

RESEARCH ARTICLE

Synthesis of some thiazolyl aminobenzothiazole derivatives as potential antibacterial, antifungal and anthelmintic agents

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Abstract

A series of 4-(6-substituted-1,3-benzothiazol-2-yl)amino-2-(4-substitutedphenyl)- amino-1,3-thiazoles, **9–24** have been synthesised from 2-chloro-*N*-(6-substituted-1,3-benzothiazol-2-yl)acetamides, **5–8**. The structures of these compounds have been elucidated by spectral (IR, ¹H NMR, Mass) and elemental (C, H, N) analysis data. All the newly synthesised compounds (**9–24**) were screened for their antibacterial, antifungal and anthelmintic activities. Almost all of these compounds showed moderate to good antimicrobial activity against two gram negative bacteria (*E. coli*, *P. aeruginosa*), two gram positive bacteria (*S. aureus*, *B. subtilis*), pathogenic fungal strains (*C. albicans*, *A. niger*) and good anthelmintic activity against earthworm species (*P. corethruses*). Compounds **18** and **20** exhibited good antibacterial and antifungal activities, while compound **22** displayed the most significant anthelmintic activity.

Keywords: Aminobenzothiazole, thiazole, antibacterial activity, antifungal activity, anthelmintic activity

Introduction

Nowadays helminth infections are a major medical problem worldwide and there has been a considerable increase in its incidence [1]. It is one of the most frequent sources of human infections particularly in tropical countries like India [2,3]. Chemotherapy is important in the treatment of parasitic infections [4]. Helminth infections often have a connection to some other disease (especially those caused by microorganisms), whenever the body system gets debilitated. Diseases caused by microbial infection are a serious menace to the health of human beings [5]. Despite the important advances in the field of anthelmintic and antimicrobials, together with the availability of drugs for these infections, there is a continuing increase in the incidence of these infectious diseases, together with a gradual rise in resistance. This has resulted in an increased morbidity and mortality with an overall increase in healthcare costs. This scenario highlights the urgent need for the design of new and effective entities with chemical structures that are different from the traditional drugs which will act both as anthelmintic and antimicrobials.

The scaffold benzothiazole and its analogue are important pharmacophores that are found in many marine compounds or natural plants [6,7]. In the past decades, 2-aminobenzothiazole and its derivatives have received much attention due to their chemotherapeutic value in the development of novel anthelmintics and antimicrobial agents [8–12]. In addition, many 2-aminobenzothiazole derivatives exhibit a wide variety of biological activities such as diuretic [13], anticancer [14], anticonvulsant [15,16], antihistamine [17] and anti-inflammatory [18]. Moreover, the thiazole ring is an important building block in medicinal chemistry and endowed with antimicrobial, anthelmintic and various other pharmacological activities [19–21]. In addition, there are a number of antimicrobial and anthelmintic thiazoles available in clinical practice (Cefixime, Cefprozime, Cefotaxime, Ceftazidime, Cefibuten, Aztreonam, Tigemonam, Thiabendazole) that make less use of the amino group.

In view of the high degree of bioactivity shown by the two heterocyclic compounds mentioned above and in our continued search for antimicrobial and anthelmintic agents, we aimed to construct a system combining both these vital

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(Received 23 August 2009; revised 08 December 2009; accepted 13 December 2009)

moieties in a single molecular framework, together with an exploration of the additive effects of their biological activities. In the present paper, we report the synthesis of some hitherto unreported 4-(6-substituted-1,3-benzothiazol-2-yl)amino-2-(4-substituted-phenyl)-amino-1,3-thiazole derivatives and also to evaluate them biologically for their antibacterial, antifungal and anthelmintic activities.

