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Synthesis and Pharmacological Evaluation of Benzothiazole Derivatives for Anti-Inflammatory Activity

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Sadaf Hashmi*[1], Abhinav Prasoon Mishra[2]

Research Scholar[1], Associate Professor[2], Department of Pharmaceutical Chemistry, Advanced Institute Of Biotech And Paramedical Sciences, Kanpur, UP, India

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ABSTRACT

A parent benzothiazole nucleus was synthesized by para amino acetanilide, then it is subjected to treatment with various substituted aromatic aldehydes to get the corresponding Schiff's bases followed by treatment with phthalic anhydride to form 2-(6-acetamidobenzo[d]thiazol-2-ylcarbamoyl)benzoic acid. The structures of 10 synthesized compounds were confirmed by Thin Layer Chromatography, melting point, and various spectroscopic methods such as IR, 1H, and NMR spectroscopy. Among the synthesized compounds, 2d and 3e showed very good anti-inflammatory activity. The products were evaluated for their anti-inflammatory activities. Some of the compounds exhibited potent activities when compared with the standards.

INTRODUCTION:

In the 1950s, several 2-aminobenzothiazole derivatives were intensively studied, as the 2-amino benzothiazole scaffold is one of the privileged structures in medicinal chemistry [1-2] and reported cytotoxic on cancer cells [3]. It must be emphasized that the combination of 2-aminobenzothiazoles with other heterocyclic is a well-known approach to designing new drug-like molecules, which allows for achieving new pharmacological profiles, action, and toxicity lowering. Numerous compounds bearing benzothiazole ring are known to possess important pharmacological activities such as antimicrobial [4-5] anticancer [6-7], antiviral [8], anti-HIV [9], and antidiabetic [10]. In addition, the benzothiazole ring is present in various marine or terrestrial natural compounds, which have useful biological activities. Due to their importance in pharmaceutical utilities, the synthesis of different benzothiazole derivatives is of considerable interest.

Benzothiazole nucleus is one of the most important heterocyclics that has received much attention due to its diversified molecular design and remarkable optical and electronic properties. Among all the benzoheterocycles, benzothiazole has a considerable place in the area of research especially in synthetic as well as in pharmaceutical chemistry due to its potent and diversified pharmacological activities The present study describes the synthesis of benzothiazole derivatives and evaluation of the *in-vivo* anti-inflammatory activity.

MATERIALS AND METHODS

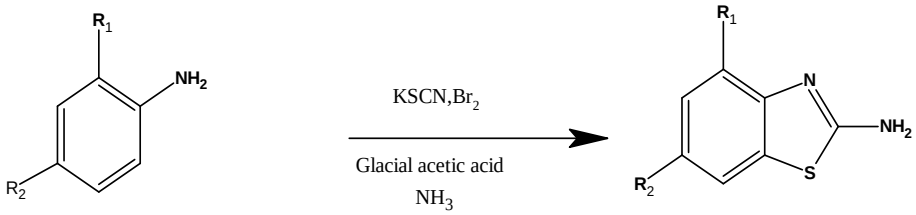
All materials used for the synthesis of benzothiazole derivatives were obtained from S. D. Fine chem. Ltd., Mumbai, and Sigma-Aldrich Chemical Co. of Lancaster, and used with no further filtration. To track the advancement of the reaction & output of the product, TLC was used. On apre-coated 0.25 mm plate of silica gel 60F254, a thin layer of chromatography of the combined chemicals is produced. Merck, Darmstadt, Germany, used a particular medium for solvents. Under UV light and in the Iodine chamber, screening is performed. Spot detection is achieved over short and long distances under a UV light. M.P. has been calculated by and is not set by the open capillary system. IR Spectra (max in cm-1) of synthetic chemicals reported in the potassium bromide Shimadzu FTIR-8400s, Perkin Elmer 881 in 400-4000 cm^{-1} range. On the Brucker ADVANCE DRX 300 MHz / 200MHz spectrometer, 1HNMR spectra (ppm, δ) were registered with TMS as an internal standard.

Design synthetic scheme of Di- substituted -1,3 benzothiazole -2 amine: [11-12]

The designed synthetic scheme was performed according to the sequence of steps given below:

1. Synthesis of Di – substituted -1,3 benzothiazole - 2 amines
2. Synthesis of Schiff base

Synthesis of Di- substituted -1,3 benzothiazole -2 amine:

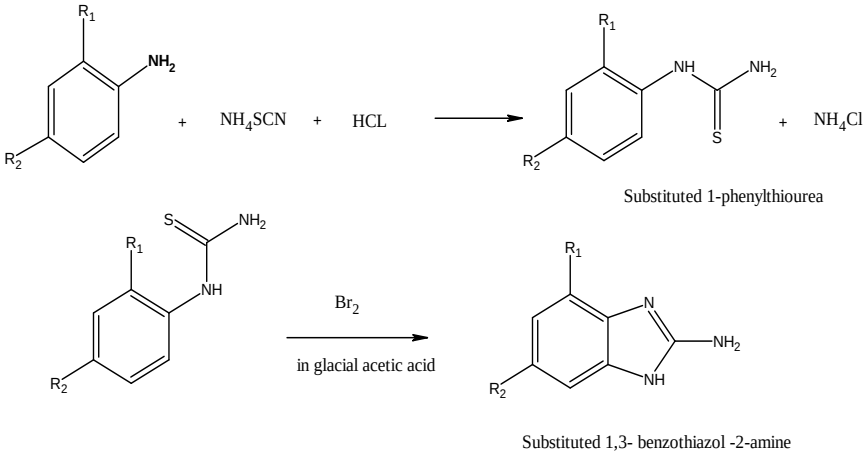


Substituted Aniline

(1a-f) (2a-f)

