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Benzothiazole derivatives of thiazole/ oxazole as potent antimicrobial agents

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Non-steroid biologically active heterocyclic compounds (Z)-N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl) oxazol-2-yl)-N-(4-substituted phenylimino)-3-substituted-2,3-dihydrobenzo[d]oxazole-2-carboxamidine **(4a-4h)** and (Z)-N'- (4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N- (4-substituted phenylimino) -3-substituted-2-hydrobenzo[d] thiazole-2-carboxamidine **(4a'-4h')** have been synthesized, tested for their antimicrobial inhibiting potential and compared with standard drugs Miconazole (antifungal) and Imipenem (antibacterial). Compound **4e'** is more potentially active than other compounds and standard drugs. The structure configuration of newly synthesized compounds has been determined by elemental analysis and various spectroscopic (IR, ^{1}H and ^{13}CNMR and GCMS) techniques.

Keywords: Antimicrobial, miconazole, imipenem, benzothiazole, oxazole, thiazole

Non-steroid biologically active compounds are most commonly used for the treatment of variety of disease. A variety of heterocyclic compounds and its derivatives have been researched in bio-organic and medicinal chemistry with the application of drug discovery. Furthermore benzothiazole and its derivatives constitute the active class of compounds having wide range of biological activity such as anti-inflammation[1-6], Analgesic[7], Anti-microbial[8-10], Anti-bacterial[11], Anti-parasite[12], Anti-oxidation[13-15], Anti-cancer[16,17] and Anti-tumor[18,19]. Ultrasonication for the synthesis such types of compounds is of great interest in synthetic organic chemistry. Ultrasounds energy help improve the liquid–liquid interfacial area through emulsification, which is important for viscous films containing gas-filled bubbles, oscillation and cavitations bubbles and may activate various mechanisms vibrational energy is confined in small volume with heating, which improve liquid-liquid interfacial area to promote the rate of reaction[20,21]. In this article our aim is to produce a new series of benzothiazole derivative processing through thiazol and oxazol with the hope to get better biological action.

Result and Discussion

Antifungal activity

The antifungal activity of newly synthesized benzothiazole derivatives exhibited a considerable enhancement against *Aspergillus sp*., *Rizoctonia sp.* and *Penicillium sp*. at 1, 1.5 and 2 mg/ml concentration. The activity is greatly enhanced at the higher concentration 2mg/ml. DMSO (control) has shown negligible activity as compare to benzothiazole derivatives. However, the thiazole derivatives (**3a'-3d'** and **4a'-4h'**) have shown better activity than the oxazole (**3a-3d** and **4a-4h**)[22,23]. The antifungal experimental results of the compounds were compared with the standard antifungal drugs Miconazole. From the data (Table I) it has been also observed that the activity depends upon the type of substituent group varies in the following order $-C_2H_5>-CH_3>-H>-C_6H_5$ (**R**) and $-Cl>-OCH_3$ (**R'**). All the compounds were highly effective against *Aspergillus sp.* at 2 mg/ml concentration. Compound **4e'** $C_{32}H_{24}Cl_2N_6S_3$ {N'-(4-(2-(4-chlorophenyl) benzo [d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-chlorophenylimino)-3-ethyl-2,3-dihydrobenzo[d] thiazole-2-carboxamidine} is the only compound who show 95% activity against *Aspergillus sp.* at 2mg/ml concentration. The effect is susceptible to the concentration of the compound used for inhibition.

Minimum inhibitory concentration (MIC)

The antibacterial screening concentrations of the compounds to be used were estimated from the minimum inhibitory concentration (MIC). Minimum inhibitory concentration is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of microorganisms after overnight incubation. The MIC of the newly synthesized

compounds was tested against bacterial strains through a macrodilution tube method[24]. The MIC values for compounds against *B. subtilis, S. aureus, E. coli, S. typhi* and *P. aeruginosa* were given in Table II.

Antibacterial activity

The results of the bactericidal study of the synthesized compounds are summarized in Table III. The benzothiazole derivatives, standard drug Imipenem ($C_{12}H_{17}N_3O_4S$) and DMSO solution control

Table I — Fungicidal screening data of the newly synthesized compounds

Compd	R	R'	% Inhibition of spore germination								
			Aspergillus sp. (mg/ml)			Penicillium sp. (mg/ml)			Rizoctonia sp. (mg/ml)		
			1.0	1.5	2.0	1.0	1.5	2.0	1.0	1.5	2.0
3c	$-C_2H_5$		28	26	35	09	16	19	25	27	30
3c'	$-C_2H_5$		31	28	38	14	21	25	29	31	33
4a	-H	-Cl	60	65	74	55	58	70	50	52	59
4b	-H	$-OCH_3$	54	61	69	47	49	59	39	41	52
4c	$-CH_3$	-Cl	73	78	81	57	59	71	50	55	67
4d	$-CH_3$	$-OCH_3$	68	70	74	51	56	69	47	51	60
4e	$-C_2H_5$	-Cl	77	79	86	66	69	78	59	67	70
4f	$-C_2H_5$	$-OCH_3$	70	73	79	60	62	71	52	56	65
4g	$-C_6H_5$	-Cl	56	60	72	47	54	67	45	48	55
4h	$-C_6H_5$	$-OCH_3$	48	55	67	45	47	58	37	40	51
4a'	-H	-Cl	65	72	81	59	63	75	55	57	65
4b'	-H	$-OCH_3$	59	66	75	53	57	70	48	49	61
4c'	$-CH_3$	-Cl	80	85	89	66	69	79	59	62	75
4d'	$-CH_3$	$-OCH_3$	76	79	82	58	61	75	53	58	69
4e'	$-C_2H_5$	-Cl	82	86	95	72	77	86	68	72	79
4f'	$-C_2H_5$	$-OCH_3$	77	81	89	67	70	79	60	64	73
4g'	$-C_6H_5$	-Cl	63	69	80	56	61	73	52	55	62
4h'	$-C_6H_5$	$-OCH_3$	55	61	73	51	55	66	45	48	58
Miconazole (standard)			57	69	100	65	78	83	76	82	94

Table II — Minimum Inhibition Concentration (MIC) values μg/ml for newly synthesized compounds and standard drug

Compd	R	R'	% Inhibition of spore germination				
			Gram-positive		Gram-negative		
			Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhi*	*Pseudomonas aeruginosa*
3c	$-C_2H_5$		63	75	105	110	110
3c'	$-C_2H_5$		60	55	75	115	115
4a	-H	-Cl	50	50	75	105	105
4b	-H	$-OCH_3$	55	65	80	100	100
4c	$-CH_3$	-Cl	40	45	70	80	75
4d	$-CH_3$	$-OCH_3$	50	50	60	100	105
4e	$-C_2H_5$	-Cl	30	30	60	60	75
4f	$-C_2H_5$	$-OCH_3$	30	40	75	60	70
4g	$-C_6H_5$	-Cl	55	55	80	100	105
4h	$-C_6H_5$	$-OCH_3$	55	70	100	105	105
4a'	-H	-Cl	55	50	50	60	60
4b'	-H	$-OCH_3$	40	30	40	50	90
4c'	$-CH_3$	-Cl	25	20	50	50	50
4d'	$-CH_3$	$-OCH_3$	20	25	40	50	70
4e'	$-C_2H_5$	-Cl	10	10	30	50	60
4f'	$-C_2H_5$	$-OCH_3$	15	15	35	50	50
4g'	$-C_6H_5$	-Cl	30	30	66	75	80
4h'	$-C_6H_5$	$-OCH_3$	25	30	80	80	90
Imipenem			8	8	6	6	6

Table III — Bactericidal screening data of the newly synthesized compounds (inhibition zone in mm)