Experimental

Chemistry

All the chemicals and solvents employed in the synthesis were supplied by Merck (Darmstadt, Germany), Fluka (Seelze, Germany) and SD Fine chemicals (Mumbai, India) and used without purification. Melting points were determined on a digital melting point apparatus Electrothermal 1A 9200 (Electrothermal Engineering Ltd., Southend-on-sea, Essex, UK) and were uncorrected. All the reactions were monitored by TLC performed on 2–6 cm aluminium sheets precoated with silica gel 60 (HF-254, E. Merck, Mumbai, India). The IR spectra were recorded on a Shimadzu FTIR 8400S spectrophotometer (Kyto, Japan) using KBr optics. ^1H NMR spectra were recorded in CDCl_3 on a Varian Mercury YH-300 MHz spectrophotometer (Palo Alto, CA) and chemical shifts (δ) are given in ppm relative to TMS. Mass spectra were recorded at 70 eV on a Jeol D-300 spectrometer (Tokyo, Japan) and a 3200 QTRAP LC/MS/MS spectrophotometer (Applied Biosystems, Foster City, CA). Elemental analyses were carried out using a FLASH EA 1112 CHN analyser (Thermo Finnigan, Milan, Italy), VARIO EL (Elementar, Hanau, Germany) and found within $\pm 0.4\%$ of theoretical values.

Synthesis

General synthesis of 4-(6-substituted-1,3-benzothiazol-2-yl)amino-2-(4-substituted-phenyl)amino-1,3-thiazole (9–24)

A mixture of 2-chloro-*N*-(6-substituted-1,3-benzothiazol-2-yl)acetamides (**5–8**), substituted phenylthiourea (0.01 mol), absolute ethanol (15 mL) and anhydrous potassium carbonate (0.01 mol) were heated under reflux in a water bath for 12 h. The excess substituted phenylthiourea and ethanol were removed by distillation and the residue treated with 5% sodium carbonate solution to remove acid impurities, filtered then washed with water and dried. The crude product was crystallised from ethanol.

4-(1,3-benzothiazol-2-yl)amino-2-phenylamino-1,3-thiazole (9)

Yellow solid; Yield: 68%; mp 171–173°C; R_f 0.54 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3284 (NH), 1552 (C=N), 1086 (C–N); ^1H NMR (CDCl_3 , ppm): δ 7.69–8.12 (m, 10H, Ar-H), 11.86 (s, 1H, NH); EI-MS: m/z [$\text{M}+\text{H}$] $^+$ 325; Anal. calcd for $\text{C}_{16}\text{H}_{12}\text{N}_4\text{S}_2$: C, 59.23; H, 3.73; N, 17.27. Found: C, 59.27; H, 3.71; N, 17.28.

4-(1,3-benzothiazol-2-yl)amino-2-(4-chlorophenyl)amino-1,3-thiazole (10)

Light yellow crystals; Yield: 59%; mp 188–190°C; R_f 0.38 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3330 (NH), 1574

(C=N), 1108 (C–N); ^1H NMR (CDCl_3 , ppm): δ 7.87–8.26 (m, 9H, Ar-H), 12.22 (s, 1H, NH); EI-MS: m/z [$\text{M}+\text{H}$] $^+$ 360; Anal. calcd for $\text{C}_{16}\text{H}_{11}\text{ClN}_4\text{S}_2$: C, 53.55; H, 3.09; N, 15.61. Found: C, 53.48; H, 3.11; N, 15.59.

4-(1,3-benzothiazol-2-yl)amino-2-(4-hydroxyphenyl)amino-1,3-thiazole (11)

Brown solid; Yield: 64%; mp 181–182°C; R_f 0.56 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3620 (OH), 3382 (NH), 1546 (C=N), 1098 (C–N); ^1H NMR (CDCl_3 , ppm): δ 7.63–8.12 (m, 9H, Ar-H), 9.8 (s, 1H, OH), 12.68 (s, 1H, NH); EI-MS: m/z [$\text{M}+\text{H}$] $^+$ 341; Anal. calcd for $\text{C}_{16}\text{H}_{12}\text{N}_4\text{OS}_2$: C, 56.45; H, 3.55; N, 16.46. Found: C, 56.42; H, 3.53; N, 16.48.