Figure 1: synthesis of parent Di- substituted -1,3 benzothiazole -2 amine:

R_1 = H, R_2 = Cl, R_1 = H, R_2 = OH, R_1 = OH, R_2 = H, R_1 = OH, R_2 = COOH, R_1 = OCH_3, R_2 = H, R_1 = H, R_2 = SO_3H



SCHEME -I: Synthesis of Substituted 1,3-benzothiazole-2-amine

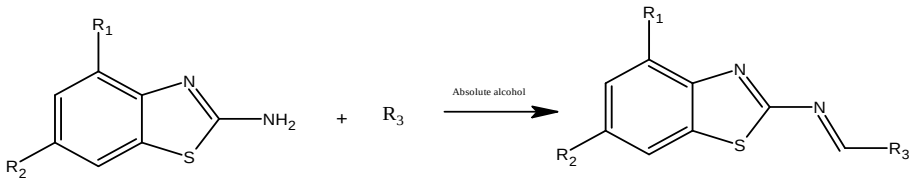
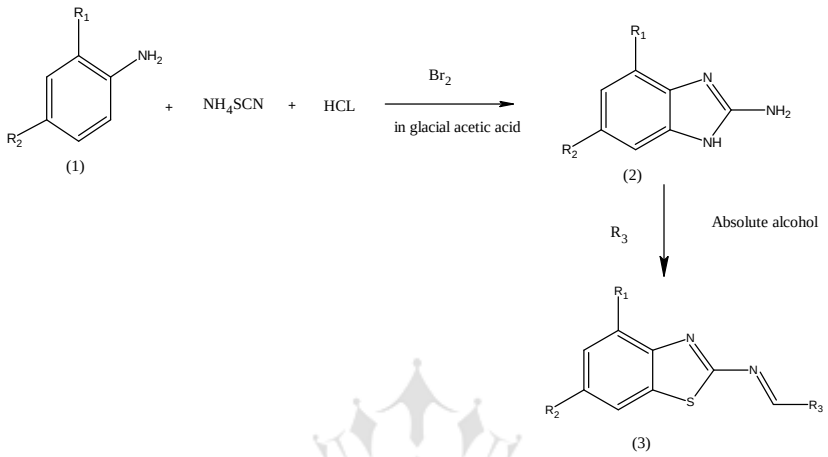


Figure 2: Synthesis of Schiff base:

Where, R3= Benzaldehyde, Vanillin



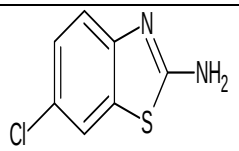
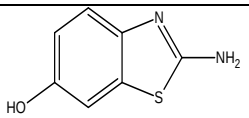
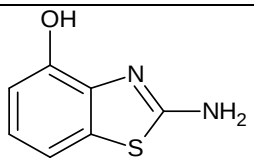
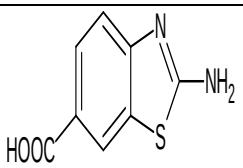
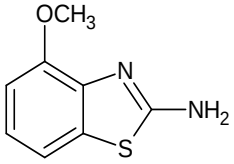
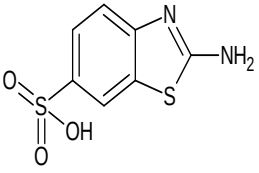
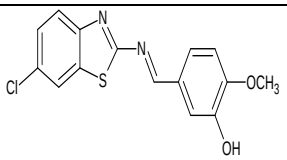
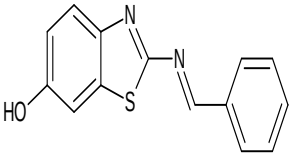
SCHEME II: Formation of Benzothiazole derivative

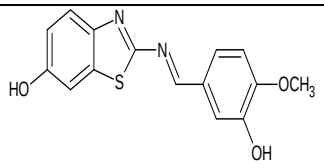
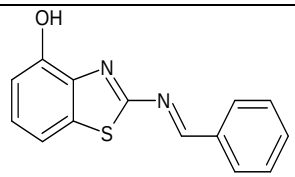
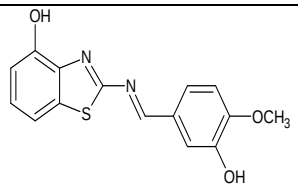
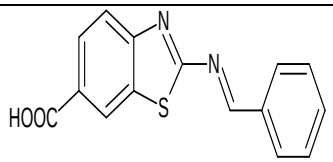
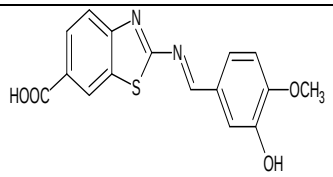
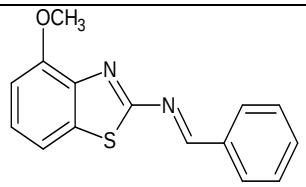
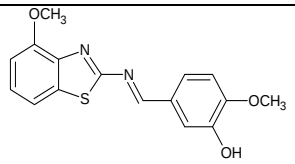
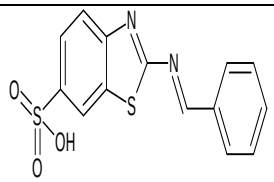
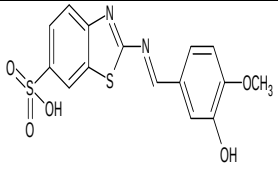
Procedure for synthesis of Step 1 compound: Glacial acetic acid (150 ml) precooled to 5 °C was added to potassium thiocyanate (0.06 mol) and substituted aniline (0.06 mol) (1a). The mixture was placed in a freezing mixture of ice and mechanically stirred while bromine (0.02 Mol) in 10 ml glacial acetic acid was added from a dropping funnel at such rate that the temperature does not rise beyond 0-5 °C. After all the bromine has been added (110 min), the solution was stirred for an additional 2 hour at 0-10 °C. The residue was filtered and dissolved in hot water (150 ml). The solution was filtered and the filtrate was neutralized with a conc ammonia solution to pH 6. The precipitates were collected and crystallized with ethanol [60].

