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Synthesis of pharmaceutically important 1,3,4-thiadiazole and imidazolinone derivatives as antimicrobials†

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Thiosemicarbazide of 6-chloro-2-aminobenzothiozole on cyclization with different aromatic carboxylic acids in $POCl_3$ and substituted azlactones in pyridine provide the corresponding 2-aryl-5-(6′-chloro-1′,3′-benzothiazole-2-yl-amino)-1,3,4-thiadiazoles **2a-j** and 4-(4′-arylidene) -2-phenyl-1-(6′-chloro-1′,3′-benzothiazol-2-yl-thiourido)-4,5-dihydroimidazolin-5-ones **3a-e**. The compounds have been characterized by elemental analysis, IR, ^{1}H NMR and mass spectral data. All the compounds have been evaluated *in vitro* for their antimicrobial activities against several microbes and show significant activity.

Keywords: Aminobenzothiazole, 1,3,4-thiadiazole, imidazolinone, antimicrobial

Benzothiazoles are bicyclic ring systems with diverse chemical reactivity and a broad spectrum of biological activities such as antimicrobial[1,2], antitumor[3,4], anti-inflammatory[5,6] and antilieshmanial[7,8]. Several 1,3,4-thiadiazole derivatives are also known to exhibit diverse biological properties like antimicrobial[9,10], antitubercular[11,12], anti-inflammatory[13] and anticonvulsant[14]. Moreover, imidazoline derivatives constitute an interesting class of organic compounds with diverse chemical and pharmacological applications[15-17]. In continuation of the work on the synthesis of biologically important heterocyclic compounds[18-20] herein is reported the synthesis and biological activities of a few benzothiazole derivatives linked with 1,3,4-thiadiazole and imidazolinone systems.

The reaction sequence leading to the formation of the desired heterocyclic compounds are outlined in **Scheme I**. 6-Chloro-1,3-benzothiazole-2-yl-thiosemicarbazide **1** was prepared by the treatment of 6-chloro-2-aminobenzothiazole with CS_2 in the presence of ethanol-ammonia solution, followed by reaction with hydrazine hydrate. 2-Aryl-5-(6′-chloro-1′,3′-benzothiazole-2-yl-amino)-1,3,4-thiadiazoles **2a-j** were prepared by treatment of 6-chloro-1,3-benzothiazole-2-yl-thiosemicarbazide **1** with various substituted aromatic carboxylic acids in the presence of phosphorous oxychloride. The reaction of 6-chloro-1,3-benzothiazole-2-yl-thiosemicarbazide **1** with 4-arylidene-2-phenyl-5-oxazolinone in pyridine, after acidic treatment, yielded a series of 4-(4′-arylidine)-2-phenyl-1-(6′-chloro-1′,3′-benzothiazol-2-yl-thiouri-do)-4,5-dihydroimidazoline-5-ones 3a-e (**Table I**).

The IR spectrum of the compound **2i** showed absorption peak at 1710 cm^{-1}due to the stretching of C=O. The N-H stretching vibration appeared at 3436 cm^{-1}. The absorption at 795 cm^{-1} was obtained due to C-Cl stretching vibration. The ^{1}H NMR ($CDCl_3$) spectrum of compound **2i** displayed one singlet at δ 2.13 showing the presence of $OCCH_3$ protons of acetyl group. A broad singlet appeared at δ 12.36 showing the presence of NH proton. The 7 aromatic protons of benzothiazole and phenyl ring were observed as a multiplet at δ 7.48-8.00. The mass spectrum of **2i** showed molecular ion peak M^+ at *m/z* 402 corresponding to molecular formula $C_{17}H_{11}ClN_4O_2S_2$.

