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Synthesis of some biologically active benzothiazole derivatives

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ABSTRACT

N-(4,5-dihydro-1*H*-imidazol-2-yl)-6-substituted-1,3-benzothiazol-2-amines and *N*-(1*H*-benzimidazol-2-yl)-6-substituted-1,3-benzothiazol-2-amines were synthesized by the reaction of 6-substituted-2-aminobenzothiazoles with carbon disulphide and methyl iodide. It was followed by the reaction with o-phenylene diamine/ ethylene diamine. All the synthesized compounds were characterized by elemental analysis, IR spectra, ^{1}H NMR and MASS spectral studies. They were screened for their anti-inflammatory, antiulcer, antitumor, entomological (antifeedant, acaricidal, contact toxicity and stomach toxicity) and antibacterial activities.

Keywords: Benzothiazole, Imidazoline, Benzimidazole, Anti-inflammatory, Antiulcer, Antitumor.

INTRODUCTION

The survey of literature related to benzothiazoles, benzimidazoles and imidazolines reveals that compounds with these nuclei are very important in the field of pharmaceutical chemistry. Benzothiazole derivatives possess a wide spectrum of biological activities such as antitumor [1-3], antihistamines [4], antibacterial [5], analgesics [6], anti-inflammatory [7], schistosomicidal [8], anti HIV [9], and antivirus [10] etc. Further a wide range of therapeutic activities of benzimidazole derivatives show that they are also used as antiviral [11, 12], anticancer, antibacterial [13], proton pump blocker [14], antiotensin II, hypertension [15], antioxidant [16], antinociceptive [17], anti-inflammatory [17, 18], analgesic [18], antiparasites [19], human cytomegalovirus (HCMV) replication inhibitor [20], fungicidal [21], and antihistamines [22, 23] etc. Similarly imidazoline derivatives also possess an array of biological activities. They are useful as anticancer [24, 25], anti-inflammatory [25], anticoagulant [26], hypnotic agents [27], antimicrobial [28, 29], and antimycobacterial [30]

etc. These reports prompted us to synthesize some new benzothiazole derivatives containing benzimidazole or imidazoline moiety. In continuation of our research work on benzothiazole derivatives [31], we are reporting the synthesis of 6-substituted- *N*-(4,5-dihydro-1*H*-imidazol-2-yl)-1,3-benzothiazol-2-amines **3** and *N*-(1H-benzimidazol-2-yl)-6-substituted-1,3-benzothiazol-2-amines **4**. Antibacterial, antiulcer, antitumor, anti-inflammatory and entomology activities have been tested for the synthesized compounds.

MATERIALS AND METHODS

2.1 Chemistry

Reagent grade chemicals were used without further purification. All the melting points were taken in open capillary tubes and are uncorrected. The purity of the synthesized compounds was checked by Thin Layer Chromatography. IR spectra were scanned on FT IR Perkins Elmer (Spectrum RX1) spectrophotometer (δ in cm^{-1}) using KBr disc. ^1H NMR spectra were recorded in DMSO with tetramethylsilane (TMS) as the internal standard at 300 MHz on a Bruker DRTX-300 spectrophotometer. The chemical shifts were reported as parts per million (ppm). Fast atom bombardment mass spectra (FABMS) were recorded on a Jeol SX-102/DA-6000 mass spectrophotometer/data system using Argon/Xenon (6 kV, 10 mA) as the FAB gas. The accelerating potential was 10 kV. The elemental analysis of compounds was performed on Elementar Vario EL III Carlo Erba-1108 elemental analyzer.

2.1.1 Synthesis of 6-substituted-2-aminobenzothiazoles (1):

6- Substituted-2-aminobenzothiazoles **1** were prepared by method reported earlier [32, 33].

2.1.2 Synthesis of dimethyl (6-substituted-1,3-benzothiazol-2-yl)dithioimidocarbonates (2)

To a well stirred ice cold solution of **1** (0.025 mol.) in dimethylformamide (10 mL), were added 10 M aq. NaOH (4 mL), carbon disulphide (0.05 mol.) and methyl iodide (0.025 mole) after an interval of 30 min. and stirring was continued for 4 hrs. The mixture was then poured in ice cold water and the resulting solid was washed with water and recrystallised from aq. ethanol. Physico-chemical data of synthesized compounds are given in Table 1.

Dimethyl (6-chloro-1,3-benzothiazol-2-yl)dithioimidocarbonates (2a)