Procedure for synthesis of step 2 compound: Derivatives of Di - substituted -1,3 benzothiazole - 2 amines (0.01 mole) and solution of aldehyde (0.01 mole) were dissolved in 50 ml absolute alcohol. To this solution, a small amount of anhydrous zinc chloride was added. The reaction mixture was refluxed for 18-20 hours in a water bath. It was cooled and

poured into compressed ice. The solid product was obtained and the product washed with water and recrystallized from ethanol.

Table No 1: Analytical data of isolated compound

code	Structure	Molecular formula	Molecular weight	M.P	color	% yield
2a		$C_7H_5ClN_2S$	184.646	179	White crystalline	69.56
2b	 2-aminobenzo[*d*]thiazol-6-ol	$C_7H_6N_2OS$	166.20034	154	Brown solid	59.45
2c		$C_7H_6N_2OS$	166.20034			
2d		$C_8H_6N_2O_2S$	194.21044	169	Pale yellow	78.49
2e		$C_8H_8N_2OS$	180.22692			
2f		$C_7H_6N_2O_3S_2$	230.26414			
3a		$C_{15}H_{11}ClN_2O_2S$	318.77804			
3b		$C_{14}H_{10}N_2OS$	254.307			

3c		$C_{15}H_{12}N_2O_3S$	300.33238			
3d		$C_{14}H_{10}N_2OS$	254.307			
3e		$C_{15}H_{12}N_2O_3S$	300.33238			
3f		$C_{15}H_{10}N_2O_2S$	282.3171		Yellow solid	
3g		$C_{16}H_{12}N_2O_4S$	328.34248			
3h		$C_{15}H_{12}N_2OS$	268.33358			
3i		$C_{16}H_{14}N_2O_3S$	314.35896			
3J		$C_{15}H_{12}N_2O_4S_2$	318.3708			
3k		$C_{15}H_{12}N_2O_5S_2$	348.39678			

CHARACTERIZATION:

Melting Point Determination: The melting point of the synthesized compound was determined using the capillary fusion method. A Capillary was taken and one end was sealed by bringing it near the burner flame. The open end of the capillary tube was pushed into a small heap of the compound so that a small plug of the powder was collected in the open end. The tube was tapped gently so that the collected materials settle down. The capillary tube was placed in the melting point determination apparatus. The temperature at which the compound starts to melt was noted with the help of a thermometer. Data came from M.P. determination by capillary fusion method. [13]

Thin Layer Chromatography: The main objective involved in performing TLC include the qualitative identification for completion of the reaction, checking the purity of the synthesized compounds, quantitative isolation of pure compounds, evaluating solvents as mobile phases for a particular separation and monitoring the progress of the synthetic reaction.

The pre-coated plates were activated at 110 °C for 90 minutes in a hot air oven and cooled in a vacuum desiccator on silica gel. The solutions of synthesized benzothiazole derivatives were prepared by dissolving a few amounts of the compound in 5ml of acetone and methanol. Then spotted on pre-coated plates with the help of a microcapillary tube. The plates were run in a pre-saturated mobile phase (solvent system) in a developing chamber using the following system given in the table.

Chromatograms were developed in a developing chamber saturated with the specific solvent system at 25 °C. The solvent front was allowed to travel approximately 8 cm. Plates were taken out, air-dried, and exposed to iodine vapor by putting them in it iodine saturated chamber. The plates were also seen under UV lamps 254 nm and 366nm. The Rf values were calculated using the following formula. [14]