The IR spectrum of the compound **3d** showed absorption peak at 1720 cm^{-1}due to the stretching of C=O. The N-H stretching vibrations appeared at 3430 cm^{-1}. The absorption at 780 cm^{-1} was obtained due to C-Cl stretching vibration. The ^{1}H NMR ($CDCl_3$) spectrum of compound **3d** displayed one singlet at δ 3.11 showing the presence of six dimethylamino protons. Two broad singlets were observed at δ 10.10

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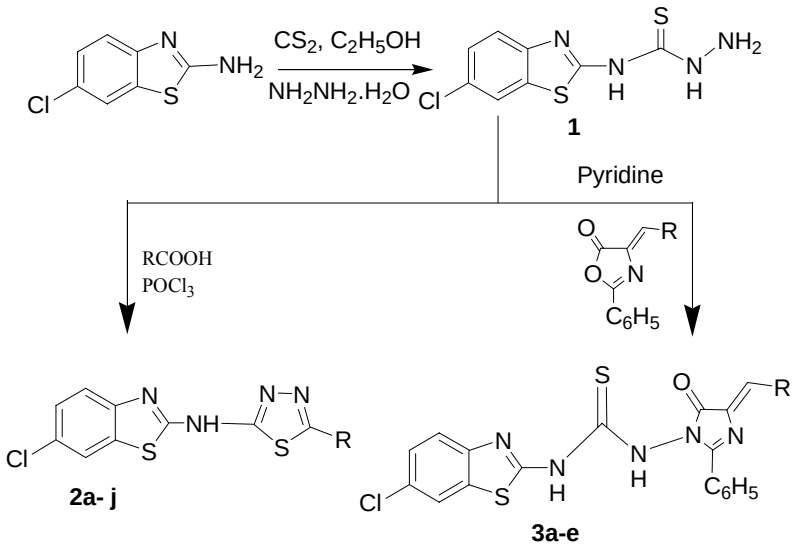

Scheme I

Table I — Physical characterization data of compounds **2a-j** and **3a-e**

Compd	R	Yield (%)	m.p. (°C)	Mol. Formula	Found (Calcd)% N
2a	Phenyl	64	220	$C_{15}H_9ClN_4S_2$	16.00 (16.25)
2b	4-Chlorophenyl	58	267	$C_{15}H_8Cl_2N_4S_2$	14.81 (14.77)
2c	2,4-Dichlorophenyl	64	280	$C_{15}H_7Cl_3N_4S_2$	13.73 (13.54)
2d	4-Nitrophenyl	68	282	$C_{15}H_8ClN_5O_2S_2$	17.84 (17.96)
2e	2-Aminophenyl	60	225	$C_{15}H_{10}ClN_5S_2$	19.59 (19.46)
2f	2,4-Dichlorophenoxymethyl	51	212	$C_{16}H_9Cl_3N_4OS_2$	12.81 (12.63)
2g	2-Naphthylmethyl	68	160	$C_{20}H_{13}ClN_4S_2$	13.86 (13.70)
2h	4-Methoxyphenyl	48	198	$C_{16}H_{11}ClN_4OS_2$	14.79 (14.95)
2i	2-Acetoxyphenyl	42	268	$C_{17}H_{11}ClN_4O_2S_2$	14.13 (13.91)
2j	3-Pyridyl	44	237	$C_{14}H_8ClN_5S_2$	20.41 (20.25)
3a	Phenyl	68	175	$C_{24}H_{16}ClN_5OS_2$	14.35 (14.29)
3b	4-Chlorophenyl	58	210	$C_{24}H_{15}Cl_2N_5OS_2$	13.05 (13.35)
3c	4-Flourophenyl	47	158	$C_{24}H_{15}ClFN_5OS_2$	13.56 (13.79)
3d	4-N,N-dimethyl phenyl	67	217	$C_{26}H_{21}ClN_6OS_2$	15.53 (15.77)
3e	3-Indolyl	62	198	$C_{26}H_{17}ClN_6OS_2$	15.71 (15.89)

and 12.56 showing the presence of two NH protons. The 12 aromatic protons of benzothiazole and phenyl ring were observed as a multiplet at δ 7.20-8.06. The singlet of CH-Ar proton was observed at δ 8.39. The mass spectrum of **3d** showed molecular ion peak M^+ at *m/z* 532 corresponding to molecular formula $C_{26}H_{21}ClN_6OS_2$.