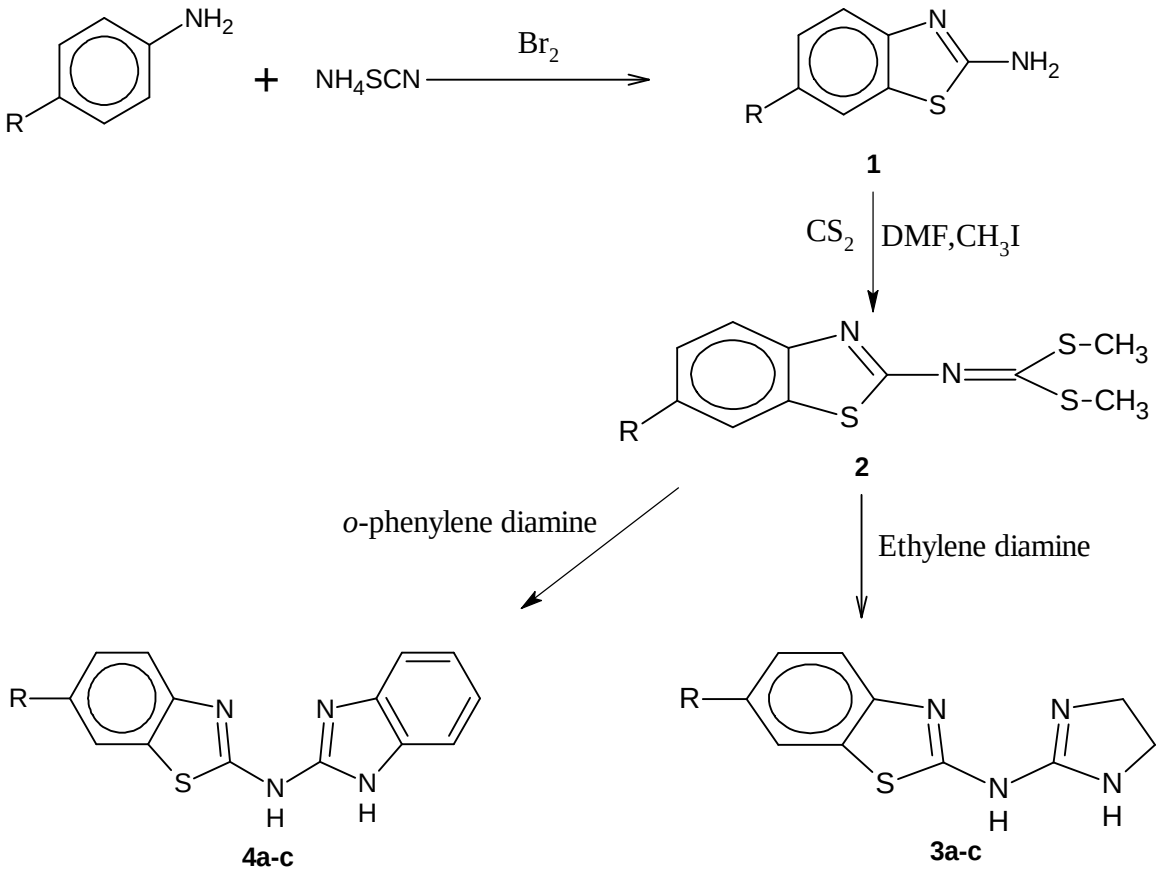
Yield 80%. Anal. Calcd for $C_{10}H_9ClN_2S_3$: C- 41.58%, H- 3.14%, N- 9.70%, Found C- 41.60%, H-3.08%, N-9.74%; IR: - 3044, 1588, 1498, 1172, 1100, 771, 676 and 623 (benzothiazole with aromatic ring), 2920 (aliphatic CH), 1633 (C=N), 1263, 1326 (C-S), 847 (C- Cl), MS 289 (M+), ^1H NMR: - 2.61 (s, 6H, 2 x SCH_3), 7.12- 7.56 (m, 3H, Ar-H).

Dimethyl (6-methoxy-1,3-benzothiazol-2-yl)dithioimidocarbonates (2b)

Yield 78%. Anal. Calcd for $C_{11}H_{12}N_2OS_3$: C- 46.45%, H- 4.25%, N- 9.85%, Found C- 46.48%, H-4.21%, N- 9.90%; IR: - 3095, 1605, 1464, 1178, 1111, 808, 710 and 615 (benzothiazole with aromatic ring), 2898 (aliphatic CH), 1643 (C=N), 1338, 1264 (C-S), 1277 (C-O-C), MS 285 (M+), ^1H NMR: - 2.51 (s, 6H, 2 x SCH_3), 3.71 (s, 3H, Ar-OCH_3), 7.15- 7.38 (m, 3H, Ar-H).

Dimethyl (6-nitro-1,3-benzothiazol-2-yl)dithioimidocarbonates (2c)

Yield 85%. Anal. Calcd for $C_{10}H_9N_3O_2S_3$: C- 40.12%, H- 3.03%, N- 14.04%, Found C- 40.20%, H- 3.00%, N-14.01%; IR: - 3090, 1594, 1100, 1176, 1123, 747, 674 and 616 (benzothiazole with aromatic ring), 2960 (aliphatic CH), 1640 (C=N), 1311, 1260 (C-S), 1331 (NO_2), MS 300 (M+),^1H NMR: - 2.63 (s, 6H, 2 x SCH_3), 7.31- 7.75 (m, 3H, Ar-H).



R: Cl, OCH_3, NO_2

Scheme: - 1

2.1.3 Synthesis of *N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-substituted-1,3-benzothiazol-2-amines (3)

To a solution of **2** (0.004 mol.) in DMF (15 mL) was added solution of ethylene diamine (0.008 mol.) in DMF (15 mL) with stirring at room temp. The reaction mixture was refluxed for 8 hrs. The mixture was poured on crushed ice. The resulting solid was dried and recrystallised from ethanol. Physico-chemical data of synthesized compounds are summarized in Table 1.