Compd	R	R'	% Inhibition of spore germination				
			Gram-positive		Gram-negative		
			Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhi*	*Pseudomonas aeruginosa*
3c	$-C_2H_5$		47	39	22	20	15
3c'	$-C_2H_5$		51	55	25	18	12
4a	-H	-Cl	60	59	40	33	29
4b	-H	$-OCH_3$	57	54	37	29	25
4c	$-CH_3$	-Cl	64	60	40	36	34
4d	$-CH_3$	$-OCH_3$	59	58	36	30	27
4e	$-C_2H_5$	-Cl	70	68	51	48	40
4f	$-C_2H_5$	$-OCH_3$	68	65	47	44	39
4g	$-C_6H_5$	-Cl	56	55	35	33	26
4h	$-C_6H_5$	$-OCH_3$	52	47	32	23	22
4a'	-H	-Cl	69	67	47	41	38
4b'	-H	$-OCH_3$	65	63	45	37	33
4c'	$-CH_3$	-Cl	72	70	49	44	41
4d'	$-CH_3$	$-OCH_3$	68	66	44	39	35
4e'	$-C_2H_5$	-Cl	81	79	59	54	51
4f'	$-C_2H_5$	$-OCH_3$	77	73	55	51	47
4g'	$-C_6H_5$	-Cl	65	64	44	37	34
4h'	$-C_6H_5$	$-OCH_3$	61	58	39	32	31
Imipenem			100	100	100	100	100

[a]Excellent activity (90-100% inhibition), Good activity (60-70% inhibition), Significant activity (30-50% inhibition), negligible activity (08-20% inhibition),
[b]Imipenem = Standard drug

were screened for their antibacterial activity against the bacteria *Staphylococcus aureus* and *Bacillus subtilis* (as gram positive bacteria) and *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* (as gram negative bacteria). From the bactericidal activity, it is apparent that the newly synthesized compounds were more toxic towards gram positive strains than gram negative strains. The reason is the difference in the structure of the cell walls. The walls of gram negative cells are more complex than those of gram positive cells. Further to it, the compounds (**3a'-3d'** & **4a'-4h'**) are moderate to highly activities as compare to the (**3a-3d** & **4a-4h**) towards the all organism and compound **4e'** $C_{32}H_{24}Cl_2N_6S_3$ {N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl) thiazol-2-yl)-N-(4-chlorophenylimino)-3-ethyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine} was more effective than standard drug. The variation in the antimicrobial activity of different compounds against different microorganisms depends on their impermeability of the cell or the differences in ribosomes in microbial cell[25]. The lipid membrane surrounding the cell favors the passage of any lipid soluble materials and it is known that liposolubility is an important factor controlling antimicrobial activity[26].

In the present study low activity of the compounds is may be due to their low lipophilicity, because of which penetration of the compounds through the lipid membrane was decreased and hence, they could neither block nor inhibit the growth of the microorganism. HPLC was used to analyze the lipophilicity of the compounds (linear regression analysis)[26]. RP-HPLC method flow rate of 1 mL/min, an injection volume of 5 µL, a column temperature of 25°C, the UV detection at 254 nm and a 25 min isocratic mobile phase methanol, 25 mmol KH_2PO_4, pH3 (20:80) was used to determine the lipophilicity of the compounds[27].

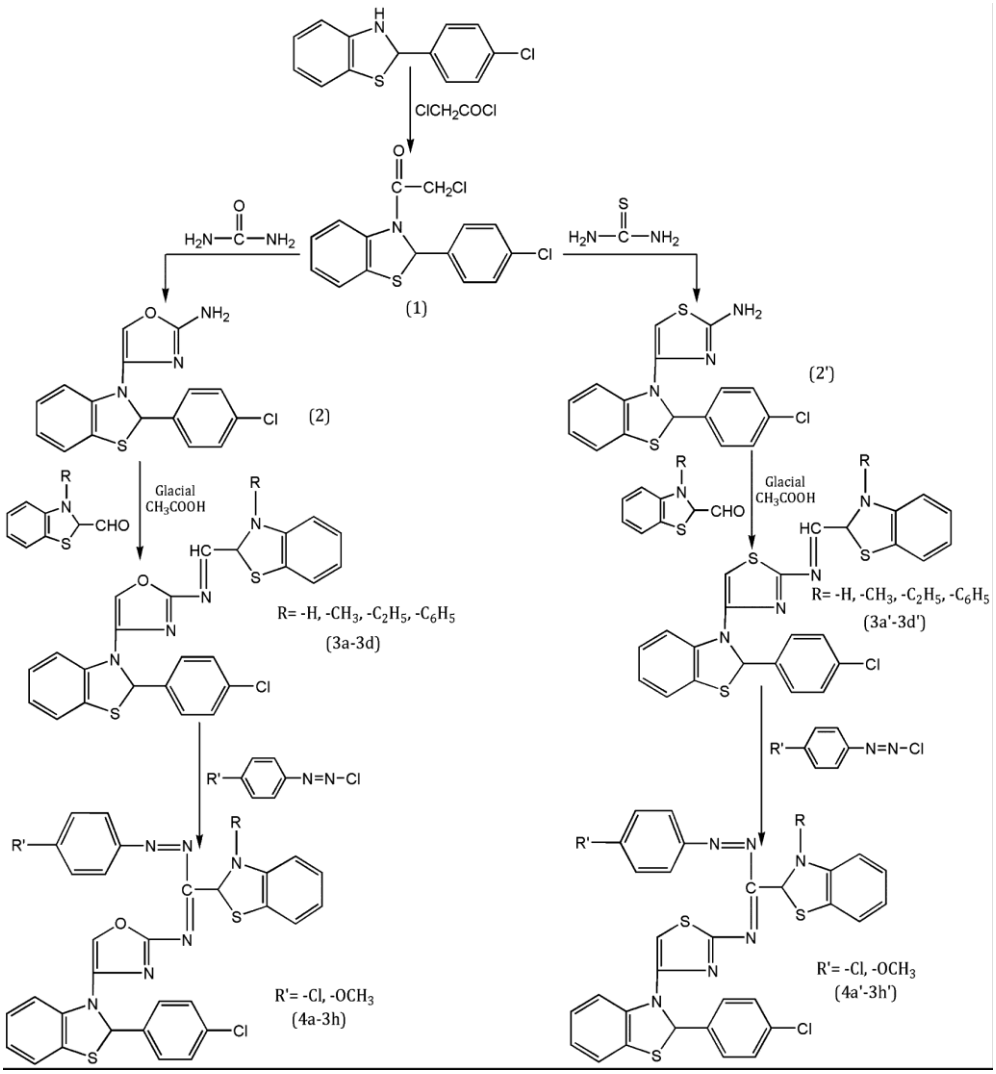
Experimental Section

The entire chemicals used were of the analytical reagent grade, 2-(4-chlorophenyl)-2,3-dihydrabenzo [d]thiazol, 2-chloroacetyl chloride, thiourea, urea and substituted aniline procured from s.d.-fine. Glacial acetic acid, HCl, ethanol, methanol and calcium chloride purchased from Merck.