Biological Evaluation

All the compounds have been screened for both antibacterial and antifungal activities using cup-plate agar diffusion method[21] by measuring the inhibition zone in mm. Ofloxacin (50 μg/mL) was used as a standard drug for antibacterial activity, and ketoconazole (50 μg/mL) as a standard drug for antifungal activity. The compounds were screened for antibacterial activity against *E. coli, S. aureus* and *P. aerugenosa* in nutrient agar medium, and for antifungal activity against *A. niger* and *C. albicans* in Sabouraud's dextrose agar medium. These sterilized agar media were poured into Petri-dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre-sterilized) was used to bore cavities. All the synthesized compounds (50 μg/mL) were placed serially in the cavities with the help of micropipette and allowed to diffuse for 1.0 hr. DMF was used as a solvent for all the compounds, and as a control. These plates were incubated at 37°C for 34 hr and 28°C for 48 hr, for antibacterial and antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured and percent inhibition of the compounds was calculated. The results are presented in **Table II** and **Table III**.

The thiadiazole derivative **2i** having a acetoxy-phenyl group showed potent activity against *S. aureus* (93.75%), whereas compound **2g** having 2-napthyl-methyl group showed maximum inhibition against *E. Coli* (94.11%), when compared with standard drug ofloxacin. The compound **2c** having 2,4-dichloro-phenyl group also showed significant antibacterial activity (87.50, 88.23 and 81.25% inhibition) against *E. coli*, *S. aureus* and *P. aerugenosa* respectively. Rest of the compounds showed moderate to good antibacterial activity. The imidazolinone derivatives **3a-e** were also found to be effective against all microorganisms at the same concentration. The compound **3a** having 4-chlorophenyl group showed maximum antibacterial activity (87.50 and 94.11% inhibition) against *E. coli* and *S. aureus* respectively, whereas compound **3c** having 4-fluorophenyl group

Table II — Antibacterial activity of 1,3,4-thiadiazole and imidazolinone derivatives

Compd	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aerugenosa*	
	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition
2a	11	68.75	11	64.70	12	75.00
2b	13	81.25	12	70.58	11	68.75
2c	14	87.50	15	88.23	13	81.25
2d	12	75.00	15	88.23	11	68.75
2e	13	81.25	12	70.58	12	75.00
2f	13	81.25	11	64.70	12	75.00
2g	12	75.00	16	94.11	13	81.25
2h	12	75.00	13	76.47	11	68.75
2i	15	93.75	12	70.58	13	81.25
2j	13	81.25	12	70.58	12	75.00
3a	14	87.50	16	94.11	13	81.25
3b	11	68.75	13	76.57	11	68.75
3c	13	81.25	15	88.23	14	87.50
3d	13	81.25	12	70.58	11	68.75
3e	13	81.25	14	82.35	12	75.00
Ofloxacin	16	100.0	17	100.0	16	100.0

Table III — Antifungal activity of 1,3,4-thiadiazole and imidazolinone derivatives

Compd	*Aspergillus niger*		*Candida albicans*	
	Zone of Inhibition (mm)	% Inhibition	Zone of Inhibition (mm)	% Inhibition
2a	18	60.00	13	65.00
2b	16	53.33	12	60.00
2c	14	46.66	12	60.00
2d	12	40.00	12	60.00
2e	26	86.66	16	80.00
2f	24	80.00	17	85.00
2g	16	53.33	13	65.00
2h	11	36.66	14	70.00
2i	14	46.66	13	65.00
2j	14	46.66	14	70.00
3a	12	60.00	16	53.33
3b	17	85.00	11	36.66
3c	13	65.00	17	56.66
3d	11	55.00	14	46.66
3e	16	80.00	12	40.00
Ketoconazole	20	100.00	30	100.00

showed maximum inhibition (87.50%) against *P. aerugenosa*. Rest of the imidazolinone derivatives showed moderate to good antibacterial activity.