***N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-Chloro-1,3-benzothiazol-2-amine (3a)**

Yield 82%. Anal. Calcd for $C_{10}H_9ClN_4S$: C- 47.53%, H- 3.59%, N- 22.17%, Found C- 47.50%, H- 3.60%, N- 22.10%; IR: - 3041, 1522, 1478, 1098, 1075, 804, 724 and 690 (benzothiazole with aromatic ring), 3303, 3281 (N-H), 1618 (C=N), 1349 (C-S), 840 (C-Cl), MS 253 (M+), ^{1}H NMR: - 5.58 (s, 4H, 2 x CH_2), 3.48 (s, 1H, acyclic N-H), 7.24- 7.58 (m, 3H, Ar-H), 8.07 (s, 1H, cyclic N-H).

***N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-Methoxy -1,3-benzothiazol-2-amine (3b)**

Yield 75%. Anal. Calcd for $C_{11}H_{12}N_2OS$: C- 53.20%, H- 4.87%, N- 22.56%, Found C- 53.15%, H- 4.80%, N- 22.54%; IR: - 3090, 1520, 1482, 1113, 1057, 820, 723 and 671 (benzothiazole with aromatic ring), 3316, 3285 (N-H), 1620 (C=N), 1265 (C-O-C), 1360 (C-S), MS 249 (M+), ^{1}H NMR: - 3.52 (s, 4H, 2 x CH_2), 3.81 (s, 3H, Ar-OCH_3), 5.60 (s, 1H, acyclic N-H), 7.15- 7.48 (m, 3H, Ar-H), 7.98 (s, 1H, cyclic N-H).

***N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-Nitro-1,3-benzothiazol-2-amine (3c)**
Yield 85%. Anal. Calcd for $C_{10}H_9N_5O_2S$: C- 45.62%, H- 3.45%, N- 26.60%, Found C- 45.58 %, H- 3.40%, N- 26.58%; IR: - 3090, 1528, 1502, 1148, 1085, 810, 724 and 624 (benzothiazole with aromatic ring), 3326, 3289 (N-H), 1626 (C=N), 1357 (C-S), 1333 (NO_2), MS 264 (M+), ^{1}H NMR: - 3.54 (s, 4H, 2 x CH_2), 5.72 (s, 1H, acyclic N-H), 7.12- 7.45 (m, 3H, Ar-H), 8.05 (s, 1H, cyclic N-H).

2.1.4 Synthesis of *N*-(1*H*-benzimidazol-2-yl)-6-substituted-1,3-benzothiazol-2-amines (4)
To a solution of **2** (0.005 mol.) in DMF (20 mL) was added in solution of *o*-phenylene diamine (0.004 mol.) in DMF (15mL) with stirring at room temp. The reaction mixture was refluxed for 8 hrs. The mixture was poured on crushed ice. The resulting solid was dried and recrystallised from ethanol. Physico-chemical data of synthesized compounds are given in Table 1.

Table 1: - Physico-chemical data of synthesized compounds

Compound	R	Mol. Formula	Melting point ^{o}C
2a	Cl	$C_{10}H_9ClN_2S_3$	180- 184
2b	OCH_3	$C_{11}H_{12}N_2OS_3$	159- 161
2c	NO_2	$C_{10}H_9N_3O_2S_3$	216- 219
3a	Cl	$C_{10}H_9ClN_4S$	230- 234
3b	OCH_3	$C_{11}H_{12}N_2OS$	196- 199
3c	NO_2	$C_{10}H_9N_5O_2S$	216- 218
4a	Cl	$C_{14}H_9ClN_4S$	250- 255
4b	OCH_3	$C_{15}H_{12}N_4OS$	150- 153
4c	NO_2	$C_{14}H_9N_5O_2S$	272- 275

***N*-(1*H*-benzimidazol-2-yl)-6-chloro-1,3-benzothiazol-2-amine (4a)**
Yield 70%. Anal. Calcd for $C_{14}H_9ClN_4S$: C- 55.90%, H- 3.02%, N- 18.62%, Found C- 55.88%, H- 3.00%, N- 18.60 %; IR: - 3076, 1597, 1539, 1156, 1098, 804, 743 and 667 (benzothiazole with aromatic ring), 3358, 3285 (N-H), 1633 (C=N), 1306 (C-S), 844 (C-Cl) , MS 301 (M+),^{1}H NMR: - 6.51 (s, 1H, acyclic N-H), 7.21- 7.60 (m, 7H, Ar-H), 7.98 (s, 1H, cyclic N-H).

***N*-(1*H*-benzimidazol-2-yl)-6-methoxy-1,3-benzothiazol-2-amine (4b)**
Yield 65%. Anal. Calcd for $C_{15}H_{12}N_4OS$: C- 60.79%, H- 4.08%, N- 18.90%, Found C- 60.75%, H- 4.04%, N- 18.85%; IR: - 3096, 1595, 1545, 1125, 1080, 806, 705 and 611 (benzothiazole with aromatic ring), 3387, 3287 (N-H) ,1641 (C=N), 1336 (C-S), 1265 (C-O-C), MS 297 (M+), ^{1}H NMR: - 3.71 (s, 3H, Ar-OCH_3), 6.54 (s, 1H, acyclic N-H), 6.95- 7.40 (m, 7H, Ar-H), 7.95 (s, 1H, cyclic N-H).