$$R_f = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent front}}$$

Table No 2: Solvent system used in TLC

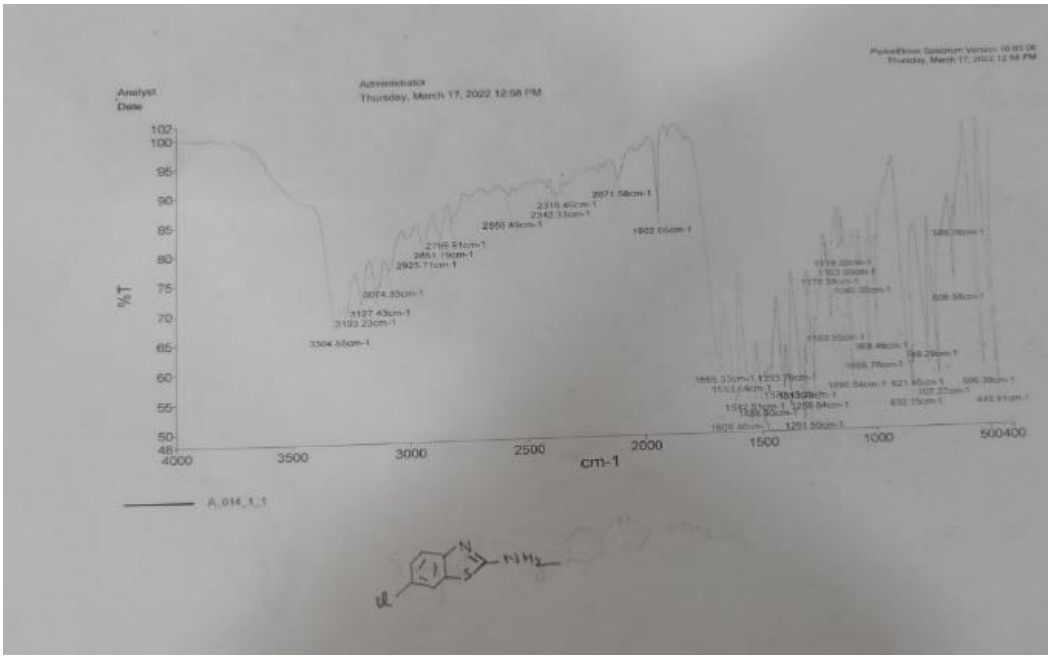
1	Chloroform: methanol: few drop of formic acid	7:3:few drops
2	n-hexane: Ethyl acetate: Methanol	6:3:1

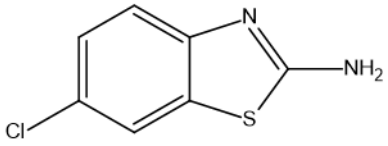
Table no 3: Rf value of synthesized compound

S. No.	code	Rf Value
1	2a	0.67
2	2b	0.68
3	2c	0.67
4	2d	0.69
5	2e	0.70
6	2f	0.69
7	3a	0.72
8	3b	0.70
9	3c	0.72
10	3d	0.71
11	3e	0.70
12	3f	0.72
13	3g	0.73
14	3h	0.71
15	3i	0.70
16	3J	0.72
17	3k	0.70

Spectroscopic study: The characterization of synthesized compounds was done by IR, and NMR spectral data.IR spectra were scanned on Shimadzu FTIR-8400s, Perkin Elmer 881 Spectrophotometer in the frequency range of 4000-400cm-1 by KBr-DRS method. 1H NMR spectral was recorded in DMF with tetramethylsilane (TMS) as the internal standard at 400 MHz on a Brucker ADVANCE DRX 300 MHz / 200MHz spectrometer. The chemical shifts are reported as parts per million (ppm). [15]

Spectral data of Compound 2a:





6-chlorobenzo[*d*]thiazol-2-amine

2a

Figure no 2.1: FTIR Spectra of Compound of 2A

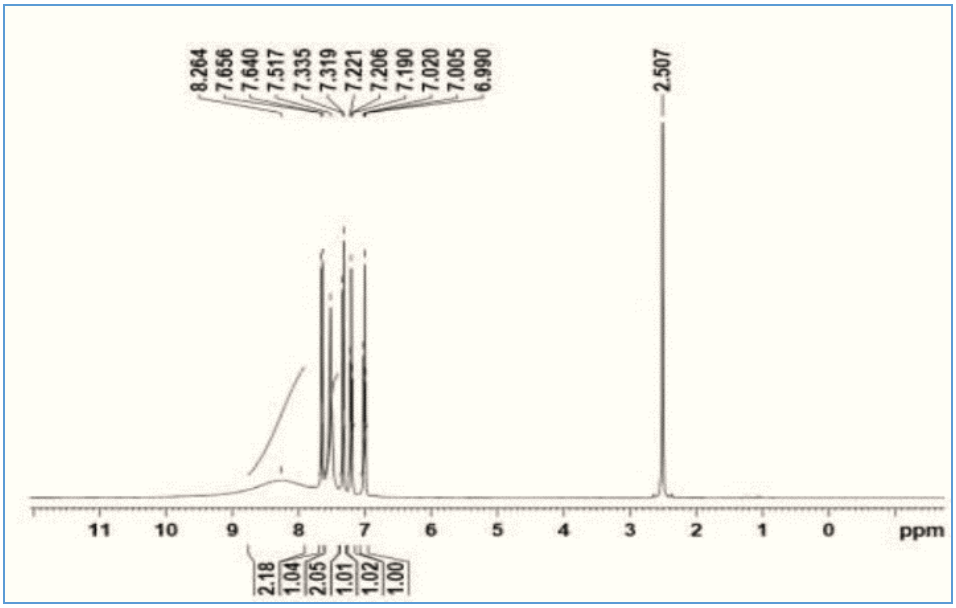
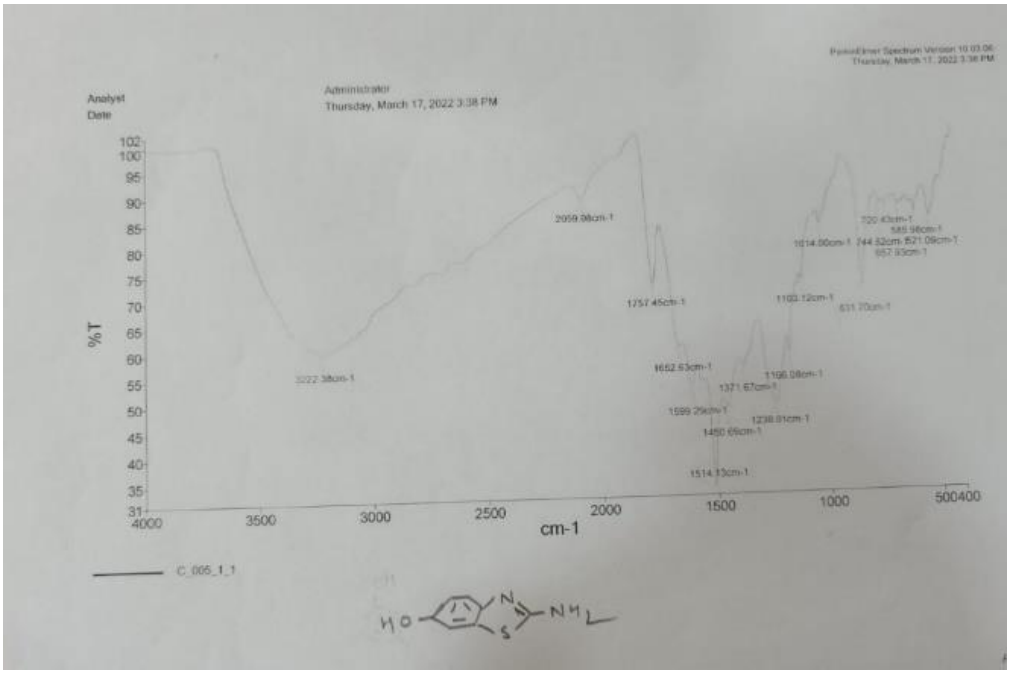
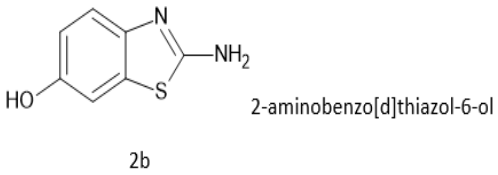