4-(1,3-benzothiazol-2-yl)amino-2-(4-methoxyphenyl)amino-1,3-thiazole (12)

Light yellow crystals; Yield: 69%; mp 193–195°C; R_f 0.63 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3336 (NH), 1580 (C=N), 1084 (C–N); ^1H NMR (CDCl_3 , ppm): δ 3.78 (s, 3H, OCH_3), 7.88–8.31 (m, 9H, Ar-H), 13.11 (s, 1H, NH); EI-MS: m/z [$\text{M}+\text{H}$] $^+$ 355; Anal. calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{OS}_2$: C, 57.61; H, 3.98; N, 15.81. Found: C, 57.65; H, 3.96; N, 15.79.

4-(6-Methyl-1,3-benzothiazol-2-yl)amino-2-phenylamino-1,3-thiazole (13)

Brown solid; Yield: 60%; mp 208–210°C; R_f 0.52 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3284 (NH), 1552 (C=N), 1124 (C–N); ^1H NMR (CDCl_3 , ppm): δ 2.48 (s, 3H, CH_3), 7.76–8.23 (m, 9H, Ar-H), 12.84 (s, 1H, NH); EI-MS: m/z [$\text{M}+\text{H}$] $^+$ 339; Anal. calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{S}_2$: C, 60.33; H, 4.17; N, 16.55. Found: C, 60.27; H, 4.15; N, 16.57.

4-(6-Methyl-1,3-benzothiazol-2-yl)amino-2-(4-chlorophenyl)amino-1,3-thiazole (14)

White crystals; Yield: 52%; mp 202–204°C; R_f 0.46 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3324 (NH), 1574 (C=N), 1105 (C–N); ^1H NMR (CDCl_3 , ppm): δ 2.34 (s, 3H, CH_3), 7.64–7.98 (m, 8H, Ar-H), 12.08 (s, 1H, NH); EI-MS: m/z [$\text{M}+\text{H}$] $^+$ 374; Anal. calcd for $\text{C}_{17}\text{H}_{13}\text{ClN}_4\text{S}_2$: C, 54.76; H, 3.51; N, 15.02. Found: C, 54.72; H, 3.53; N, 14.99.

4-(6-Methyl-1,3-benzothiazol-2-yl)amino-2-(4-hydroxyphenyl)amino-1,3-thiazole (15)

Brown solid; Yield: 58%; mp 233–235°C; R_f 0.55 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3570 (OH), 3295 (NH), 1568 (C=N), 1092 (C–N); ^1H NMR (CDCl_3 , ppm): δ 2.56 (s, 3H, CH_3), 7.74–8.05 (m, 8H, Ar-H), 10.2 (s, 1H, OH), 11.76 (s, 1H, NH); EI-MS: m/z [$\text{M}+\text{H}$] $^+$ 355; Anal. Calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{OS}_2$: C, 57.61; H, 3.98; N, 15.81. Found: C, 57.64; H, 3.96; N, 15.81.

4-(6-Methyl-1,3-benzothiazol-2-yl)amino-2-(4-methoxyphenyl)amino-1,3-thiazole (16)

White crystals; Yield: 64%; mp 213–215°C; R_f 0.61 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3330 (NH), 1552 (C=N), 1122 (C–N); ^1H NMR (CDCl_3 , ppm): δ 2.35 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 7.68–7.94 (m, 8H, Ar-H),

12.33 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 355; Anal. Calcd for $C_{18}H_{16}N_4O_2S_2$: C, 58.67; H, 4.38; N, 15.21. Found: C, 58.63; H, 4.38; N, 15.22.

4-(6-Fluoro-1,3-benzothiazol-2-yl)amino-2-phenylamino-1,3-thiazole (17)

Brown solid; Yield: 54%; mp 245–247°C; R_f 0.52 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3308 (NH), 1574 (C=N), 1096 (C–N); 1H NMR ($CDCl_3$, ppm): δ 7.72–8.16 (m, 9H, Ar-H), 11.98 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 343; Anal. Calcd for $C_{16}H_{11}FN_4S_2$: C, 56.12; H, 3.24; N, 16.36. Found: C, 56.16; H, 3.22; N, 16.35.