***N*-(1*H*-benzimidazol-2-yl)-6-nitro-1,3-benzothiazol-2-amine (4c)**
Yield 80%. Anal. Calcd for $C_{14}H_9N_5O_2S$: C-54.01%, H- 2.91%, N- 22.49%, Found C- 54.00, H-2.87%, N- 22.40; IR: - 3073, 1580, 1498, 1122, 1079, 820, 744 and 610 (benzothiazole with aromatic ring), 3386, 3290 (N-H), 1648 (C=N), 1329 (NO_2), 1330(C-S), MS 312 (M+), ^{1}H NMR: - 6.49 (s, 1H, acyclic N-H), 7.21- 7.72 (m, 7H, Ar-H), 8.07 (s, 1H, cyclic N-H).

2.2. Antibacterial activity: All the synthesized compounds were tested against gram positive bacteria *Staphylococcus aureus* and *Micrococcus luteus and* gram negative bacteria *Escherichia coli* and *Klebsiella species* using paper disc method [34]. Muller Hinton Agar (Hi-Media Pvt. Ltd. Mumbai, India) was used to culture the test bacteria. The microbial

culture were grown at 37 ^{0}C for 8 hours and then appropriately diluted with sterile 0.8% saline solution. The concentration of test drugs was kept 100 µg/mL and tested compounds at 200 µg/mL and 100 µg/mL in DMF. Standard drugs Novobiocin, Kanamycin and Amikacin were used for comparison. The antimicrobial activity was evaluated by measuring the zones of growth inhibition around disc of test organism and results are given in Table 2.

2.3. Antiulcer activity:

Aspirin (ASP) Induced Ulcers: Aspirin in dose of 200mg/ kg (20mg/mL) was administered to the animals on the day of the experiment and ulcers were scored after four hrs. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5 mL of 0.9% NaCl and ulcers were scored by a person unaware by the experimental protocol in the glandular portion of the stomach. Ulcer index was calculated by adding the total number of ulcers/ stomach and total severity of ulcers/stomach. The pooled group ulcer score was then calculated by reported method.

Ethanol (EtOH) induced Ulcers: The gastric ulcers were induced in rats by administering ethanol (1mL/ 200gm/kg for 1 hr) and the animals were sacrificed by cervical dislocation and the stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm^2/ rats). The results of antiulcer activity are summarized in Table 3.

2.4. Anti-inflammatory activity: The anti-inflammatory activity (% inhibition) of the test samples was evaluated *in-vivo* using carrageenan-induced paw edema bioassay method in rats. The % inhibition values were determined for each samples using phenyl butanone as a reference standard drug. The freshly prepared suspension of carrageenan (0.2 mL, 1.0% in 0.9% saline) was injected subcutaneously into the planter aponeurosis of the hind paw of the rats of both genders (male/ female) of about 120-140 g of body weight. One group of five rats was kept as a control and the animals of other group of five each were penetrated with the test compounds given orally 30 min before the carrageenan injection. The paw volume was measured by a water plethysmometer socrel at the time of treatment and then at an interval of one hour for four hour. The mean increase of paw volume at each time interval was compared with that of control groups and % anti-inflammatory values was calculated as given below:

$$\% \text{ anti-inflammatory} = (1-DT/DC) \times 100$$

Here, DT= volume of paw edema in drug treated, DC= volume of paw edema in drug control. The results of anti-inflammatory activity are summarized in Table 4.

2.5. Antitumor activity: This method was carried out to estimate the effect of test compound on the growth of tumor cells. The human breast cancer cells lines (MCF-7) were employed. The human breast cancer cell line (MCF-7) and mammary cancer cell line (EVSA-7),were co-incubated with the test compounds at 1 µg/mL doses for 96 hrs and the cell growth count was measured by MTT assay [35]. The basic principle involved in this assay depends upon the reduction of tetrazoleum salt. The yellow colored tetrazoleum MTT, [3-(4, 5-dimethylthiazol-2-yl)-2, 5,-diphenyltetrazoleumbromide] is reduced by metabolically active cells in part by the action of dehydrogenase enzymes to generate reducing equivalents such as NADH and NADPH. The resulting intra cellular purple colour zones were solubilized and quantified by spectrophotometer method. The MTT was dissolved in PBS (Phosphate Buffer Saline) at a concentration of 5 mg/ml.

Table 2: - The zone of inhibition of the compound as well as standard drugs tested for antibacterial activity

Compound	R	*E. Coli* *200* µg/mL, 100µg/mL		*K. species* *200* µg/mL, 100 µg/mL		*M. luteus* *200* µg/mL, 100 µg/mL		*S. aureus* *200* µg/mL, 100µg/mL	
2a	Cl	++	++	++	++	++	++	++	++
2b	OCH_3	++	++	++	++	++	++	++	++
2c	NO_2	++	++	++	++	++	++	++	++
3a	Cl	+++	++	+++	+++	++	++	++	++
3b	OCH_3	++	++	++	++	+++	++	+++	++
3c	NO_2	+++	++	+++	++	+++	++	+++	++
4a	Cl	+++	++	+++	++	++	++	+++	++
4b	OCH_3	++	++	++	++	++	++	++	++
4c	NO_2	++	++	++	++	+++	+++	+++	+++
DMF	------	+		+		+		+	
Novobiocin	------	+++		+++		++++		+++	
Kanamycin	------	++++		+++		++++		+++	
Amikacin	------	++++		+++		++++		+++	

Data represent zones of inhibition (mm) as follows: + 00, ++ 7-13 mm; +++ 14-19 mm; ++++ 20-26 mm

Then 50 µL of the MTT solution was added to each well of the 96 well culture plates, containing the 100 µL culture along with test compound and incubated at 37^{o}C for 4 hrs. The medium was then removed carefully without disturbing the purple colored crystals. Then, 50 mL of dimethylsulfoxide (DMSO) was added to each well and mixed thoroughly to dissolve the crystals. The plates were then read on ELISA plate reader at a wavelength of 570 nm. The readings were presented as optical density/cell count. The results of antitumor activity are summarized in Table 5.