Synthesis

Starting compound **(1)** 2-chloro-1-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl) ethanone was

synthesized by reaction between 2-(4-chlorophenyl)-2,3-dihydrobenzo[d]thiazole and 2-chloroacetyl chloride. Resulting compound on reaction with urea/ thiourea produced 4-(2-(4-chlorophenyl)benzo [d]thiazol-3(2H)-yl)oxazol-2-amine **(2)** and 4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-amine **(2')**, respectively. Synthesized compounds **2** and **2'** on reaction with 3-substituted-2-hydrobenzo [d]thiazole-2-carbaldehyde in the presence of glacial acetic acid produced 4-(2-(4-chlorophenyl) benzo[d]thiazol-3(2H)-yl)-N-((3-substituted-2, 3-dihydrobenzo[d]thiazol-2-yl)methylene)oxazol-2-amine **(3a-3d)** and 4-(2-(4-chlorophenyl) benzo [d]thiazol-3(2H)-yl)-N-((3-substituted-2,3-dihydrobenzo[d]thiazol-2-yl)methylene)thiazol-2-amine **(3a'-3d')** which on further reaction with substituted aniline (diazonium salt) synthesized the next product (Z)-N'-(4-(2-(4-chlorophenyl) benzo[d]thiazol-3(2H)-yl)oxazol-2-yl)-N-(4-substituted phenylimino)-3-substituted-2,3-dihydrobenzo[d] thiazole-2-carboxamidine **(4a-4h)** and Synthesis of (Z)-N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl) thiazol-2-yl)-N-(4-substituted phenylimino)-3-substituted-2,3-dihydrobenzo[d]thiazole-2-carboxamidine **(4a'-4h').** A S7 type sonotrode was submerged up to 25 mm into the reactant. The ultrasonic wave cycle, its amplitude as well as the time of the reaction was adjusted by the controller. Under these parameters reactions were carried out for 5 to 12 minutes. After completion of the reaction, the unreacted solvent was recovered in a rotarvapour flask under reduce pressure. The precipitate was separated washed with ethanol and recryltalized by suitable solvent. Synthetic root of the compound is given in Scheme I.



Scheme I — Synthesis of oxazol/ thiaxol substituted benzothiazole derivative

Synthesis of 2-chloro-1-(2-(4-chlorophenyl)benzo[d] thiazol-3(2H)-yl) ethanone (1)

Synthesis of compound (1): A solution of 2-(4-chlorophenyl)-2,3-dihydrobenzo[d]thiazole (0.01 mol) in dioxane (15 mL) was added dropwise to a hot solution (40°C) of 2-chloroacetyl chloride (0.01 mol) in dioxane (20 mL). The reaction mixture was taken in an Erlenmeyer type flask and assisted by ultrasonic irradiation for 08 min. the progress of the reaction was monitored by TLC. After complete the reaction product was cooled and poured into ice cold water a precipitate was appeared. The resulting precipitate was filtered off, washed and recrystallised by ethanol. Analytical data for $C_{15}H_{11}Cl_2NOS$ (324.22) Calcd C, 55.57; H, 3.42; N, 4.32; Found: C, 55.55; H, 3.50; N, 4.36, M.p. 253°C. IR (KBr) ν_{max} in cm^{-1} 665 cm^{-1} (C—Cl), 760 cm^{-1} (C—C), 1245 cm^{-1} (C—N), 1540 cm^{-1} (C=C for aromatic compound), 1720 cm^{-1} (C=O), 1270 cm^{-1} (C—S), 3040 cm^{-1} (C—H for aromatic compound), ^{1}H NMR (DMSO-d_6) δ in ppm 3.40 (s, 2H, —CH_2Cl), 7.65-6.85 (m, 8H, Ar—H), 4.95 (s, IH, CH of benzothiazole), ^{13}C-NMR (75 MHz, $CDCl_3$) δ 165.8, 140.0, 134.1, 132.7, 129.2, 128.9, 127.0, 125.4, 124.6, 121.8, 57.5, 40.2, FAB mass peaks [M^+] m/e 323.22, 288.03, 276.76, 246.74, 136.20, 111.55, 77.49, 48.48, 28.02.

Synthesis of 4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)oxazol-2-amine (2)

Synthesis of compound (2): The solution of 2-chloro-1-(2-(4-chlorophenyl) benzo[d]thiazol-3(2H)-yl)ethanone (compound 1) (0.01 mol) in ethanol (25 mL) was added to urea (0.01 mol). The reaction mixture was taken in an Erlenmeyer type flask and assisted by ultrasonic irradiation for 06 min. the progress of the reaction was monitored by TLC. After completion of the reaction product was cooled and poured into ice cold water. The resulting precipitate was filtered off, washed with ethanol and recrystallised by ethanol/water. The elemental analysis (CHN) and physical characterization data of the compounds is given in Table IV. IR (KBr) ν_{max} in cm^{-1}662 cm^{-1} (C—Cl), 762 cm^{-1} (C—C), 1241 cm^{-1} (C—N), 1544 cm^{-1} (C=C for aromatic compound), 1075 cm^{-1} (C—O—C), 3040 cm^{-1} (C—H for aromatic compound), 1579 cm^{-1} (C=N), 3333 cm^{-1} (—NH_2), ^{1}H NMR (DMSO-d_6) δ in ppm 4.94 (s, IH, CH of benzothiazole), 6.12 (s, 2H, —NH_2), 7.65-6.85 (m, 8H, Ar—H), 7.74 (s, H, —CH of oxazole). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 162.3, 141.6, 140.0, 138.1, 132.7, 129.2, 128.9, 127.6, 126.0,125.4, 117.4, 113.7, 65.5, 40.0, FAB mass peaks [M^+] m/e 328.80, 313.78, 294.35, 279.32, 246.01, 203.23, 136.20, 111.55, 83.06, 77.09, 68.04, 16.03.

Synthesis of 4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-substituted-2,3-dihydrobenzo [d]thiazol-2-yl)methylene)oxazol-2-amine (3a-3d)

Synthesis of compound (3a-3d): A solution of 4-(2-(4-chlorophenyl)benzo[d] thiazol-3(2H)-yl) oxazol-2-amine (compound 2) (0.01 mol) in ethanol (75 mL) was added to 3-substituted-2-hydrobenzo[d]thiazole-2-carbaldehyde (0.01 mol) in the presence of 2-3 drop of glacial acetic acid. The reaction mixture was taken in an Erlenmeyer type flask and assisted by ultrasonic irradiation for 10 min. The progress of the reaction was monitored by TLC. The solvents were recovered under reduce pressure, then the product was cooled and poured in to ice cold water, The resulting precipitate was filtered off, washed with ethanol and recrystallised by ethanol/water. The CHN and physical characterization data of the compounds is given in 4.

4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((2,3-dihydrobenzo[d]thiazol-2-yl)methylene) oxazol-2-amine (3a)

IR (KBr) ν_{max} in cm^{-1} 661 cm^{-1} (C—Cl), 762 cm^{-1} (C—C), 1244 cm^{-1} (C—N), 1545 cm^{-1} (C=C for aromatic compound), 1052 cm^{-1} (C—O—C), 3040 cm^{-1} (C—H for aromatic compound), 1577 cm^{-1}

Table IV — Physical characterization and elemental analysis of 4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2*H*)-yl)-N-((3-substituted-2,3-dihydrobenzo[d]thiazol-2-yl)methylene)oxazol-2-amine (**3a-3d**)

Compd	R	Mol. formula	Mol. Wt.	m.p. (°C)	Recrystallising solvent	Yield %	Elemental analysis					
							C%		H%		N%	
							Calcd	Found	Calcd	Found	Calcd	Found
2		$C_{16}H_{12}ClN_3OS$	329.80	152	Ethanol/water	60	58.27	58.23	3.67	3.66	12.74	12.77
3a	-H	$C_{24}H_{17}ClN_4OS_2$	477.00	160	Ethanol	62	60.43	60.44	3.59	3.56	11.75	11.77
3b	$-CH_3$	$C_{25}H_{19}ClN_4OS_2$	491.02	166	Ethanol/water	61	61.15	61.16	3.90	3.93	11.41	11.45
3c	$-C_2H_5$	$C_{26}H_{21}ClN_4OS_2$	505.05	170	Methanol	55	61.83	61.88	4.19	4.18	11.09	11.12
3d	$-C_6H_5$	$C_{30}H_{21}ClN_4OS_2$	553.09	178	Ethanol/water	59	65.15	65.16	3.83	3.86	10.13	10.17

(C=N), 3240 cm^{-1} (—NH). ^{1}H NMR (DMSO-d_6) δ 4.90 (s, 2H, —CH of benzothiazole), 7.71 (s, H, —CH of oxazole), 7.65-6.85 (m, 12H, Ar—H), 8.12 (N=CH—Ar), 9.30 (s, 1H, —NH of benzothiazole exchangeable with D_2O). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.1, 163.7, 150.6, 146.6, 141.6, 140.0, 138.1, 132.7, 129.2, 128.9, 127.6, 126.0, 125.4, 124.3, 117.4, 113.7, 66.5, 57.5. FAB mass peaks [M^+] m/e 476.00, 427.52, 313.02, 279.32, 246.74, 230.26, 203.23, 163.21, 111.50, 95.07, 77.09, 68.04, 28.02.

