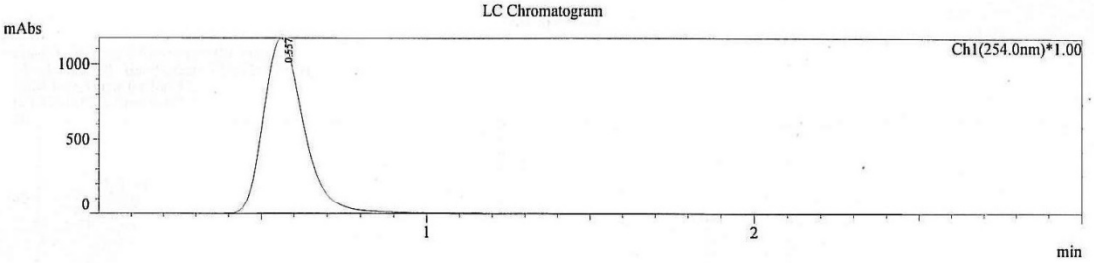
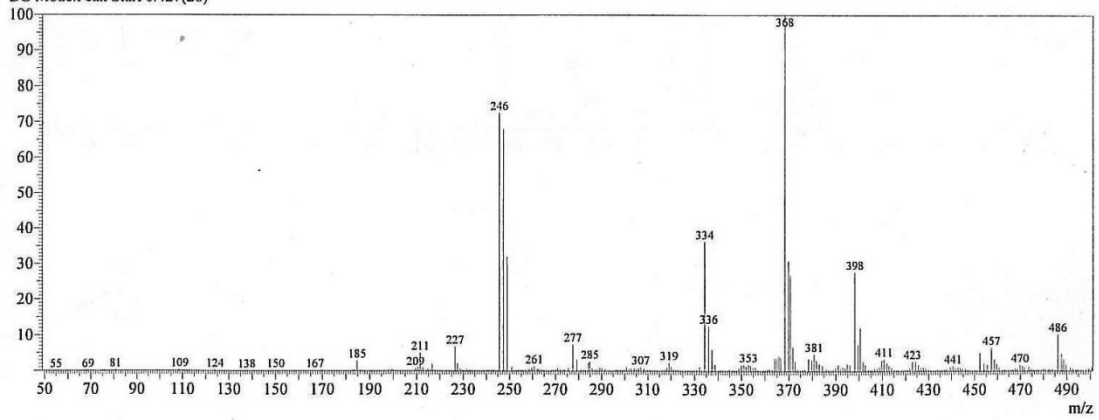
LC Chromatogram



MS Spectrum

Line#:1 R.Time:0.626(Scan#:38) Positive
MassPeaks:338 BasePeak:368.35(12603901)
RawMode:Single 0.626(38)
BG Mode:Peak Start 0.427(26)



LCMS-2010A DATA REPORT
SHIMADZU

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Inj. Volume : 5.000
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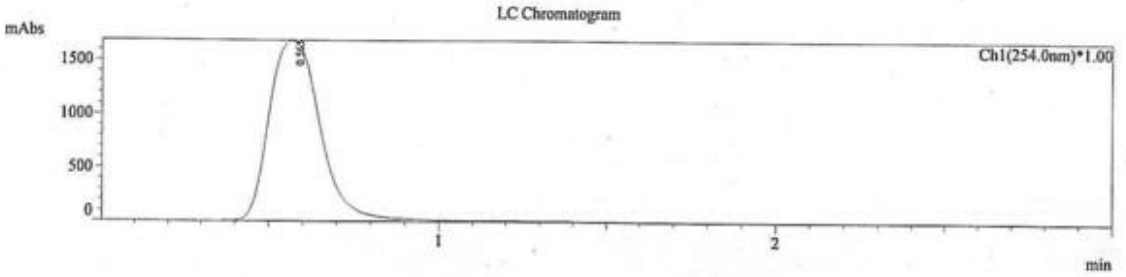
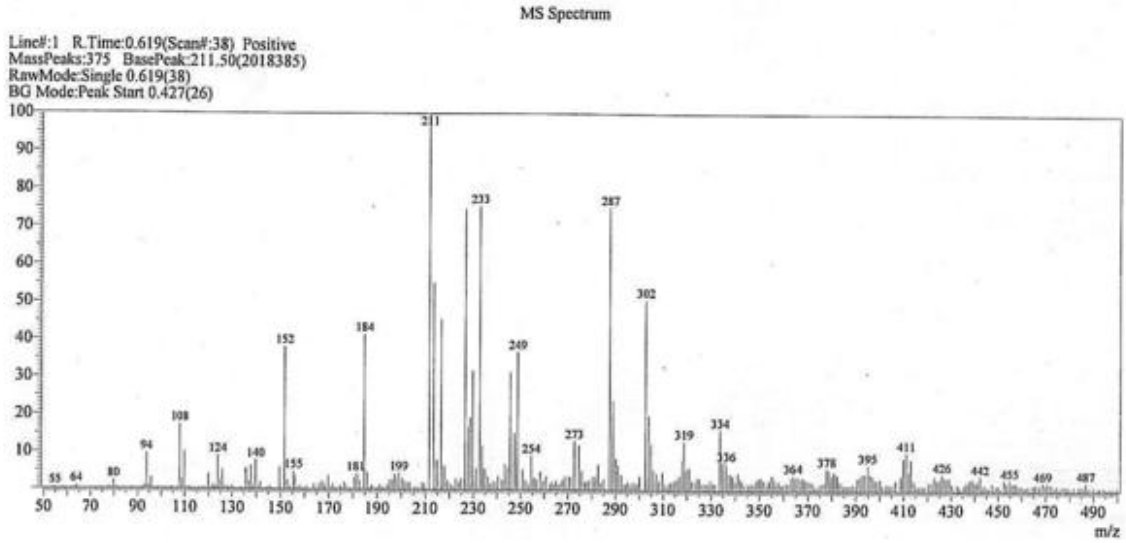
Method Name : C:\LCMSsolution\User\Method\Copy of JAY-4-APCI.qlm





DISCUSSION

All the synthesized substituted benzothiazoles were evaluated for anti-inflammatory activity. Their ability to prevent inflammation has been identified. Also, the co-relation between the structure and anti-inflammatory activity among the set of the compounds was carried out. The rats were kept on fasting for 12hrs. They were provided only with water during this time. Inflammation was induced with the help of Carrageenan and anti-inflammatory activity was enquired in comparisons with that of standard drug.

The synthesized benzothiazole derivatives were screened for anti-inflammatory activity and they showed significant activity at the given concentration levels. Hence, compounds appear to be promising as anti-inflammatory agents. The obtained results of the activity of benzothiazole derivatives can be further studied for the improvement of anti-inflammatory agents.

CONCLUSION

The synthesis of benzothiazole phenyl derivatives has been done. They have a significant anti inflammatory activity. Being a chemical compound, Benzothiazole finds use in research and as a starting material in the synthesis of larger, usually active substances. Substitution of methyl or nitro group to benzothiazoles will increase the potency of anti inflammatory activity.

Evidence show that the prepared benzothiazole derivatives i.e.3-Methyl phenyl benzothiazole shows more potent anti-inflammatory activity followed by 4-Nitro phenyl benzothiazole, 2-Acetoxy phenyl benzothiazole, Meta-Hydroxy phenyl benzothiazole, Para-chloro phenyl benzothiazole.

The above result establishes the fact that benzothiazoles are a rich source of anti-inflammatory drugs. Therefore, in search for a new generation of anti-inflammatory agents, it may be worthwhile to explore the benzothiazole derivatives by different substitutions to increase the potency.

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