Figure 3: 1H-NMR Spectra of Compound 2a

Spectral data of Compound 2b:





2-aminobenzo[d]thiazol-6-ol

2b

Figure 4: FTIR Spectra of Compound 2b

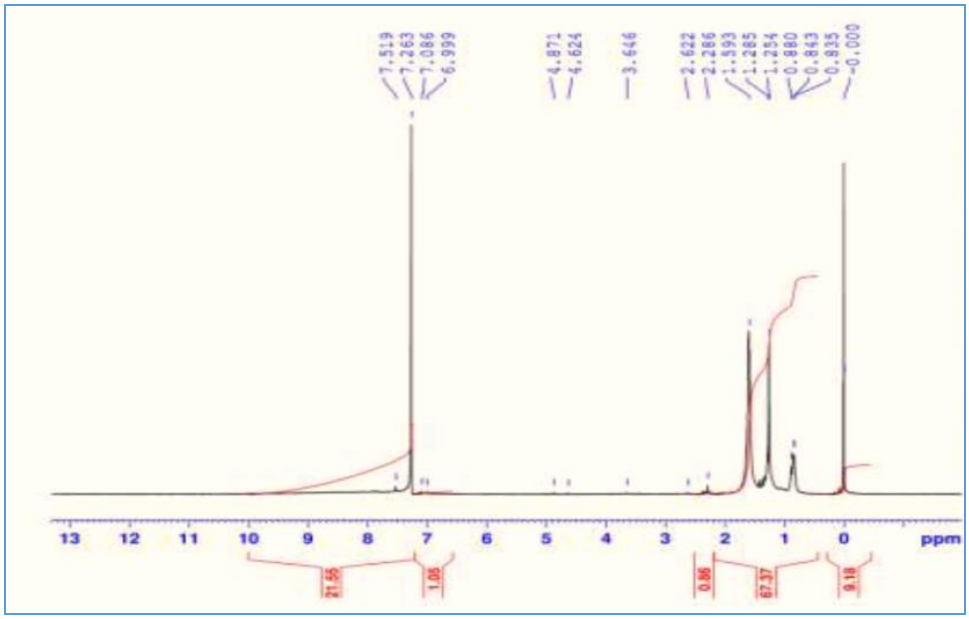
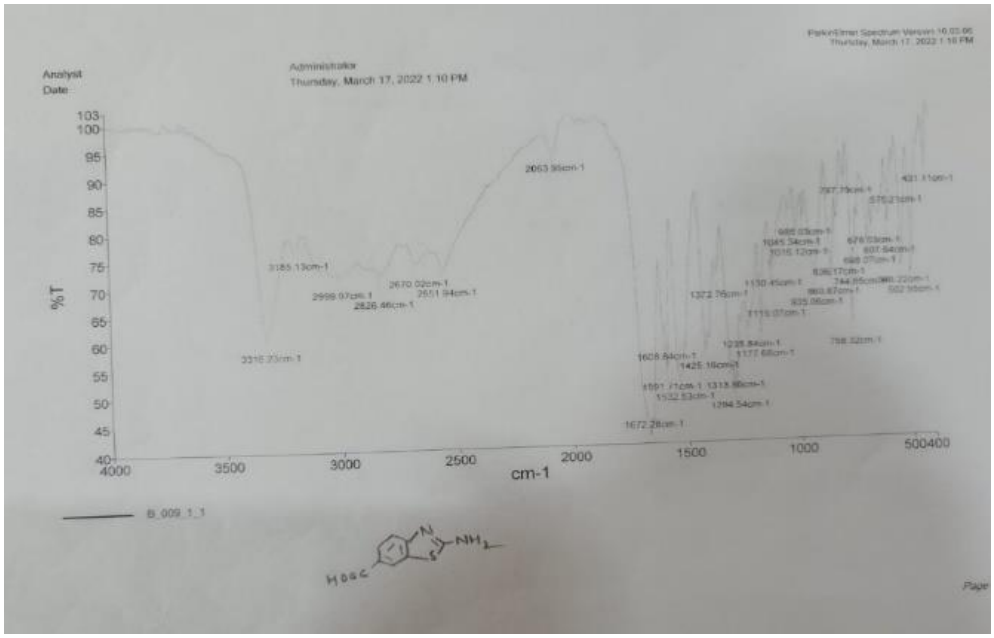
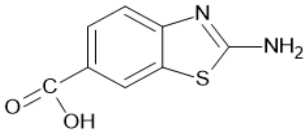