2.6. Antifeedant activity: The antifeedant activity of these compounds was also carried out by leaf dip method [36, 37] using fourth instars larvae of Spodoptera litura. The leaf discs of about 25 cm^2 were prepared and dipped for thirty seconds in various test compounds. The leaf discs were air dried to evaporate the excess acetone and offered for feeding. The insects were allowed to feed for 24 hrs. After 24 hrs. leaf area uneaten was measured by using leaf area meter. The difference between leaf area provided and the leaf area uneaten is taken as amount of leaf area consumed. The feeding inhibition was calculated and used for calculation of effective concentration (EC_{50}/ LD_{50}) using Maximum Likelihood Programmer MLP 3.01. The results of antifeedant activity are summarized in Table 6.

2.7. Acaricidal activity: The acaricidal activity of these compounds was carried out by leaf dip method [36, 37]. Leaf discs of Mulberry (5 cm^2 diameter) were dipped in different test compounds for 30 seconds. The leaf discs were air dried to evaporate the excess acetone and placed over wet cotton in petri plate. The adult female mites were released on treated leaf discs and mortality data were recorded after 24 hours. Mites released on leaf treated only with acetone and tween 20 emulsifier served as control. The mortality data were used for calculation of LC_{50}/ LD_{50} using Maximum Likelihood Programmer MLP 3.01.The results of acaricidal activity are summarized in Table 7.

2.8. Contact toxicity: The contact toxicity of test compounds was carried out by topical application method [38, 39] against larvae of *Spodoptera litura*, which are harmful for Indian crops. First the test compounds were dissolved in acetone and than each compound were applied on the dorsal surface of the larvae. About 10 µL of each concentration was applied on each larva. Some of the larvae of insect were treated by acetone alone, work as control. Now the mortality data was recorded after 24 hrs, and the treated mortality was corrected with control morality. These corrected mortality data were used for calculation of LC_{50}/ LD_{50} using Maximum Likelihood Programmer MLP 3.01. The results of contact toxicity are summarized in Table 8.

2.9. Stomach toxicity: The stomach toxicity of test compounds was carried out by leaf dip method [36, 37]. In this method we used fourth instar larvae of *Spodoptera litura* of an insect which is responsible for the damage of Indian agricultural crops. Ten larvae were used for each replication and three replications were maintained for each compound. The test compounds were dissolved in acetone. The leaf disc were prepared out of caster leaf and dipped in various solutions of the test compounds for thirty seconds. The leaf discs were air dried to evaporate the excess acetone (The leaf discs dipped only in acetone served as control). The mortality data were recorded after 24 hrs, and the treatment mortality was corrected with control mortality. These mortality data was used for calculation of LC_{50}/LD_{50} using Maximum Likelihood Programmer, MLP 3.01. The results of stomach toxicity are summarized in Table 9.

RESULTS AND DISCUSSION

6-Substituted-2-aminobenzothiazoles **1** were prepared by thiocyanogenation of *p*-substituted anilines using method reported earlier [32, 33]. Compound **1** was reacted with carbon disulphide and methyl iodide in presence of concentrated NaOH leading to the formation of Dimethyl (6-substituted-1,3-benzothiazol-2-yl) dithioimidocarbonates **2.** It on treatment with bisnucleophiles viz *o*-phenylene diamine and ethylene diamine gave **3** and **4** (Scheme 1). The synthesized compounds were characterized by elemental analysis, IR, ^{1}HNMR and mass spectral studies. The structures of all the synthesized compounds were established on the basis of spectroscopic and analytical data. The elemental analysis (C, N and H) found for all the condensed products were in close agreement with the calculated values. The infrared (IR) spectrum of compounds **3** and **4** display two characteristic bands at 3350-3250 cm^{-1} and 2910- 2860 cm^{-1} due to N-H and CH_2 vibration, respectively.

Table 3: - Antiulcer (Gastro protective) activity of some selected compounds

Compound	Aspirin Induced		Ethanol Induced	
	Ulcer Index (mm^2/rat)	Protective Ratio (%)	Ulcer Index (mm^2/rat)	Protective Ratio (%)
3a	7.3±0.58	61.72	19.8±5.4	31.24
3b	7.2±0.54	61.58	19.6±5.3	33.72
3c	7.2±0.56	61.70	19.6±5.2	31.20
4a	7.2±0.58	61.68	19.9±5.4	18.21
4b	7.1±0.54	61.21	19.8±5.5	18.18
4c	7.2±0.54	61.68	19.7±5.2	18.17
Ranitidine	7.6±0.53	58.46	10.3±3.3	57.43
Aspirin	18.3±1.6	-	-	-
Ethanol	-	-	24.2±6.5	-

3.1. Antibacterial activity

The antibacterial activity of all the synthesized compounds was tested *in-vitro* against pathogenic *Escherichia coli, Klebsiella species, Micrococcus luteus and Staphylococcus aureus.* The results were compared with some standard drugs like Novobiocin, Kanamycin and Amikacin. In case of *Escherichia coli* Compounds **3a**, **3c** and **4a** exhibited higher activity at 200 µg/mL while rest of the compounds showed moderate activity. In case of *Klebsiellia* compounds **3a**, **3c** and **4a** showed higher activity than the rest of the compounds. In case of *Micrococcus luteus and Staphylococcus aureus* compounds **3b**, **3c** and **4c** showed higher activity than the rest of the compounds (Table 2).