4-(6-Fluoro-1,3-benzothiazol-2-yl)amino-2-(4-chlorophenyl)amino-1,3-thiazole (18)

Brown solid; Yield: 66%; mp 222–224°C; R_f 0.49 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3274 (NH), 1556 (C=N), 1108 (C–N); 1H NMR ($CDCl_3$, ppm): δ 7.68–8.06 (m, 8H, Ar-H), 12.65 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 378; Anal. Calcd for $C_{16}H_{10}ClFN_4S_2$: C, 50.99; H, 2.67; N, 14.87. Found: C, 50.92; H, 2.69; N, 14.87.

4-(6-Fluoro-1,3-benzothiazol-2-yl)amino-2-(4-hydroxyphenyl)amino-1,3-thiazole (19)

Yellow solid; Yield: 72%; mp 254–256°C; R_f 0.61 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3475 (OH), 3326 (NH), 1543 (C=N), 1121 (C–N); 1H NMR ($CDCl_3$, ppm): δ 7.64–7.92 (m, 8H, Ar-H), 9.6 (s, 1H, OH), 12.24 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 359; Anal. Calcd for $C_{16}H_{11}FN_4O_2S_2$: C, 53.62; H, 3.09; N, 15.63. Found: C, 53.59; H, 3.09; N, 15.61.

4-(6-Fluoro-1,3-benzothiazol-2-yl)amino-2-(4-methoxyphenyl)amino-1,3-thiazole (20)

Orange-brown crystalline solid; Yield: 56%; mp 231–233°C; R_f 0.53 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3284 (NH), 1562 (C=N), 1110 (C–N); 1H NMR ($CDCl_3$, ppm): δ 3.86 (s, 3H, OCH_3), 7.78–8.21 (m, 8H, Ar-H), 11.92 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 373; Anal. Calcd for $C_{17}H_{13}FN_4O_2S_2$: C, 54.82; H, 3.52; N, 15.04. Found: C, 54.85; H, 3.53; N, 15.01.

4-(6-Ethoxy-1,3-benzothiazol-2-yl)amino-2-phenylamino-1,3-thiazole (21)

Brown solid; Yield: 63%; mp 256–258°C; R_f 0.35 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3328 (NH), 1544 (C=N), 1077 (C–N); 1H NMR ($CDCl_3$, ppm): δ 1.42 (t, $J=8.4$, 3H, OCH_2-CH_3 , methyl), 4.09 (q, 2H, OCH_2), 7.72–8.14 (m, 9H, Ar-H), 12.43 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 369; Anal. Calcd for $C_{18}H_{16}N_4O_2S_2$: C, 58.67; H, 4.38; N, 15.21. Found: C, 58.62; H, 4.36; N, 15.23.

4-(6-Ethoxy-1,3-benzothiazol-2-yl)amino-2-(4-chlorophenyl)amino-1,3-thiazole (22)

White crystalline solid; Yield: 56%; mp 279–281°C; R_f 0.62 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3342 (NH), 1580 (C=N), 1074 (C–N); 1H NMR ($CDCl_3$, ppm): δ 1.34 (t, $J=7.72$, 3H, OCH_2-CH_3 , methyl), 4.09 (q, 2H, OCH_2), 7.89–8.36 (m, 8H, Ar-H), 13.12 (s, 1H, NH); EI-MS: m/z

$[M+H]^+$ 403; Anal. Calcd for $C_{18}H_{15}ClN_4O_2S_2$: C, 53.66; H, 3.75; N, 13.91. Found: C, 53.63; H, 3.72; N, 13.92.

4-(6-Ethoxy-1,3-benzothiazol-2-yl)amino-2-(4-hydroxyphenyl)amino-1,3-thiazole (23)

Grey solid; Yield: 61%; mp 262–264°C; R_f 0.35 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3640 (OH), 3286 (NH), 1538 (C=N), 1125 (C–N); 1H NMR ($CDCl_3$, ppm): δ 1.54 (t, $J=8.12$, 3H, OCH_2-CH_3 , methyl), 4.22 (q, 2H, OCH_2), 7.64–8.11 (m, 8H, Ar-H), 10.4 (s, 1H, OH), 13.06 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 385; Anal. Calcd for $C_{18}H_{16}N_4O_2S_2$: C, 56.23; H, 4.19; N, 14.57. Found: C, 56.28; H, 4.17; N, 14.56.