Figure 5: 1H-NMR Spectra of Compound 2b

Spectra data of compound 2d:





2-aminobenzo[d]thiazole-6-carboxylic acid

2d

Figure 6: FTIR Spectra of Compound 2d

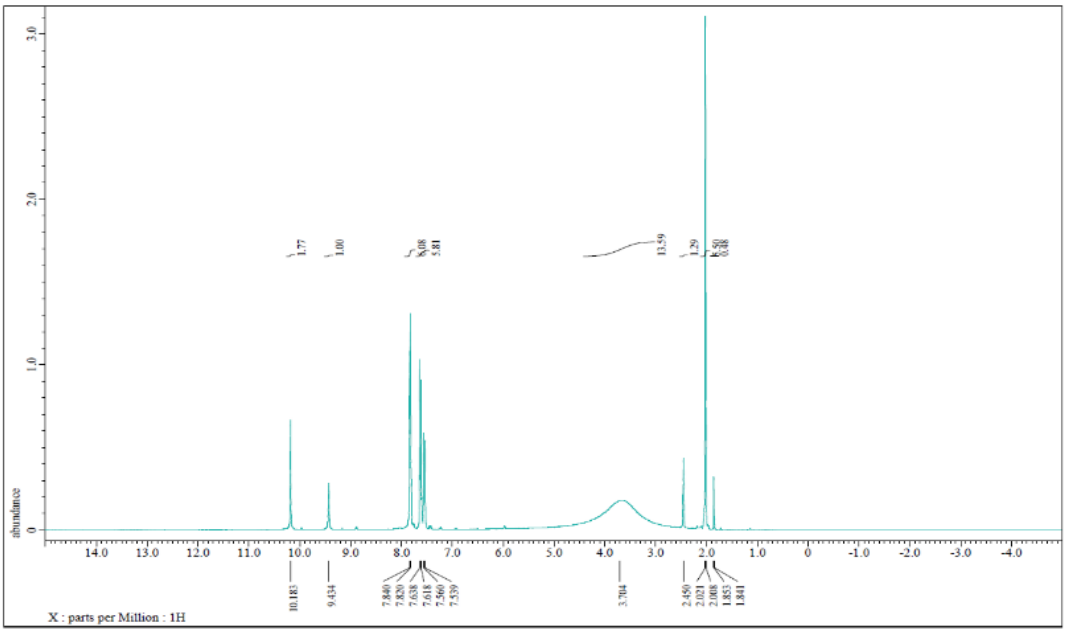
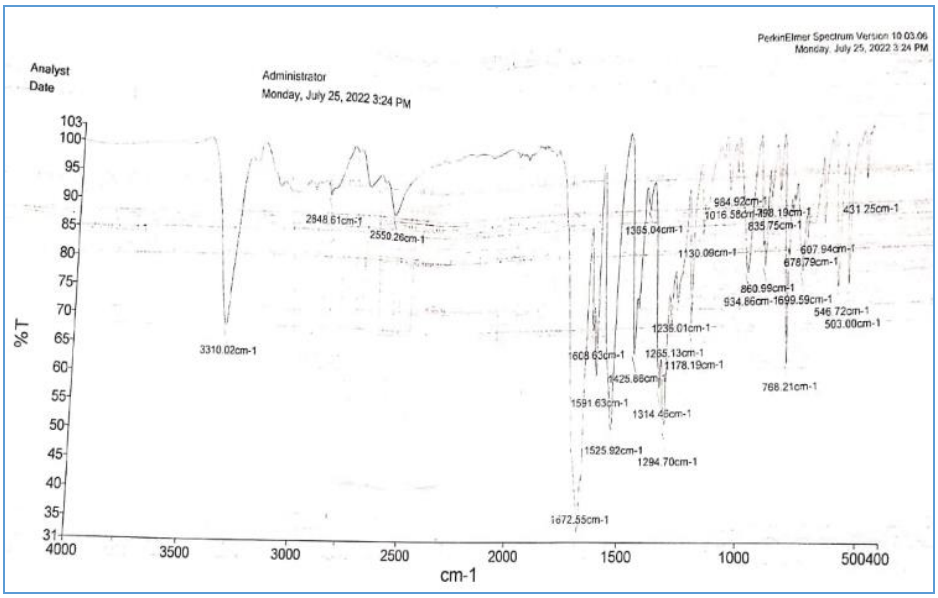
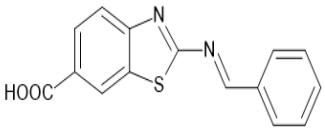