The pattern of result of the antifungal activity of the test compounds was quite different from their antibacterial activity. The 1,3,4-thiadiazole derivative **2e** having 2-aminophenyl group showed maximum inhibition (86.66%) against *A. niger* whereas **2f** having 2,4-dichlorophenoxymethyl group showed maximum inhibition (85%) against *C. albicans*. Furthermore 1,3,4-thiadiazole derivative **2e** showed 80% inhibition against *C. albicans*, whereas **2f** showed 80% inhibition against *A. niger*. Rest of the thiadiazole derivatives showed moderate to good antifungal activity. The imidazolinone derivatives **3b** and **3e** having 4-chlorophenyl and 3-indolyl groups showed significant antifungal activity against *A. niger* (85 and 80% inhibition respectively). Rest of the imidazolinone derivatives showed moderate to low antifungal activity against both *A. niger* and *C. albicans*. Thus, it is concluded from the screening results that 1,3,4-thidiazole derivatives were most effective against all microorganisms at a concentration of 50 μg/mL.

Experimental Section

Melting points were measured in open capillary tubes and are uncorrected. Elemental analysis (N) was performed on the CHNS Elementar (Analysen Systime, GmbH) Germany Vario EL III. IR (KBr) spectra were recorded on a Nicolet, 5PC FT-IR spectrometer and ^{1}H NMR spectra on a Bruker DRX-300 FT NMR spectrometer using TMS as internal reference (chemical shift in δ ppm). Mass spectra were recorded on a Jeol-JMS-D-300 mass spectrometer (70 eV). Homogeneity of the compounds was checked on silica gel G plates using iodine vapor as visualizing agents. 6-Chloro-2-aminobenzothiazole[22] and 4-arylidene-2-phenyl-5-oxazolinone[23] were synthesized by the procedure given in literature.

6-Chloro-1,3-benzothiazol-2-yl-thiosemicarbazide 1. 6-Chloro-2-aminobenzothiazole **1** (0.10 mole) was dissolved in ethanol (95%, 50 mL) and ammonia solution (20 mL). Then CS_2 (20 mL) was added slowly within 15 min. with shaking, and the solution was allowed to stand for 1.0 hr. To it were added sodium chloroacetate (0.10 mole), and 50% hydrazine hydrate (20 mL). The reaction-mixture was warmed gently, filtered and evaporated to half of its volume and kept overnight. The solid thus obtained was filtered and purified by recrystallization from ethanol. IR (KBr): 3359 (NH), 3079 (CH), 757 cm^{-1} (C-Cl); ^{1}H NMR (DMSO-d_6): δ 4.48 (s, 2H, NH_2), 7.19 (d, 1H, 4-ArH), 7.29 (d, 1H, 5-ArH), 7.70 (s, 1H, 7-ArH), 8.64 (bs, 2H, NH-CS-NH).