4-(6-Ethoxy-1,3-benzothiazol-2-yl)amino-2-(4-methoxyphenyl)amino-1,3-thiazole (24)

Greenish-grey solid; Yield: 74%; mp 283–285°C; R_f 0.72 (ethyl acetate:ammonia); IR (KBr, cm^{-1}): 3342 (NH), 1574 (C=N), 1085 (C–N); 1H NMR ($CDCl_3$, ppm): δ 1.46 (t, $J=8.87$, 3H, OCH_2-CH_3 , methyl), 3.94 (s, 3H, OCH_3), 4.17 (q, 2H, OCH_2), 7.75–8.19 (m, 8H, Ar-H), 13.12 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 399; Anal. Calcd for $C_{19}H_{18}N_4O_2S_2$: C, 57.27; H, 4.55; N, 14.06. Found: C, 57.21; H, 4.53; N, 14.05.

Biological activity

Antibacterial activity

All the newly synthesised compounds were screened for their antibacterial activity against two different strains of Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) bacteria. The minimum inhibitory concentration (MIC) was determined by the test tube dilution technique using Muller-Hinton nutrient broth [22]. The MIC values were also tested for the well-known antibiotics (ciprofloxacin and ampicillin) to compare the antibacterial activity of our test compounds with currently used antibiotics. The stock solution of the test compounds (2–4 $\mu g/mL$) was prepared in dimethylsulphoxide (DMSO). The stock solution was sterilised by passing through a 0.2 μm polycarbonate sterile membrane (Nucleopore Corporation, Pleasanton, CA, USA) filters. Serial dilution of the test compounds was carried out and the following concentrations were used: 1000, 500, 250, 125, 62, 32, 16, 8, 4, down to 1 $\mu g/mL$. Test compounds at various concentrations were added to culture medium in a sterilised borosilicate test tube and different bacterial strains were inoculated at concentration of 10^6 bacilli/mL. The tubes were incubated at 37°C for 24h and then examined for the presence or absence of growth of the test organisms. All experiments were performed in triplicate. The MIC values were obtained from the lowest concentration of the test compounds where the tubes remained clear (i.e. no turbidity), indicating that the bacterial growth was completely inhibited at this concentration. The MIC values were expressed in $\mu g/mL$ and summarised in Table 1.

Table 1. Antimicrobial activity data for the tested compounds.

Compound	Gram negative bacteria ^a		Gram positive bacteria ^a		Fungi ^a	
	<i>E. coli</i>	<i>P.aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>A. niger</i>
9	15	21	39	28	81	72
10	12	13	21	18	41	52
11	22	16	27	23	63	34
12	27	29	18	12	29	32
13	37	26	53	62	57	44
14	28	21	39	42	35	32
15	44	33	51	54	48	61
16	59	54	32	38	26	22
17	13	15	28	21	14	12
18	6	8	18	11	9	4
19	16	21	19	17	11	8
20	22	29	8	6	4	3
21	56	49	74	95	29	43
22	42	36	48	56	17	13
23	64	78	56	74	24	19
24	76	84	41	42	12	9
Ciprofloxacin	3	4	6	2	–	–
Ampicillin	2	2	1	1	–	–
Fluconazole	–	–	–	–	1	2

^aData are given as MIC (µg/mL).**Antifungal activity**

The newly synthesised compounds were screened for their antifungal activity against *Candida albicans* (NCIM no. 3471) and *Aspergillus niger* (NCIM no. 1196) in DMSO by the serial plate dilution method [23]. Sabour and Dextrose Agar (Merck) media were used for the cultivation of the fungi. A spore suspension was made for each fungal strain using normal saline. A loopful of fungal strain was transferred to 3 mL saline to produce a suspension. Agar media (20 mL) were poured into each petri dish and the excess suspension was decanted. The plates were dried by placing in an incubator at 37°C for 1h. The MIC values were noted and the activity of each compound was compared with fluconazole as the standard drug. The MIC values were in µg/mL and shown in Table 1.