4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-methyl-2,3-dihydrobenzo[d] thiazol-2-yl) methylene)oxazol-2-amine (3b)

IR (KBr) ν_{max} in cm^{-1} 665 cm^{-1} (C—Cl), 760 cm^{-1} (C—C), 1240 cm^{-1} (C—N), 1540 cm^{-1} (C=C for aromatic compound), 1060 cm^{-1} (C—O—C), 3040 cm^{-1} (C—H for aromatic compound), 1572 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.90 (s, 2H, CH of benzothiazole), 7.69 (s, H, —CH of oxazole), 7.71-6.85 (m, 12H, Ar—H), 8.13 (N=CH—Ar). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.1, 163.7, 146.6, 141.6, 140.0, 138.1, 132.7, 129.2, 128.9, 127.6, 126.1, 125.4, 125.1, 124.3, 118.5, 114.5, 66.5, 61.9, 40.0, 34.3. FAB mass peaks [M^+] m/e 490.06, 455.10, 428.52, 313.78, 279.32, 246.74, 203.02, 177.25, 150.22, 136.20, 111.55, 95.07, 77.09, 68.04, 28.02, 14.03.

4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-ethyl-2,3-dihydrobenzo[d]thiazol-2-yl)methylene) oxazol-2-amine (3c)

IR (KBr) ν_{max} in cm^{-1} 660 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1245 cm^{-1} (C—N), 1545 cm^{-1} (C=C for aromatic compound), 1080 cm^{-1} (C—O—C), 3044 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.35 (t, 3H, CH_3), 4.22 (q, 2H, CH_2), 4.90 (s, 2H, CH of benzothiazole), 7.68 (s, H, —CH of oxazole), 7.75-6.75 (m, 12H, Ar—H), 8.11 (N=CH—Ar). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.1, 163.7, 146.6, 142.6, 141.6, 140.0, 138.1, 132.7, 129.2, 128.9, 127.6, 126.1, 125.4, 125.1, 124.3, 118.5, 114.5, 66.5, 59.4, 41.2, 12.3. FAB mass peaks [M^+] m/e 504.05, 490.01, 475.99, 469.60, 313.78, 279.02, 258.31, 246.74, 203.02, 191.28, 177.23, 136.20, 111.55, 95.14, 77.03, 68.04, 29.06, 14.03.

4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-phenyl-2,3-dihydrobenzo[d]thiazol -2-yl) methylene) oxazol-2-amine (3d)

IR (KBr) ν_{max} in cm^{-1} 660 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1245 cm^{-1} (C—N), 1545 cm^{-1} (C=C for aromatic compound), 1070 cm^{-1} (C—O—C), 3044 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 4.90 (s, 2H, CH of benzothiazole), 7.67 (s, H, —CH of oxazole), 7.85-6.85 (m, 17H, Ar—H), 8.11 (N=CH—Ar). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.1, 163.7, 149.1, 141.3, 140.0, 138.1, 125.4, 132.7, 129.9, 129.7, 129.2, 128.9, 127.7, 126.1, 125.4, 124.3, 118.3, 117.4, 113.7, 66.5, 61.8. FAB mass peaks [M^+] m/e 552.09, 517.74, 475.99, 441.54, 313.78, 306.07, 246.74, 239.32, 212.29, 203.23, 136.20, 111.55, 95.07, 77.10, 68.04, 28.05.

Synthesis of (Z)-N'-(4-(2-(4-chlorophenyl)benzo[d] thiazol-3(2H)-yl)oxazol-2-yl)-N- (4-substituted phenylimino)-3-substituted-2-hydrobenzo[d]thiazole-2-carboxamidine (4a-4h)

Synthesis of compound (4a-4h): A solution of the compound 3a-3d (0.1 mol) in ethanol was added to a solution of diazonium salt {(prepared by chloro/ methoxy substituted aniline (0.1 mol) in glacial acetic (4mL) was added to conc. HCl (2mL) at 0-4^{o}C and 20% sodium nitrite solution (2mL)} with constant stirring in pyridine (20 mL) below 0^{o}C. The reaction mixture was taken in an Erlenmeyer type flask and assisted by ultrasonic irradiation for 05 min. in ice bath. The progress of the reaction was monitored by TLC. The resulting solids were washed with water, recrystallised from ethanol and dried under vacuum over anhydrous $CaCl_2$. Their CHN and physical characterization data of the compounds is given in Table V.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl) oxazol-2-yl)-N-(4-chlorophenylimino)-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4a)

IR (KBr)ν_{max} in cm^{-1}, 665 cm^{-1} (C—Cl), 766 cm^{-1} (C—C), 1242 cm^{-1} (C—N), 1435 cm^{-1} (N=N), 1560 cm^{-1} (C=C for aromatic compound), 1075 cm^{-1} (C—O—C), 3044 cm^{-1} (C—H for aromatic compound), 1577 cm^{-1} (C=N), 3145 cm^{-1} (—NH). ^{1}H NMR (DMSO-d_6) δ 4.90 (s, 2H, —CH of benzothiazole), 7.65-6.75 (m, 16H, Ar—H), 7.72 (s, H, —CH of oxazole), 8.14 (N=CH—Ar), 9.30 (s, 1H, —NH of benzothiazole exchangeable with D_2O). ^{13}C-NMR (75 MHz, CDCl3) δ 150.6, 146.6, 141.6, 140.0, 138.1, 134.3, 132.7, 130.2, 129.2, 128.9, 127.6, 126.8, 126.0, 125.4, 124.3, 117.4, 113.7, 66.5, 58.0. FAB mass peaks [M^+] m/e 614.55, 567.08, 445.64, 368.03, 313.78, 301.77, 246.01, 230.24, 233.62, 203.02, 191.25, 163.21, 158.27, 136.18, 123.07, 111.54, 77.09, 68.04, 56.03.

Table V — Physical characterization and elemental analysis of (Z)-N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)oxazol-2-yl)-N-(4-substituted phenylimino)-3-substituted-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (**4a-4h**)

Compd	R	R'	Mol. formula	Mol. Wt.	m.p. (°C)	Recrystallising solvent	Yield %	Elemental analysis					
								C%		H%		N%	
								Calcd	Found	Calcd	Found	Calcd	Found
4a	-H	-Cl	$C_{30}H_{20}Cl_2N_6OS_2$	615.55	242	Ethanol	56	58.54	55.55	3.27	3.29	13.65	13.67
4b	-H	$-OCH_3$	$C_{31}H_{23}ClN_6O_2S_2$	611.13	248	Methanol/water	58	60.92	60.91	3.79	3.80	13.75	13.77
4c	$-CH_3$	-Cl	$C_{31}H_{22}Cl_2N_6OS_2$	629.58	244	Ethanol/water	62	59.14	59.16	3.52	3.55	13.35	13.37
4d	$-CH_3$	$-OCH_3$	$C_{32}H_{25}ClN_6O_2S_2$	625.16	252	Methanol	66	61.48	61.52	4.03	4.02	13.44	13.48
4e	$-C_2H_5$	-Cl	$C_{32}H_{24}Cl_2N_6OS_2$	643.60	258	Pet. ether	61	59.72	59.75	3.76	3.78	13.06	13.10
4f	$-C_2H_5$	$-OCH_3$	$C_{33}H_{27}ClN_6O_2S_2$	639.18	262	Acetone	52	62.01	62.04	4.26	4.25	13.15	13.17
4g	$-C_6H_5$	-Cl	$C_{36}H_{24}Cl_2N_6OS_2$	691.65	266	n-Hexane	56	62.51	62.55	3.50	3.55	12.15	12.18
4h	$-C_6H_5$	$-OCH_3$	$C_{37}H_{27}ClN_6O_2S_2$	687.23	268	n-hexane	60	64.66	64.69	3.96	3.99	12.23	12.25