Figure 7: 1H-NMR Spectra of compound 2d

Spectra data of Compound 3f:





3f

Figure 8: FTIR Spectra of Compound 3f

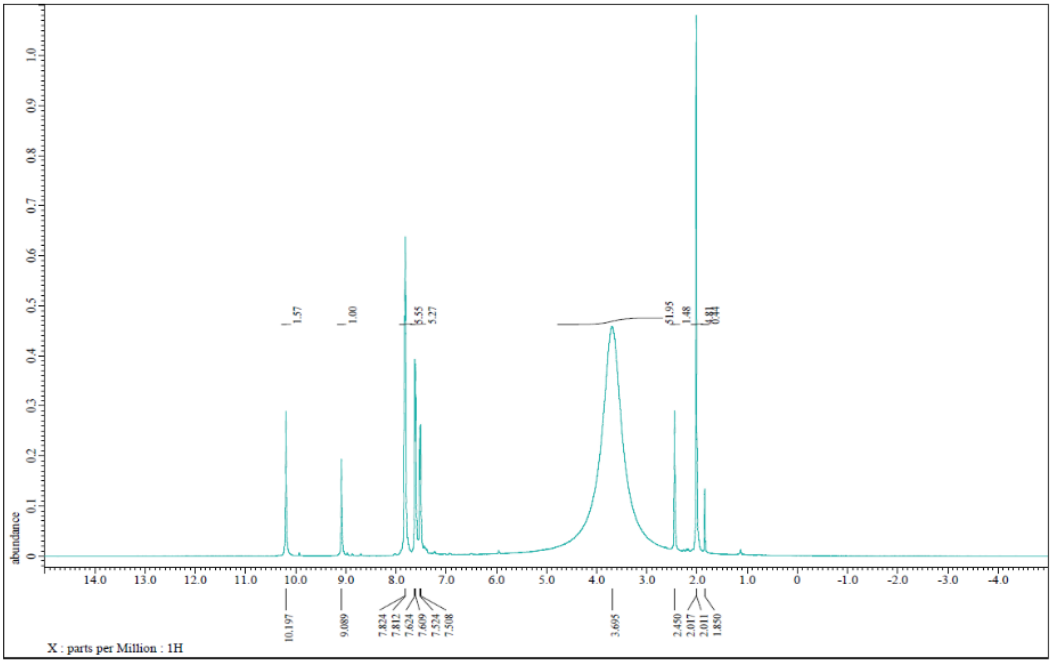


Figure 9: 1H-NMR Spectra of compound 3f

PHARMACOLOGICAL EVALUATION

Acute Toxicity Studies:(16, 17) The method was followed using OECD 420 (Acute Toxic Class Method). The acute toxic class process is a step-wise procedure with three rats of a single-sex per step. Depending on the mortality of the rats and the standard two to three steps may be essential to allow a conclusion on the acute toxicity of the test material. This method results in the use of rats while allowing for satisfactory data-based systematic conclusions. The process is used to define doses (1500, 1000, 500, and 250 mg/kg) the reports allow material to be ranked and classified based on the Globally Harmonized System (GHS) for the categorization of chemicals which produce acute toxicity.

Materials: Vehicle - .5% DMSO + Phosphate buffer saline, Oral needle, Derivative 1 and 2

Experimental Animals: Albino rats (weighing 150-200 g) were housed at the animal house of the Advanced Institute of Biotech and Paramedical sciences under a controlled environment (23–25° C). Animals were kept in plastic cages and were fasted for 24 h before starting the experiment. Animals were provided with tap water ad libitum and a standard pellet diet. Experiments performed complied with the rules of the Institute of Laboratory Animal Resource, National Research Council (1996) and were approved by the Ethical Committee of Advanced Institute of Biotech and Paramedical sciences, Dr. A.P.J. Abdul Kalam Technical University. (AEC) clearance was done with reference no: 1122/PO/RE/S/2007/CPCSEA.

Experimental Procedure: (17) The two different derivatives were selected for acute oral toxicity study. The initial dose level of derivatives was 1500 mg/kg, p.o. for different groups. The derivatives have LD50 higher than 1500 mg/kg; p.o. A single dose of derivatives was given 1ml/100 gm body weight to rats which are kept fasted overnight. Food was withdrawn for the next 3-4 hours after administration of the derivative. Weight was noted down before and after the administration of derivatives. The signs of changes in skin and fur, mucous membranes, eyes, circulatory, respiratory, autonomic, and central nervous system, motor activity, and behavior patterns were noted. The toxicity signs of fits, excessive salivation, tremors, diarrhea, lethargy, sleep, and coma, as well as the appearance of toxicity and signs of toxicity, were too noted and given in Tables no 4 and 5.