Anthelmintic activity

The *in vitro* trial for anthelmintic activity was carried out against earthworm *Pontoscotex corethruses* (ICARBC 408) species [24]. The test compound suspensions were prepared by triturating the newly synthesised compounds with 0.5% Tween 80 and distilled water. The resulting mixture was stirred for 30 minutes using a mechanical stirrer. The suspensions were diluted to contain 0.2% w/v of test samples. A suspension of the standard drug, mebendazole, was prepared at the same concentration in a similar way. Three sets of five earthworms of almost similar sizes were placed in petri plates of 4 inch diameter containing 50 mL of suspension of the test sample and the standard drug at room temperature. Another set of five earthworms were kept as controls in a 50 mL suspension of distilled water and 0.5% Tween 80. The paralyzing and

Table 2. Anthelmintic activity of tested compounds.

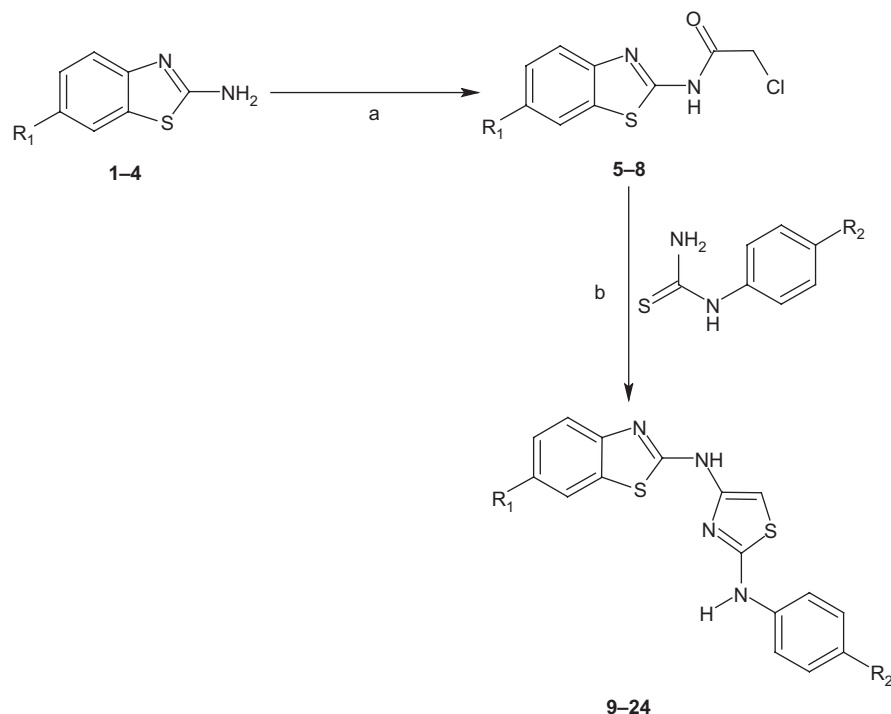
Compound	Mean death time	
	Mean paralyzing time (min) ^a	(min) ^a
9	69.63 ± 0.41	82.61 ± 0.77
10	62.56 ± 0.31	75.83 ± 0.29
11	64.58 ± 0.37	73.63 ± 0.46
12	75.09 ± 0.51	82.57 ± 0.27
13	41.87 ± 0.40	49.12 ± 0.60
14	34.79 ± 0.33	50.92 ± 0.36
15	38.60 ± 0.32	48.61 ± 0.46
16	47.08 ± 0.30	61.00 ± 0.44
17	55.26 ± 0.13	65.16 ± 0.56
18	50.94 ± 0.39	68.46 ± 0.33
19	53.98 ± 0.36	71.46 ± 0.35
20	58.92 ± 0.41	68.25 ± 0.15
21	30.82 ± 0.37	47.28 ± 0.14
22	22.26 ± 0.58	35.83 ± 0.34
23	28.00 ± 0.30	40.55 ± 0.41
24	32.74 ± 0.60	39.93 ± 0.61
Mebendazole	19.84 ± 1.24	31.39 ± 0.34

^aData are given as mean ± S.E.M. (n=3) against earthworm *P. corethruses* species

death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water at 50°C, which stimulated movement if the worm was still alive. The results are depicted in Table 2.