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)oxazol-2-yl)-N-(4-methoxyphenylimino)-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4b)

IR (KBr)ν_{max} in cm^{-1} 666 cm^{-1} (C—Cl), 777 cm^{-1} (C—C), 1233 cm^{-1} (C—N), 1444 cm^{-1} (N=N), 1560 cm^{-1} (C=C for aromatic compound), 1070 cm^{-1} (C—O—C), 3041 cm^{-1} (C—H for aromatic compound), 1561 cm^{-1} (C=N), 3150 cm^{-1} (—NH). ^{1}H NMR (DMSO-d_6) δ 3.39 (s, 3H, —OCH_3), 4.90 (s, 2H, —CH of benzothiazole), 7.65-6.75 (m, 16H, Ar—H), 7.71 (s, H, —CH of oxazole), 8.17 (N=CH—Ar), 9.30 (s, 1H, —NH of benzothiazole exchangeable with D_2O). ^{13}C-NMR (75 MHz, CDCl3) δ 150.6, 146.6, 142.6, 141.6, 140.0, 138.1, 134.3, 132.7, 130.2, 129.2, 128.9, 127.6, 126.8, 126.0, 125.4, 124.3, 117.4, 113.7, 66.5, 57.4, 34.3. FAB mass peaks [M^+] m/e 610.13, 580.10, 575.68, 475.99, 393.45, 364.40, 313.78, 297.35, 246.01, 230.24, 203.02, 163.21, 136.18, 123.07, 111.54, 77.09, 68.04, 56.03, 31.03.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)oxazol-2-yl)-N-(4-chlorophenylimino)-3-methyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4c).

IR (KBr) ν_{max} in cm^{-1} 662 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1240 cm^{-1} (C—N), 1430 cm^{-1} (N=N), 1565 cm^{-1} (C=C for aromatic compound), 1080 cm^{-1} (C—O—C), 3054 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, —CH_3), 4.90 (s, 2H, —CH of benzothiazole), 7.65-6.75 (m, 16H, Ar—H), 7.69 (s, H, —CH of oxazole), 8.12 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 150.8, 142.6, 141.6, 140.0. 138.1, 134.3, 132.7, 130.2, 129.2, 128.9, 127.6, 126.8, 126.1,126.0, 125.4, 125.1, 18.5, 117.4, 114.5, 113.7,66.5, 54.9, 41.2, 12.3. FAB mass peaks [M^+] m/e 628.58, 594.12, 518.03, 463.98, 382.05, 313.78, 272.12, 246.01, 244.28, 230.24, 203.02, 177.23, 150.22, 139.56, 136.18, 123.07, 111.54, 77.09, 68.04, 56.03, 15.03.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)oxazol-2-yl)-N-(4-methoxyphenylimino)-3-methyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4d)

IR (KBr) ν_{max} in cm^{-1} 662 cm^{-1} (C—Cl), 760 cm^{-1} (C—C), 1244 cm^{-1} (C—N), 1441 cm^{-1} (N=N), 1566 cm^{-1} (C=C for aromatic compound), 1072 cm^{-1} (C—O—C), 3044 cm^{-1} (C—H for aromatic compound), 1568 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H, —CH_3), 3.41 (s, 3H, —OCH_3), 4.91 (s, 2H, —CH of benzothiazole), 7.69 (s, H, —CH of oxazole), 7.75-6.75 (m, 16H, Ar—H), 8.11 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 150.6, 149.1, 141.6, 140.0. 138.1, 134.3, 132.7, 130.2, 129.7, 129.2, 128.9, 127.7, 126.8, 126.1, 126.0, 125.4, 125.1, 119.3, 118.5, 118.3, 117.4, 114.6, 113.7, 66.5, 57.3. FAB mass peaks [M^+] m/e 624.16, 594.12, 589.70, 513.61, 378.42, 313.78, 311.38, 246.01, 244.28, 230.24, 203.02, 177.23, 162.16, 150.22, 136.18, 123.07, 111.54, 77.09, 68.04, 56.03, 31.03, 15.03.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)oxazol-2-yl)-N-(4-chlorophenylimino)-3-ethyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4e)

IR (KBr) ν_{max} in cm^{-1} 665 cm^{-1} (C—Cl), 761 cm^{-1} (C—C), 1241 cm^{-1} (C—N), 1432 cm^{-1} (N=N), 1566 cm^{-1} (C=C for aromatic compound), 1085 cm^{-1} (C—O—C), 3044 cm^{-1} (C—H for aromatic compound), 1577 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, —CH_3), 4.24 (q, 2H, —CH_2), 4.91 (s, 2H, —CH of benzothiazole), 7.67 (s, H, —CH of oxazole), 7.75-6.75 (m, 16H, Ar—H), 8.13 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 160.7, 150.7, 146.6, 141.6, 140.0, 138.1, 129.8, 129.2, 128.9, 127.6, 126.0, 125.4, 124.3, 117.4, 114.3, 113.7, 66.5,

55.9, 53. FAB mass peaks [M^+] m/e 642.60, 608.15, 532.05, 504.04, 421.51, 396.87, 368.81, 329.82, 313.78, 246.01, 230.24, 203.02, 191.26, 164.24, 136.18, 123.07, 111.54, 77.09, 68.04, 56.03, 29.06.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)oxazol-2-yl)-3-ethyl-N-(4-methoxyphenylimino)-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4f)

IR (KBr) ν_{max} in cm^{-1} 662 cm^{-1} (C—Cl), 760 cm^{-1} (C—C), 1244 cm^{-1} (C—N), 1441 cm^{-1} (N=N), 1566 cm^{-1} (C=C for aromatic compound), 1080 cm^{-1} (C—O—C), 3045 cm^{-1} (C—H for aromatic compound), 1566 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H, —CH_3), 3.39 (s, 3H, —OCH_3), 4.21 (q, 2H, —CH_2), 4.90 (s, 2H, —CH of benzothiazole), 7.65-6.65 (m, 16H, Ar—H), 7.66 (s, H, —CH of oxazole), 8.12 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 160.7, 150.6, 142.6, 141.6, 140.0, 138.1, 132.7, 129.8, 129.2, 128.9, 127.7, 126.1, 126.0, 125.1, 121.0, 119, 118.5, 117.4, 114.5, 114.3, 113.7, 66.5, 57.4, 55.9, 34.3. FAB mass peaks [M^+] m/e 638.18, 607.15, 603.73, 527.64, 504.04, 392.45, 325.40, 313.78, 246.01, 230.24, 203.02, 191.02, 164.24, 136.18, 123.07, 111.54, 77.09, 68.04, 56.03, 31.02, 29.06.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)oxazol-2-yl)-N-(4-chlorophenylimino)-3-phenyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4g)