3.2. Antiulcer activity

All the synthesized compounds and reference drug ranitidine have been examined for their antiulcer activity. The pharmacological results of synthesized compounds have been reported in Table 3. The entire synthesized compounds showed good activity as compared with standard drug (ranitidine) some compounds **3a**, **3c, 4a** and **4c** showed good activity, compound **3b** showed moderate activity in Aspirin Induced Ulcer (ASP). Synthesized compounds were also tested for Ethanol Induced Ulcer. Compound **3a, 3b** and **3c** showed moderate activity and compound **4a, 4b** and **4c** showed less activity.

3.3. Anti-inflammatory activity

All the newly synthesized compounds and reference drug phenylbutanone have been examined for their anti-inflammatory activity. The pharmacological results of synthesized compounds have been reported in Table 4. All the compounds have shown anti-inflammatory activity ranging from 22.2- 26.5% at the dose of 50 mg/kg body weight. The results obtained clearly infer that compound **3a** shows the highest Anti-inflammatory activity with respect to the other compounds. Compound **3b, 4a** and **4b** showed moderate activity and compounds **3c** and **4c** showed less activity.

Table 4: - Anti-inflammatory activity (% Inhibition) of some selected compounds

Compound	% Inhibition (50 mg/kg body weight)
3a	26.5
3b	24.5
3c	22.7
4a	25.5
4b	24.7
4c	22.2
Phenyl butanone	38.9

3.4. Antitumor activity

This activity was carried out to estimate the effect of test compound on the growth of tumor cells. The human breast cancer cell line (MCF-7) and mammary cancer cell line (EVSA-7) were employed. Their results are summarized in Table 5 respectively. Compound **3a, 3b** and **4a** exhibit good result against human breast cancer cells lines (MCF-7) and compounds **3a, 3b** and **4a** show good results against mammary cancer cell line (EVSA-7).

Table 5: - Antitumor activity of some selected compounds

Compound	Cell No. x 10^4 (MCF-7)	Cell No. x 10^4 (EVSA-7)
3a	11.89±1.12	10.68±1.08
3b	11.58±1.02	10.62±1.06
3c	9.17±0.87	9.69±0.92
4a	11.69±1.02	10.68±1.08
4b	9.17±0.87	9.69±0.92
4c	9.22±0.72	9.62±0.88
Negative Control	10.21±1.01	10.23±1.03
Positive Control	40.26±3.23	42.24±4.22

3.5. Antifeedant activity

The antifeedant activity of the newly synthesized compounds was tested by a leaf dip method [36, 37] against larvae of *Spodoptera litura*. The results clearly indicate that the compounds **4a**, **4b** and **4c** show higher activity, compounds **3a**, **3b** and **3c** show moderate activity against the larvae of the insect. It was found that these compounds may cause a spasm condition in insects by interacting with the active site of the enzyme responsible for nervous breakdown in insects (Table 6).

Table 6: - Antifeedant activity

Compound	Fiducial Limits	Slope ±	Chi. Sq. (3)	LC_{50}/ LD_{50} At 24 hrs.
3a	0.68–1.72	1.03±0.14	0.66 (3)	0.98
3b	0.82–3.41	0.81±0.14	0.43 (3)	1.35
3c	0.62–1.46	1.05±0.14	1.09 (3)	0.87
4a	0.32–0.53	1.15±0.14	7.53 (3)	0.40
4b	0.21–0.32	1.31±0.14	5.70 (3)	0.25
4c	0.33–0.61	1.00±0.13	0.68 (3)	0.43

3.6. Acaricidal activity

The acaricidal activity of these compounds was performed by the same method, as in the case of antifeedant activity, against *Tetranychus urticae*, a species of mite using acetone as a standard. The results obtained clearly show that compound **3c** shows the highest acaricidal activity with respect to the other compounds. Compounds **3a**, **3b** and **4b** show moderate activity and the rest of the compounds show lower acaricidal activity against the mites (Table 7).

Table 7: - Acaricidal activity

Compound	Fiducial Limits	Slope ±	Chi. Sq. (3)	LC_{50}/ LD_{50} At 24 hrs.
3a	0.07–0.22	0.76±0.06	5.63 (3)	0.14
3b	0.08–0.23	0.65±0.7	6.12 (3)	0.13
3c	0.05–0.09	1.16±0.09	12.67 (3)	0.07
4a	0.12–0.30	0.78±0.88	1.70 (3)	0.18
4b	0.10–0.23	0.88±0.08	2.14 (3)	0.15
4c	0.03–0.06	0.76±0.07	15. 89 (3)	0.40

3.7. Contact toxicity

The contact toxicity of these compounds was carried out by topical application method [38, 39] against larvae of *Spodoptera litura.* The results clearly indicate that the compounds show higher, moderate and less contact toxicity against the larvae of the insect. Compounds **3c**, **3b** and **3a** show higher activity, compounds **4b** show moderate activity and the rest of the compounds show lower to moderate activity against the mites (Table 8).