Results and discussion

In the present work a series of 16 new aminobenzothiazole derivatives were synthesised. The synthetic route leading to the formation of 4-(6-substituted-1,3-benzothiazol-



Compound		R ₁	Compound	R ₁	R ₂	Compound	R ₁	R ₂
1	5	H	9	H	H	17	F	H
2	6	CH ₃	10	H	Cl	18	F	Cl
3	7	F	11	H	OH	19	F	OH
4	8	OC ₂ H ₅	12	H	OCH ₃	20	F	OCH ₃
			13	CH ₃	H	21	OC ₂ H ₅	H
			14	CH ₃	Cl	22	OC ₂ H ₅	Cl
			15	CH ₃	OH	23	OC ₂ H ₅	OH
			16	CH ₃	OCH ₃	24	OC ₂ H ₅	OCH ₃

Scheme 1. General scheme for the synthesis of compounds **9-24**. Reagents and conditions: (a) dry benzene, ClCOCH₂Cl, TEA, reflux, 3h, 80°C; (b) dry EtOH, RNHCSNH₂, reflux, 10-12h.

2-yl)amino-2-(4-substitutedphenyl)amino-1,3-thiazole **9-24** is shown in Scheme 1. The compounds 6-substituted-1,3-benzothiazol-2-amine **1-4** were synthesised with an excellent yield following the methodology as described by Jimonet et al. [25] from aryl amines. The compounds **1-4** on reaction with chloroacetyl chloride afforded 2-chloro-N-(6-substituted-1,3-benzothiazol-2-yl)acetamide **5-8** on refluxing the reaction mixture at 120°C for 5h [26]. Final compounds 4-(6-substituted-1,3-benzothiazol-2-yl)amino-2-(4-substituted-phenyl)amino-1,3-thiazole **9-24** were obtained by the cyclisation of 2-chloroacetamides **5-8** with commercially available 4-substituted phenyl thioureas in absolute ethanol. All the reaction products were obtained in moderate to very good yield. The structures of the final compounds were elucidated by elemental analyses, Fourier transform infrared spectroscopy (FTIR), ¹H NMR and electron impact mass spectrometry (EI-MS) analyses.

The IR spectra of the compounds **9-24** showed, in each case, stretching band of N-H and C=N group in the region 3342–3270 and 1580–1538 cm⁻¹, respectively. The ¹H NMR spectra showed, in each case, the signals as multiplet at δ 7.64–8.36 ppm attributed to Ar-H in addition to the singlet of N-H group in the region 11.34–13.12 ppm. The disappearance of the C=O band at 1732–1685 cm⁻¹, provided confirmatory evidence for ring closure from the 2-chloroacetamides (**5-8**). The ¹H NMR spectra also lacked the CO-CH₂ signal at δ 4.38–4.74 ppm. EI-MS of all compounds displayed the (M+H)⁺ which confirmed their molecular weights.

The MIC (μg/mL) of tested compounds are shown in Table 1. The antibacterial test results indicated that most title compounds exhibited noticeable activities against all the strains of bacteria tested (Gram-negative, Gram-positive) *in vitro*. Seven compounds of the newly synthesised series showed high *in vitro* antimicrobial activity.