IR (KBr) ν_{max} in cm^{-1} 661 cm^{-1} (C—Cl), 760 cm^{-1} (C—C), 1242 cm^{-1} (C—N), 1433 cm^{-1} (N=N), 1556 cm^{-1} (C=C for aromatic compound), 1077 cm^{-1} (C—O—C), 3048 cm^{-1} (C—H for aromatic compound), 1572 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 4.91 (s, 2H, —CH of benzothiazole), 7.68 (s, H, —CH of oxazole), 8.10-6.75 (m, 21H, Ar—H), 8.14 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 160.7, 150.6, 142.6, 141.6, 140.0. 138.1, 132.7, 129.8, 129.2, 128.9, 127.7, 126.8, 126.1,126.0, 125.4, 125.1, 124.3, 121.0, 119, 118.5, 117.4, 114.5, 114.3, 113.7,66.5, 55.9, 54.9, 41.2, 12.3. FAB mass peaks [M^+] m/e 690.65, 656.19, 580.10, 566.08, 444.91, 441.54, 377.87, 368.81, 313.78, 306.35, 246.01, 239.30, 230.24, 212.29, 203.02, 139.56, 136.18, 123.07, 111.54, 95.23, 77.09, 68.04, 56.03.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl) oxazol-2-yl)-N-(4-methoxyphenylimino)-3-phenyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4h)

IR (KBr) ν_{max} in cm^{-1} 663 cm^{-1} (C—Cl), 762 cm^{-1} (C—C), 1248 cm^{-1} (C—N), 1451 cm^{-1} (N=N), 1576 cm^{-1} (C=C for aromatic compound), 1080 cm^{-1} (C—O—C), 3041 cm^{-1} (C—H for aromatic compound), 1566 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 3.36 (s, 3H, —OCH_3), 4.92 (s, 2H, —CH of benzothiazole), 7.65-6.65 (m, 21H, Ar—H), 7.69 (s, H, —CH of oxazole), 8.12 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 160.7, 150.6, 149.1, 141.6, 140.0. 138.1, 132.7, 129.9, 129.7, 129.2, 128.9, 127.7, 127.6, 126.8, 126.1, 126.0, 125.4, 124.3, 121.0, 119.1, 119.0, 118.5, 118.3, 117.4, 114.3, 113.7, 66.5, 57.3, 55.9. FAB mass peaks [M^+] m/e 686.23, 656.19, 575.68, 552.08, 469.55, 440.11, 373.45, 364.39, 313.78, 306.35, 246.01, 239.30, 230.24, 212.29, 203.02, 136.18, 123.07, 111.54, 77.09, 68.04, 56.03, 31.03.

Synthesis of 4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-amine (2')

Synthesis of (compound 2'): A solution of 2-chloro-1-(2-(4-chlorophenyl) benzo[d]thiazol-3(2H)-yl)ethanone (compound 1) (0.01 mol) in ethanol (10 mL) in absolute ethanol (22 mL) was added to thiourea (0.01 mol). The reaction mixture was taken in an Erlenmeyer type flask and assisted by ultrasonic irradiation for 06 min. The progress of the reaction was monitored by TLC. After completion of the reaction product was cooled and poured into ice cold water. The resulting precipitate was filtered off, washed with ethanol and recrystallised from ethanol/water. The CHN and physical characterization data of the compounds is given in Table VI. IR (KBr) ν_{max} in cm^{-1} 662 cm^{-1} (C—Cl), 762 cm^{-1} (C—C), 1241 cm^{-1} (C—N), 1544 cm^{-1} (C=C for aromatic compound), 681 cm^{-1} (C—S—C), 3040 cm^{-1} (C—H for aromatic compound), 1579 cm^{-1} (C=N), 3333 cm^{-1} (—NH_2). ^{1}H NMR (DMSO-d_6) δ 4.94 (s, IH, CH of benzothiazole), 6.12 (s, 2H, —NH_2), 7.50 (s, H, —CH of thiazole), 7.65-6.85 (m, 8H, Ar—H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.0, 141.6, 140.0, 139.0, 132.7, 129.9, 128.9, 127.6, 126.4, 124.3, 113.7, 108, 66.4, 40.0. FAB mass peaks [M^+] m/e 344.86, 310.42, 296.40, 246.74, 234.32, 219.31, 212.29, 136.20, 111.55, 99.14, 84.12, 16.03.

Synthesis of 4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-substituted-2,3-dihydrobenzo [d]thiazol-2-yl)methylene)thiazol-2-amine (3a'-3d')

Synthesis of compound (3a'-3d'): A solution of 4-(2-(4-chlorophenyl)benzo[d] thiazol-3(2H)-yl)thiazol-2-amine (compound 2') (0.01 mol) in ethanol (75 mL) was added 3-substituted-2-hydrobenzo[d]thiazole-2-

Table VI — Physical characterization and elemental analysis of 4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-substituted-2,3-dihydrobenzo [d]thiazol-2-yl)methylene)thiazol-2-amine (**3a'-3d'**)

Compd	R	Mol. formula	Mol. Wt.	m.p. (°C)	Recrystallising solvent	Yeld %	Elemental analysis					
							C%		H%		N%	
							Calcd	Found	Calcd	Found	Calcd	Found
2'		$C_{16}H_{12}ClN_3S_2$	345.01	158	Ethanol/water	58	55.56	55.63	3.50	3.52	12.15	12.16
3a'	-H	$C_{24}H_{17}ClN_4S_3$	493.06	168	Ethanol	60	58.46	58.51	3.48	3.49	11.36	11.39
3b'	$-CH_3$	$C_{25}H_{19}ClN_4S_3$	507.09	172	Ethanol/water	62	59.21	59.24	3.78	3.79	11.05	11.09
3c'	$-C_2H_5$	$C_{26}H_{21}ClN_4S_3$	521.11	177	Methanol	66	59.92	59.88	4.06	4.08	10.75	10.77
3d'	$-C_6H_5$	$C_{30}H_{21}ClN_4S_3$	569.16	185	Ethanol/water	61	63.31	63.32	3.72	3.78	9.84	9.87

carbaldehyde (0.01 mol) in ethanol (15 mL) in the presence of 2-3 drop of glacial acetic acid. The reaction mixture was taken in an Erlenmeyer type flask and assisted by ultrasonic irradiation for 10 min. The progress of the reaction was monitored by TLC. The solvents were recovered under reduce pressure, then the product was cooled and poured in to ice cold water, The resulting precipitate was filtered off, washed with ethanol and recrystallised from ethanol/water. The CHN and physical characterization data of the compounds is given in Table VI.

4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((2,3-dihydrobenzo[d]thiazol-2-yl)methylene) thiazol-2-amine (3a')

IR (KBr) ν_{max} in cm^{-1} 660 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1245 cm^{-1} (C—N), 1545 cm^{-1} (C=C for aromatic compound), 685 cm^{-1} (C—S—C), 3044 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N), 3244 cm^{-1} (—NH). ^{1}H NMR (DMSO-d_6) δ 4.90 (s, 2H, —CH of benzothiazole), 7.50 (s, H, —CH of thiazole), 7.65-6.85 (m, 12H, Ar—H), 8.12 (N=CH—Ar), 9.30 (s, 1H, —NH of benzothiazole exchangeable with D_2O). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.1, 163.7, 146.6, 141.6, 140.0, 132.7, 129.2, 128.9, 127.6, 126.0, 124.3, 119.4, 117.4, 113.7, 66.5, 57.5, 40.0. FAB mass peaks [M^+] m/e 492.06, 382.52, 356.88, 344.86, 310.42, 296.40, 246.74, 234.32, 219.31, 212.29, 163.04, 136.20, 136.20, 111.55, 99.14, 84.12.

4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-methyl-2,3-dihydrobenzo[d]thiazol -2-yl) methylene) thiazol-2-amine (3b')

IR (KBr) ν_{max} in cm^{-1} 660 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1245 cm^{-1} (C—N), 1545 cm^{-1} (C=C for aromatic compound), 685 cm^{-1} (C—S—C), 3044 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 4.90 (s, 2H, CH of benzothiazole), 7.48 (s, H, —CH of thiazole), 7.65-6.85 (m, 12H, Ar—H), 8.12 (N=CH—Ar). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.1, 163.7, 146.6, 141.6, 140.0, 132.7, 129.2, 128.9, 127.6, 126.1, 125.1, 124.3, 119.4, 118.5, 114.5, 66.5, 61.9, 40.0, 34.3. FAB mass peaks [M^+] m/e 506.04, 492.06, 396.55, 382.52, 260.03, 356.88, 344.86, 310.42, 296.40, 246.74, 234.32, 219.31, 212.29, 177.25, 163.04, 136.20, 150.22, 136.20, 111.55, 99.14, 84.12, 14.03.