2-Aryl-5-(6′-chloro-1′,3′-benzothiazol-2-yl-amino)-1,3,4-thiadiazoles 2a-j. A mixture of 6-chloro-1,3-benzothiazole-2-yl-thiosemicarbazide **1** (0.01 mole), an aromatic acid (0.01 mole) and phosphorous oxychloride (25 mL) was refluxed for 18-22 hr. After cooling to RT the reaction-mixture was slowly poured over crushed ice and kept overnight. The solid thus separated was filtered, washed with water, dried and purified by recrystallization from methanol. **2a**: IR (KBr): 3360 (NH), 3030 (CH), 750 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 7.35-7.90 (m, 8H, ArH), 8.27 (s, 1H, NH); MS: *m/z* 344 (M^+), 345 (M^+ +1), 346 (M^+ +2). **2b**: IR (KBr): 3369 (NH), 3032 (C-H), 755 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 7.30-7.97 (m, 7H, ArH), 10.49 (s, 1H, NH). **2c**: IR (KBr): 3386 (NH), 3060 (C-H), 765 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 7.35-7.60 (m, 6H, ArH), 10.49 (s, 1H, NH). **2d**: IR (KBr): 3366 (NH), 3033 (C-H), 752 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 7.38-8.36 (m, 7H, ArH), 13.36 (s, 1H, NH); MS: *m/z* 389 (M^+), 390 (M^+ +1), 391 (M^+ +2). **2e:** IR (KBr): 3411 (NH), 3033 (C-H), 752 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 4.5 (s, 2H, NH_2), 7.08-7.89 (m, 7H, ArH), 12.58 (s, 1H, NH). **2f:** IR (KBr): 3411 (NH), 3033 (C-H), 752 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 4.98 (s, 2H, CH_2-O), 7.08-7.91 (m, 6H, ArH), 12.58 (s, 1H, NH). **2g:** IR (KBr): 3400 (NH), 3021 (C-H), 752 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 3.27 (s, 2H, CH_2), 7.50-7.90 (m, 10H, ArH), 12.36 (s, 1H, NH). **2h**: IR (KBr): 3399 (NH), 3033 (C-H), 752 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 3.85 (s, 3H, OCH_3), 7.02-7.83 (m, 7H, ArH), 12.52 (s, 1H, NH); MS: *m/z* 374 (M^+), 375 (M^+ +1), 376 (M^+ +2). **2i**: IR (KBr): 3436 (NH), 3038 (C-H), 1710 (C=O), 795 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 2.13 (s, 3H, $OCCH_3$), 7.48-8.00 (m, 7H, ArH), 12.36 (s, 1H, NH); MS: *m/z* 402 (M^+), 403 (M^+ +1), 404 (M^+ +2). **2j**: IR (KBr): 3421 (NH), 3036 (C-H), 745 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 7.08-7.85 (m, 7H, ArH), 12.59 (s, 1H, NH).

4-(4′-Arylidene)-2-phenyl-1-(6′-chloro-1′,3′-benzothiazol-2-yl-thiourido)-4,5-dihydro-imidazolin-5-ones 3a-e. A mixture of 6-chloro-1,3-benzothiazole-2-yl-thiosemicarbazide **1** (0.01 mole) and 4-arylidene-2-phenyl-5-oxazolinone (0.01 mole) was refluxed in pyridine (30 mL) for 6-8 hr. After cooling to RT the reaction-mixture was slowly poured over crushed ice and excess pyridine was neutralized with dilute HCl. The solid thus separated out was filtered, dried and purified by recrystallization from methanol. **3a**: IR (KBr): 3398 (NH), 3058 (C-H), 1699 (C=O), 768 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 7.23-8.12 (m, 13H, ArH), 8.14 (s, 1H, =CH-Ar), 10.22 (s, 1H, N*H*-CS), 12.89 (s, 1H, CS-N*H*); **3b**: IR (KBr): 3433 (NH), 3062 (C-H), 1698 (C=O), 799 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 7.39-7.75 (m, 12H, ArH), 8.16 (s, 1H, =CH-Ar), 10.29 (s, 1H, N*H*-CS), 12.87 (s, 1H, CS-N*H*); MS: *m/z* 523 (M^+), 524 (M^+ +1), 525 (M^+ +2). **3c**: IR (KBr): 3403 (NH), 3008 (C-H), 1703 (C=O), 741 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.89-7.85 (m, 12H, ArH), 7.91 (s, 1H, =CH-Ar), 10.20 (s, 1H, N*H*-CS), 12.93 (s, 1H, CS-N*H*). **3d**: IR (KBr): 3430 (NH), 3038 (C-H), 1720 (C=O), 780 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 3.11 (s, 6H, $(CH_3)_2$), 7.20-8.06 (m, 12H, ArH), 8.39 (s, 1H, =CH-Ar), 10.10 (s, 1H, N*H*-CS), 12.56 (s, 1H, CS-N*H*); MS: *m/z* 532 (M^+), 533 (M^+ +1), 534 (M^+ +2). **3e**: IR (KBr): 3439 (NH), 3068 (C-H), 1699 (C=O), 752 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 7.39-7.75 (m, 13H, ArH), 8.15 (s, 1H, =CH-Ar), 10.22 (s, 1H, N*H*-CS), 11.89 (s, 1H, NH), 12.89 (s, 1H, CS-N*H*).

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