Compound **18** (MIC of 6–8 µg/mL) exhibited remarkable antibacterial activity against Gram-negative (*E. coli* and *P. aeruginosa*) bacteria. Compound **20** (MIC of 6–8 µg/mL) showed excellent activity against Gram-positive (*S. aureus* and *B. subtilis*) bacteria, while compounds **18** (MIC of 11–18 µg/mL) and **12** (MIC of 12–18 µg/mL) showed respectable antibacterial activity. Most of the compounds were found to be between 2- and 50- fold less active on comparison with the standard. Investigation of the structure- activity relationship revealed that the compounds with electron withdrawing (fluoro) substituents at the 6-position of benzothiazole ring favours the activity against both Gram-negative and Gram-positive bacteria. The substitutions with an electron withdrawing groups (chloro) at the 4-position of phenyl ring showed more potency against Gram-negative bacteria whereas the electron donating groups (methoxy) exhibited better activity against Gram-positive bacteria.

Many of the newly synthesised compounds were found to show good antifungal activity. From the antifungal activity data (Table 2), it was observed that **18** (MIC of 4–9 µg/mL) and **20** (MIC of 3–4 µg/mL) were the most active compounds against both the tested pathogens (*C. albicans* and *A. niger*). Compounds **17** (MIC of 12–14 µg/mL), **19** (MIC of 8–11 µg/mL) and **22** (MIC of 13–17 µg/mL) showed moderate to good antifungal activity. Substitution with an electron withdrawing group (fluoro) at 6-position of benzothiazole increases the antifungal activity compared with an electron donating group (methyl and ethoxy) and unsubstituted benzothiazoles. Introduction of an electron donating group (methoxy) on the 4-position of the phenyl ring shows marginal enhancement of activity compared to other groups and the unsubstituted phenyl ring against both the species.

All newly synthesised derivatives **9–24** showed good anthelmintic activity at 0.2% w/v. Compound **22**, with a mean paralyzing time of 22.26 min, showed maximum activity against *P. corethruses* species of earthworm on comparison with standard mebendazole (mean paralyzing time of 19.84 min) at the same concentration. Compounds **14** (mean paralyzing time of 34.79 min), **23** (mean paralyzing time of 28.00 min) and **24** (mean paralyzing time of 32.74 min) showed moderate activity while compound **12** (mean paralyzing time of 75.09 min) showed least activity amongst all the tested compounds. The substitution of an electron donating group (ethoxy) on the 6-position of benzothiazole increases the activity compared to an electron withdrawing group (fluoro) and unsubstituted benzothiazoles. On the other hand, substitution with an electron withdrawing group (chloro) on the 4-position of phenyl ring shows a remarkable increase in activity compared to other groups and the unsubstituted phenyl ring.

The statistical analyses were carried out using one way ANOVA (Dunnet's test) at a 95% confidence interval and all the activity data on comparison with vehicle control reaches statistical significance with $p < 0.05$.

Conclusion

This study reports the successful synthesis of 4-(6-substituted-1,3-benzothiazol-2-yl)amino-2-(4-substitutedphenyl)amino-1,3-thiazoles through an easy, convenient and efficient synthetic method. All these new compounds were confirmed by IR, NMR, MS spectra and elemental analyses and were evaluated for their antibacterial, antifungal and anthelmintic activities. The results showed that most of the synthesised aminobenzothiazole derivatives exhibited significant antibacterial, antifungal and anthelmintic activities. The 6-fluoro substituted aminobenzothiazoles exhibited good antibacterial and antifungal activities. Substitution with a chloro group in the phenyl ring confers greater antibacterial activity against Gram-negative bacteria whereas derivatives having a methoxy group possessed more antibacterial activity against Gram-positive bacteria and fungal strains. The results of anthelmintic studies revealed that substitution with an ethoxy group on aminobenzothiazole and a chloro group on the phenyl ring exhibited greater activity. Thus, 2-aminobenzothiazole serves as important pharmacophore for the design of new antibacterial, antifungal and anthelmintic agents and may lead to compounds with better pharmacological profiles than the standard drugs.

Acknowledgements

The authors are thankful to Dr. Bhoomendra Bhongade, International Medical University, Kuala Lumpur, Malaysia for his help and valuable suggestions and SAIL, School of Pharmaceutical Sciences, RGPV, Bhopal for mass spectra and CHN analysis.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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