4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-ethyl-2,3-dihydrobenzo[d]thiazol-2-yl) methylene) thiazol-2-amine (3c')

IR (KBr) ν_{max} in cm^{-1} 660 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1245 cm^{-1} (C—N), 1545 cm^{-1} (C=C for aromatic compound), 685 cm^{-1} (C—S—C), 3044 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.35 (t, 3H, CH_3), 4.22 (q, 2H, CH_2), 4.90 (s, 2H, CH of benzothiazole), 7.46 (s, H, —CH of thiazole), 7.75-6.75 (m, 12H, Ar—H), 8.11 (N=CH—Ar). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.1, 163.7, 146.6, 142.6, 141.6, 140.0, 132.7, 129.2, 128.9, 127.6, 126.1, 125.1, 124.3, 119.4, 118.5, 114.5, 66.5, 59.4, 41.2, 12.3. FAB mass peaks [M^+] m/e 520.11, 492.06, 485.66, 409.06, 382.52, 274.39, 356.88, 344.86, 310.42, 296.40, 246.74, 234.32, 219.31, 212.29, 191.28, 163.04, 136.20, 150.22, 111.55, 99.14, 84.12, 28.06, 14.03.

4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)-N-((3-phenyl-2,3-dihydrobenzo[d]thiazol -2-yl) methylene) thiazol-2-amine (3d')

IR (KBr) ν_{max} in cm^{-1} 660 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1245 cm^{-1} (C—N), 1545 cm^{-1} (C=C for aromatic compound), 685 cm^{-1} (C—S—C), 3044 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 4.90 (s, 2H, CH of benzothiazole), 7.45 (s, H, —CH of thiazol), 7.85-6.85 (m, 17H, Ar—H), 8.11 (N=CH—Ar). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.1, 163.7, 149.1, 141.3, 140.0, 132.7, 129.9, 129.7, 129.2, 128.9, 127.7, 126.1, 124.3, 119.3, 118.3, 117.4, 113.7, 66.5, 61.8. FAB mass peaks [M^+] m/e 568.16, 533.71, 522.23, 492.06, 457.62, 382.52, 356.88, 344.86, 339.32, 322.05, 274.39, ,

246.74, 239.32, 219.31, 212.29, 191.28, 163.04, 150.22, 136.20, 111.55, 99.14, 84.12, 77.11, 28.05.

Synthesis of (Z)-N'-(4-(2-(4-chlorophenyl)benzo[d] thiazol-3(2H)-yl)thiazol-2-yl)-N- (4-substituted phenylimino)-3-substituted-2-hydrobenzo[d]thiazole-2-carboxamidine (4a'-4h').

Synthesis of compound (4a'-4h'): A solution of the compound 3a'-3d' (0.1 mol) in ethanol was added to a solution of diazonium salt {(prepared by chloro/ methoxy substituted aniline (0.1 mol) in glacial acetic (4mL) was added to conc. HCl (2 mL) at 0-4^{o}C and 20% sodium nitrite solution (2 mL)} with constant stirring in pyridine (20 mL) below 0^{o}C. The reaction mixture was taken in an Erlenmeyer type flask and assisted by ultrasonication for 05 min. in ice bath. The progress of the reaction was monitored by TLC. The resulting solids were washed with water, recrystallised from suitable solvent and dried under vacuum over anhydrous $CaCl_2$. Their CHN and physical characterization data of the compounds are given in Table VII.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-chlorophenylimino) -2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4a')

IR (KBr) ν_{max} in cm^{-1} 662 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1240 cm^{-1} (C—N), 1430 cm^{-1} (N=N), 1565 cm^{-1} (C=C for aromatic compound), 685 cm^{-1} (C—S—C), 3054 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N), 3144 cm^{-1} (—NH). ^{1}H NMR (DMSO-d_6) δ 4.90 (s, 2H, —CH of benzothiazole), 7.65-6.75 (m, 16H, Ar—H), 7.54 (s, H, —CH of thiazole), 8.15 (N=CH—Ar), 9.30 (s, 1H, —NH of benzothiazole exchangeable with D_2O). ^{13}C-NMR (75 MHz, CDCl3) δ 171.7, 146.6, 141.6, 140.0, 134.3, 132.7, 130.2, 129.2, 128.9, 127.6, 126.0, 124.3, 119.0, 117.4, 113.7, 66.5, 58.0. FAB mass peaks [M$^+$] m/e 630.62, 597.17, 562.73, 520.08, 495.01, 409.53, 384.89, 329.85, 301.78, 246.74, 234.32, 219.31, 212.29, 191.24, 163.04, 136.20, 136.20, 111.55, 99.14, 84.12, 56.05.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-methoxyphenylimino)-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4b')

IR (KBr) ν_{max} in cm^{-1} 660 cm^{-1} (C—Cl), 766 cm^{-1} (C—C), 1242 cm^{-1} (C—N), 1444 cm^{-1} (N=N), 1560 cm^{-1} (C=C for aromatic compound), 695 cm^{-1} (C—S—C), 3044 cm^{-1} (C—H for aromatic compound), 1565 cm^{-1} (C=N), 3154 cm^{-1} (—NH). ^{1}H NMR (DMSO-d_6) δ 3.39 (s, 3H, —OCH_3), 4.90 (s, 2H, —CH of benzothiazole), 7.52 (s, H, —CH of thiazole), 7.65-6.75 (m, 16H, Ar—H), 8.11 (N=CH—Ar), 9.30 (s, 1H, —NH of benzothiazole exchangeable with D_2O). ^{13}C-NMR (75 MHz, CDCl3) δ 171.7, 142.6, 141.6, 140.0. 134.3, 132.7, 130.2, 129.2, 128.9, 127.7, 126.1, 125.1, 119, 118.5, 117.4, 114.5, 113.7,66.5, 57.4, 34.3. FAB mass peaks [M$^+$] m/e 627.20, 597.17, 562.73, 520.08, 495.01, 409.53, 384.89, 329.85, 301.78, 246.74, 219.29, 205.25, 177.23, 150.22, 135.14, 111.55, 84.12, 15.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-chlorophenylimino)-3-methyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4c')

IR (KBr) ν_{max} in cm^{-1} 662 cm^{-1} (C—Cl), 765 cm^{-1} (C—C), 1240 cm^{-1} (C—N), 1430 cm^{-1} (N=N), 1565 cm^{-1} (C=C for aromatic compound), 685 cm^{-1} (C—S—C), 3054 cm^{-1} (C—H for aromatic compound), 1575 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, —CH_3), 4.90 (s, 2H, —CH of benzothiazole), 7.50 (s, H, —CH of thiazole), 7.65-6.75 (m, 16H, Ar—H), 8.13 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 171.7, 142.6, 141.6, 140.0. 134.3,

Table VII — Physical characterization and elemental analysis of (Z)-N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-substituted phenylimino) -3-substituted-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (**4a'-4h'**)