Table 8: - Contact toxicity

Compound	Fiducial Limits	Slope ±	Chi. Sq. (3)	LC_{50}/ LD_{50} At 24 hrs.
3a	0.36–0.53	1.61±0.04	3.13 (3)	0.43
3b	0.31–0.42	1.88±0.16	2.50 (3)	0.36
3c	0.29–0.39	1.97±0.16	4.39 (3)	0.34
4a	1.61–2.54	1.17±0.19	0.226(3)	2.37
4b	0.69–1.32	1.40±0.67	1.67(3)	0.90
4c	1.72–7.94	1.31±0.21	0.166 (3)	2.93

3.8. Stomach toxicity

The stomach toxicity of these compounds was carried out by leaf dip method [36, 37]. In this method we used fourth instar larvae of *Spodoptera litura*. The results clearly indicate that the compounds show higher, moderate and less stomach toxicity against the larvae of the insect. Compounds **3c, 3a** and **3b** show moderate activity and the rest of the compounds show lower to moderate activity against the mites (Table 9).

Table 9: - Stomach toxicity

Compound	Fiducial Limits	Slope ±	Chi. Sq. (3)	LC_{50}/ LD_{50} At 24 hrs.
3a	0.55–0.89	1.58±0.16	9.01 (3)	0.68
3b	0.56–1.09	1.21±0.15	2.09 (3)	0.74
3c	0.49–0.77	1.57±0.16	2.79 (3)	0.60
4a	1.65–6.93	1.35±0.21	0.29 (3)	2.75
4b	2.49–39.65	0.93±0.18	0.501 (3)	5.88
4c	1.47–5.17	1.38±0.21	0.35 (3)	2.33

CONCLUSION

All the newly synthesized compounds were screened for antibacterial activity at a concentration of 200 µg/mL and 100 µg/mL using DMF as a control. Novobiocin, Kanamycin and Amikacin were used as standard drugs against gram positive and gram negative bacteria. The data in the Table 2 indicates that among the synthesized compounds **3a** and **4a** compounds were found to possess good activity. However, the activities of the remaining compounds were much less than those of standard antibacterial drugs used. The compounds also showed potent antiulcer, anti-inflammatory and antitumor. Antifeedant activity, contact toxicity and stomach toxicity tested against *Spodoptera litura* and acaricidal activity tested against *Tetranychus urticae.* From the results, it is clear that these compounds would be better used in drug development to combat bacterial infections, and would be better used as antiulcer, anti-inflammatory and pesticides in future.

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REFERENCES

[1] M. Yoshida, Hayakawa, N. Hayashi, T. Agatsuma, Y. Oda, F. Tanzawa, S. Iwasaki, K. Koyama, H. Furukawa, S. Kurakata, Y. Sugano, *Bioorg. Med. Chem. Lett.*, **2005,** 15(14), 3328.
[2] K. Suvarna, S. P. Swain, A. M. Gandhi, *Indian J. Pharm. Sci.*, **2007,** 69(1), 46.
[3] L. Racane , R. Stojkovic, V. T. Kulenovic, G. K. Zamola, *Molecules*, 2006, 11, 325.
[4] G. Giorgioni, B. Accorroni, A. D. Stefano, G. Marucci, A. Siniscalchi, F. Claudi, *Bioorg. Med. Chem. Lett*., **2005**, 14(2), 57.
[5] B. Rajeeva, N. Srinivasulu, S. M. Shantakumar, *E- J. Chem.*, **2009**, 6(3), 775.