Table No 4: Acute toxicity of compound 2d

S. No.	Group	Dose (mg/kg)	Weight of rats		Mortality		Toxic symptoms
			Before	After	D/T	Latency (h)	
1	I	50	159± 3.21	164± 3.24	0/3	-	none
2	II	50	162± 4.32	168± 4.56	0/3	-	none
3	I	300	157± 3.45	163± 3.39	1/3	<2h	Anorexia, hypoactivity
4	II	300	162± 4.65	166± 3.45	1/3	<2h	Anorexia, hypoactivity
5	I	2000	159± 2.21	169± 2.45	2/3	<1	Anorexia, hypoactivity
6	II	2000	163± 2.21	169± 3.56	2/3	<1	Anorexia, hypoactivity

Table 5: Acute toxicity of compound 3f

S. No.	Group	Dose (mg/kg)	Weight of rats		Mortality		Toxic symptoms
			Before	After	D/T	Latency (h)	
1	I	50	163± 2.67	168± 4.87	0/3	-	none
2	II	50	164± 3.03	169± 4.92	0/3	-	none
3	I	300	159± 2.89	164± 3.73	1/3	<h	Anorexia, hypoactivity
4	II	300	161± 3.89	166± 2.87	1/3	<2h	Anorexia, hypoactivity
5	I	2000	158± 2.76	164± 3.09	2/3	<1	Anorexia, hypoactivity
6	II	2000	160± 3.45	127± 3.87	2/3	<1	Anorexia, hypoactivity

Anti-inflammatory Activity:

- **Animals:** Wister rats (150-200gm)
- **Drug:** Nimesulide
- **Chemical:** Carrageenan (1%), sodium Chloride, CMC

Anti-inflammatory activity (carrageenan-induced paw edema method in rats): Anti-inflammatory activity [18, 19] was performed by the carrageenan-induced paw edema method in rats carried out using Digital Plethysmometer. The tested compound and reference drug (Nimesulide)were administered orally at a dose level of 10 mg/kg, 0.5 h before carrageenan injection at the right hind paw of albino male rats, the thickness of both paws was measured at different time intervals of zero, 15, 30, 45, 60 min and 120 min after carrageenan injection. The anti-inflammatory activity of the tested compound and Nimesulide was calculated as the percentage decrease in edema thickness induced by carrageenan. It was determined with the following formula, The percentage inhibition of paw edema was calculated by using the following formula and the results are depicted in the table.

Percentage inhibition =[(Control-Test) \Control] × 10

Table 6: The anti-inflammatory activity of compounds 2 and 3f at different intervals using carrageenan-induced paw edema compared to nimesulide

Comp.	Dose (mg/ kg)	15 min		30 min		60 min		120 min	
		Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%
2d	10	0.58±0.43	55.7	0.62±0.43	57.9	0.67±0.43	62.5	0.62±0.43	59.6
3f	10	0.50±0.032	45.7	0.53±0.042	48.7	0.56±0.087	55.7	0.55±0.07	54.7
Control	-	1.03 ±0.003		1.16 ±0.001		1.21 ±0.002		1.24 ±0.001	
Nim.	10	0.53±0.03	54.4	0.57±0.12	57.5	0.59±0.87	63.7	0.52±0.82	59.64

All synthesized compounds (2d, 3f) were screened for their in-vivo anti-inflammatory activity using the carrageenan-induced rat paw edema model and exhibited protection against carrageenan-induced edema. The protection range was up to 63.7 % for the reference drug (nimesulide) after 1 hrs and 59.64 % after 2 hrs, while the synthesized compound 2d protected up to 62.5 after 1 hr at an equivalent dose. The result of this study clearly showed

that % inhibition for the standard drug nimesulide was slightly decreased after 2 hrs. Among all the tested compounds 2d showed remarkable anti-inflammatory activity compared to the standard drug nimesulide.

4. RESULTS AND DISCUSSION

The exhaustive literature survey revealed that Benzothiazole derivatives possess various biological activities like antifungal, anti-inflammatory, antiproteolytic, antibacterial, anti-viral, anti-depressant, anticonvulsant, etc. The aim of the work is the synthesis, characterization & evaluation of the anti-inflammatory efficacy of benzothiazole derivatives.

The benzothiazole synthesis was performed in 2 stages. In the first step, The benzothiazole synthesis was performed in 2 stages. In the first step, 6- substituted aniline with potassium thiocyanate and bromine in presence of glacial acetic acid ammonia which yield substituted 2 amino benzothiazole derivatives.

Substituted benzothiazole then reacts with different heterocyclic aldehydes to form corresponding imines in absolute ethanol and GAA. Finally, in the presence of anhydrous ZnCl2, corresponding imine (Schiff base).

Physical, spectral, and elemental research has characterized all the synthesized compounds. TLC using silica gel G pre-coated plates has established the purity of the compounds. The spots of both short and long wavelengths were observed under UV light. In the iodine chamber, the spots were further identified. The melting range has been calculated and is uncorrected by the open capillary process.

The presence of C = S at 1593-1608 cm^{-1} and S-H at 3413-3415 cm^{-1} respectively was shown by the IR spectrum of Benzothiazole (2a,2b, 2d, and 3f). In a range of 3.37-3.75 for methyl proton and 7.26-7.68 for benzyl proton, the δ value was found in the 1H-NMR spectra in various synthesized compounds. For the measured presence of carbon, hydrogen, and nitrogen, elemental analysis was performed. The IR, NMR, and elemental study details for all synthesized compounds (2d and 3f) were shown.

Future Prospect:

Substitution at the 2-position and 6-position of the Benzothiazole nucleus can lead to a series of compounds having different biological activities. The change in the indole nucleus may

also lead to different compounds. The replacement of Cl in Isolated compounds by other groups may vary the physiochemical and biological activities of the compounds.

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