[6] H. Kaur, S. Kumar, I. Singh, K. K. Saxena, A. Kumar, *Digest J Nanomaterials and Biostructures* , **2010**, 5(1), 67.
[7] R. Paramashivappa, K. P. Phani, P. V. S. Rao, S. Rao, *Bioorg Med Chem Lett.* , **2003**, 13, 657.
[8] M. A. Mahran, S. William, F. Ramzy, A. M. Sembel, *Molecules*, **2007**, 12, 622.
[9] S. R. Nagarajan, G. A. D. Crescenzo, D. P. Getman, H. F. Lu, J. A. Sikorski, J. L. Walker, J. J. McDonald, K. A. Houseman, G. P. Kocan, N. Kishore, P. P. Mehta, C. L. Funkes-Shippy, L. Blystone, *Bioorg Med Chem lett*., **2003**, 11, 4769.
[10] I. Tamm, K. Folkers, H. S. Clifford, H. Dorothea, L. F. Horsfall, *The J. Exp. Med.*, **1953** , 98, 245.
[11] A. K. Tewari, A. Mishra, *Indian J. Chem.*, **2006,** 45(B), 489.
[12] R. Zou, K. R. Ayres, J. C. Drach, L. B. Townsend, *J. Med. Chem*., **1996**, 39(18), 3477.
[13] B. Q. Yang, P. H. Yang, A. L. Zhu, *Cheniese Chemical letters.*, **2003**, 14(9), 901.
[14] S. S. Mahajan, R. G. Nandre, *Indian. J. Chem.*, 2006, 45(B), 1759.
[15] J. S. Saggu, R. Sharma, H. Dureja, V. Kumar, *J Indian Inst. Sci.*, **2002**, (82), 177.
[16] G. A. Kilcigil, C. Kus, T. Coban, B. C. Eke, M. Iscan, *J. Enzyme Inhib. Med. Chem*., **2004**, 19(2), 129.
[17] H. T. Le, I. B. Lemaire, A. K. Gilbert, F. Jolicoeur, L. Yang, N. Leduc, *JPET*, **2004**, 309, 146.
[18] A. D. Settimo , G. Primofiore, F. D. Settimo, A. M. Marini, *Farmaco*., **1992** , 47(10), 1293.
[19] V. G. Navarrete, R. Cedillo, C. A. Hernandez, L. Yepez, J. Valdez, R. Morales, R. Coryes, R. Catillo, *Bioorg. Med. Chem. Lett*., **2001**, 11(2), 187.
[20] D. L. Evers, G. Komazin, R. G. Ptak, D. Shin, B. T. Emmer, L. B. Townsend, J. C. Drach, *Antimicrobial agents & chemotherapy*, **2004**, 48(10), 3918.
[21] G. A. Kilcigil, N. Altanlar, *Turk. J. Chem.*, **2006,** 30, 223.
[22] W. Nawrocka, *Boll Chim. Farm*., **1996**, 135(1), 18.
[23] M. A. Siracusa , L. Salerno , M. N. Modica , V. Pittalà , G. Romeo , M. E. Amato , M. Nowak, A. J. Bojarski , I. Mereghetti , C. A. agnotto , T. Mennini, *J Med Chem.*, **2008**, 51(15), 4529
[24] Y. A. R. Shaya, M. S. Ahmed, Elsharief, M. E. K. Saied, M. A. A. Abdulkhaliq, *Heteroatom Chem.*, 2006, 17(7), 634 and *ChemInform,* **2007**, 38, 12.
[25] W. Nawrocka , B. Sztuba, M. W. Kowalska, H. Liszkiewicz, J. Wietrzyk, A. Nasulewicz, M. Pełczyńska, A. Opolski, *Farmaco*, **2004**, 59(2), 83.
[26] A. J. K. Atia**,** *Molecules*, **2009**, 14, 2431.
[27] N. Ulusoy, E. Gursu, S. Ozkirimil, A. C. Ekinci, I. Dikici, *Indian J. Chem*., **2003**, 42(B), 2853.
[28] R. Dahiya, A. Kumar, *E- J. Chem.*, **2008**, 5, 1133.
[29] E. Banfi, G. Scialino, D. Zampieri, M. G. Mamolo, L. Vio, M. Ferrone, M. Fermeglia, M.S. Paneni, S. Pricl, *J. Antimicrob. Chemother*., **2006**, 58, 76.
[30] P. O. Miranda, L. L. Gundersen, *Arch. Pharm. Chem. Life Sci*., **2010**, 343, 40.
[31] P. K. Pareek, Mithlesh, P. Kriplani, R. Tiwari, K. G. Ojha, *Phosphorus, Sulfur, and Silicon*, **2010**, 185, 279; (b) Mithlesh, P. K. Pareek, R. Kant, S. K. Shukla, K. G. Ojha, *Cent. Eur. J. Chem.*, **2010**, 8(1), 163; (c) J. E. Drake, M. B. Hursthours, P. kriplani, M. E. Light, Mithlesh, K. G. Ojha, P. K. Pareek, R. Ratnani, *Main group Chemistry*., **2009**, 8, 21; (d) K. G. Ojha, N. Jaisinghani, H. Tahiliani, *J.Indian Chem. Soc.*, **2002**, 79, 191; (e) V. P. Mehta, R. Sharma, K. G. Ojha, *Tenside Surf. Det.*, **2003**, 40, 99; (f) P. Kriplani, P. Swarnkar, K. G. Ojha, *Heterocyclic Comm.*, **2005**, 11, 527; (g) N. Mathur, K. G. Ojha, A. Imran, S. Pooja, *Tenside Surf. Det.*, **2009**, 46, 24.
[32] K. G. Ojha, S. K. Jain, R. R. Gupta, *Synth. Commun*., **1979**, 9 (6), 457.

[33] R. R. Gupta, K. G. Ojha, M. Kumar, *J. Heterocycl. Chem*., **1980**, 17, 1325.
[34] R. Cruickshank, J. P. Duguid, B. P. Marmion, H. A. Swam, The Practice of Medical Microbiology, 12th Ed., Churchill Livingstone, London, **1975**, 544.
[35] S. Tyagi, N. Singh, S. M. Singh, U. P. Singh, Synth. React. *Inorg. Metorg. Chem.*, **2004**, 34, 573.
[36] A. M. Shelton, J. L. Robertson, J. D. Tang, *J. Econ., Entomol*., **1993**, 86(9), 697.
[37] H. Jinfeng, L. Pei, S. Xueyan, G. Xiwu, *J. Insect., Sci*., **2008**, 8(3), 9.
[38] M. G. Leonardi, S. Cappelloza, P. Ianne, L. Cappelloza, P. Parenti, B. Giordana, *Compedium of Biochemistry and Physiology*, **1996**, 113(B), 361.
[39] G. F. Ludvik, *J. Econ. Entomol.*, **1953**, 46, 364.