Compd	R	R'	Mol. formula	Mol. Wt.	m.p. (°C)	Recrystallising solvent	Yeld %	Elemental analysis					
								C%		H%		N%	
								Calcd	Found	Calcd	Found	Calcd	Found
4a'	-H	-Cl	$C_{30}H_{20}Cl_2N_6S_3$	631.62	244	Ethanol	55	57.05	57.12	3.19	3.18	13.31	13.17
4b'	-H	-OCH_3	$C_{31}H_{23}ClN_6OS_3$	627.20	251	Methanol/water	54	59.36	59.32	3.70	3.72	13.40	13.44
4c'	-CH_3	-Cl	$C_{31}H_{22}Cl_2N_6S_3$	645.64	248	Ethanol/water	58	57.69	57.71	3.43	3.48	13.02	13.07
4d'	-CH_3	-OCH_3	$C_{32}H_{25}ClN_6OS_3$	641.22	258	Methanol	62	59.94	59.98	3.93	3.92	13.11	13.15
4e'	-C_2H_5	-Cl	$C_{32}H_{24}Cl_2N_6S_3$	659.67	264	Pet. ether	59	58.26	58.28	3.67	3.68	12.74	12.77
4f'	-C_2H_5	-OCH_3	$C_{33}H_{27}ClN_6OS_3$	655.25	268	Acetone	51	60.49	60.52	4.15	4.18	12.83	12.84
4g'	-C_6H_5	-Cl	$C_{36}H_{24}Cl_2N_6S_3$	707.71	264	n-Hexane	50	61.10	61.12	3.42	3.48	11.87	11.90
4h'	-C_6H_5	-OCH_3	$C_{37}H_{27}ClN_6OS_3$	703.29	270	n-hexane	56	63.19	63.22	3.87	3.90	11.95	11.97

132.7, 130.2, 129.2, 128.9, 127.7, 126.8, 126.1,126.0, 125.1, 119, 18.5, 117.4, 114.5, 113.7,66.5, 54.9, 41.2, 12.3. FAB mass peaks [M^+] m/e 644.64, 610.19, 575.74, 534.09, 506.08, 471.63, 409.53, 397.91, 330.84, 329.85, 288.36, 246.74, 215.80, 205.25, 150.22, 139.56, 136.20, 134.18, 111.55, 84.12, 56.05, 15.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-methoxyphenylimino)-3-methyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4d')

IR (KBr) ν_{max} in cm^{-1} 662 cm^{-1} (C—Cl), 760 cm^{-1} (C—C), 1244 cm^{-1} (C—N), 1441 cm^{-1} (N=N), 1566 cm^{-1} (C=C for aromatic compound), 695 cm^{-1} (C—S—C), 3045 cm^{-1} (C—H for aromatic compound), 1566 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 3H, —CH_3), 3.39 (s, 3H, —OCH_3), 4.92 (s, 2H, —CH of benzothiazole), 7.50 (s, H, —CH of thiazol), 7.65-6.75 (m, 16H, Ar—H), 8.13 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 171.7, 149.1, 141.6, 140.0. 134.3, 132.7, 130.2, 129.7, 129.2, 128.9, 127.7, 126.8, 126.1,126.0, 125.1, 119.1, 119.0, 118.5, 118.3, 117.4, 114.5, 113.7, 66.5, 57.3. FAB mass peaks [M^+] m/e 640.22, 610.19, 605.77, 575.74, 534.04, 506.08, 394.49, 329.84, 284.35, 260.35, 246.74, 246.31, 219.29, 205.25, 177.23, 150.22, 136.20, 135.14, 111.55, 84.12, 56.05, 15.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-chlorophenylimino)-3-ethyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4e')

IR (KBr) ν_{max} in cm^{-1} 665 cm^{-1} (C—Cl), 761 cm^{-1} (C—C), 1241 cm^{-1} (C—N), 1432 cm^{-1} (N=N), 1566 cm^{-1} (C=C for aromatic compound), 688 cm^{-1} (C—S—C), 3044 cm^{-1} (C—H for aromatic compound), 1577 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, —CH_3), 4.24 (q, 2H, —CH_2), 4.91 (s, 2H, —CH of benzothiazole exchangeable), 7.49 (s, H, —CH of thiazole), 7.75-6.75 (m, 16H, Ar—H), 8.12 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 171.7, 160.7, 146.6, 141.6, 140.0, 134.3, 132.7, 130.2, 129.8, 129.2, 128.9, 127.6, 126.0, 124.3, 121.0, 119.0, 117.4, 114.3, 113.7, 66.5, 58.0, 55.9, 53.0. FAB mass peaks [M^+] m/e 658.67, 624.22, 589.76, 561.70, 519.05, 409.53, 384.87, 329.00, 274.37, 246.02, 219.01, 212.06, 191.07, 205.25, 191.26, 164.06, 162.03, 150.22, 136.02, 111.00, 83.99, 77.09, 56.05, 29.06.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-3-ethyl-N-(4-methoxyphenylimino)-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4f')

IR (KBr) ν_{max} in cm^{-1} 662 cm^{-1} (C—Cl), 760 cm^{-1} (C—C), 1244 cm^{-1} (C—N), 1441 cm^{-1} (N=N), 1566 cm^{-1} (C=C for aromatic compound), 695 cm^{-1} (C—S—C), 3045 cm^{-1} (C—H for aromatic compound), 1566 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H, —CH_3), 3.39 (s, 3H, —OCH_3), 4.21 (q, 2H, —CH_2), 4.90 (s, 2H, —CH of benzothiazole), 7.48 (s, H, —CH of thiazole), 7.65-6.65 (m, 16H, Ar—H), 8.16 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 171.7, 160.7, 142.6, 141.6, 140.0, 132.7, 129.8, 129.2, 128.9, 127.7, 126.1, 126.0, 125.1, 121.0, 119, 118.5, 117.4, 114.5, 114.3, 113.7,66.5, 57.4, 55.9, 34.3. FAB mass peaks [M^+] m/e 654.25, 619.80, 589.76, 561.70, 519.05, 408.51, 380.45, 329.00, 325.40, 274.32, 246.31, 219.01, 212.05, 191.07, 164.06, 162.02, 135.06, 136.02, 111.55, 107.05, 83.99, 56.05, 29.11. The pathway fragmentation pattern of the mass spectrum of the compound **4f'** is depicted in Figure 1.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-chlorophenylimino)-3-phenyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4g')

IR (KBr) ν_{max} in cm^{-1} 661 cm^{-1} (C—Cl), 760 cm^{-1} (C—C), 1242 cm^{-1} (C—N), 1433 cm^{-1} (N=N), 1556 cm^{-1} (C=C for aromatic compound), 677 cm^{-1} (C—S—C), 3048 cm^{-1} (C—H for aromatic compound), 1572 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 4.91 (s, 2H, —CH of benzothiazole), 7.46 (s, H, —CH of thiazole), 8.10-6.75 (m, 21H, Ar—H), 8.17 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 171.7, 160.7, 142.6, 141.6, 140.0. 132.7, 129.8, 129.2, 128.9, 127.6, 126.8, 126.1,126.0, 125.1, 124.3, 121.0, 119, 118.5, 117.4, 114.5, 114.3, 113.7,66.5, 55.9, 54.9, 41.2, 12.3. FAB mass peaks [M^+] m/e 706.71, 672.26, 637.81, 630.61, 596.16, 520.11, 460.98, 384.87, 377.87, 329.84, 301.76, 267.31, 246.74, 239.30, 219.29, 212.05, 166.67, 164.20, 135.18, 111.55, 84.12, 77.09, 56.05.

N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-methoxyphenylimino)-3-phenyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine (4h')

IR (KBr) ν_{max} in cm^{-1} 663 cm^{-1} (C—Cl), 762 cm^{-1} (C—C), 1248 cm^{-1} (C—N), 1451 cm^{-1} (N=N), 1576 cm^{-1} (C=C for aromatic compound), 690 cm^{-1} (C—S—C), 3041 cm^{-1} (C—H for aromatic compound), 1566 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 3.36 (s, 3H, —OCH_3), 4.92 (s, 2H, —CH of benzothiazole), 7.65-6.65 (m, 21H, Ar—H), 7.45 (s, H, —CH of thiazole), 8.17 (N=CH—Ar). ^{13}C-NMR (75 MHz, CDCl3) δ 171.7, 160.7, 149.1, 141.6, 141.3, 140.0. 132.7, 129.9, 129.2, 128.9, 127.7, 127.6, 126.8,

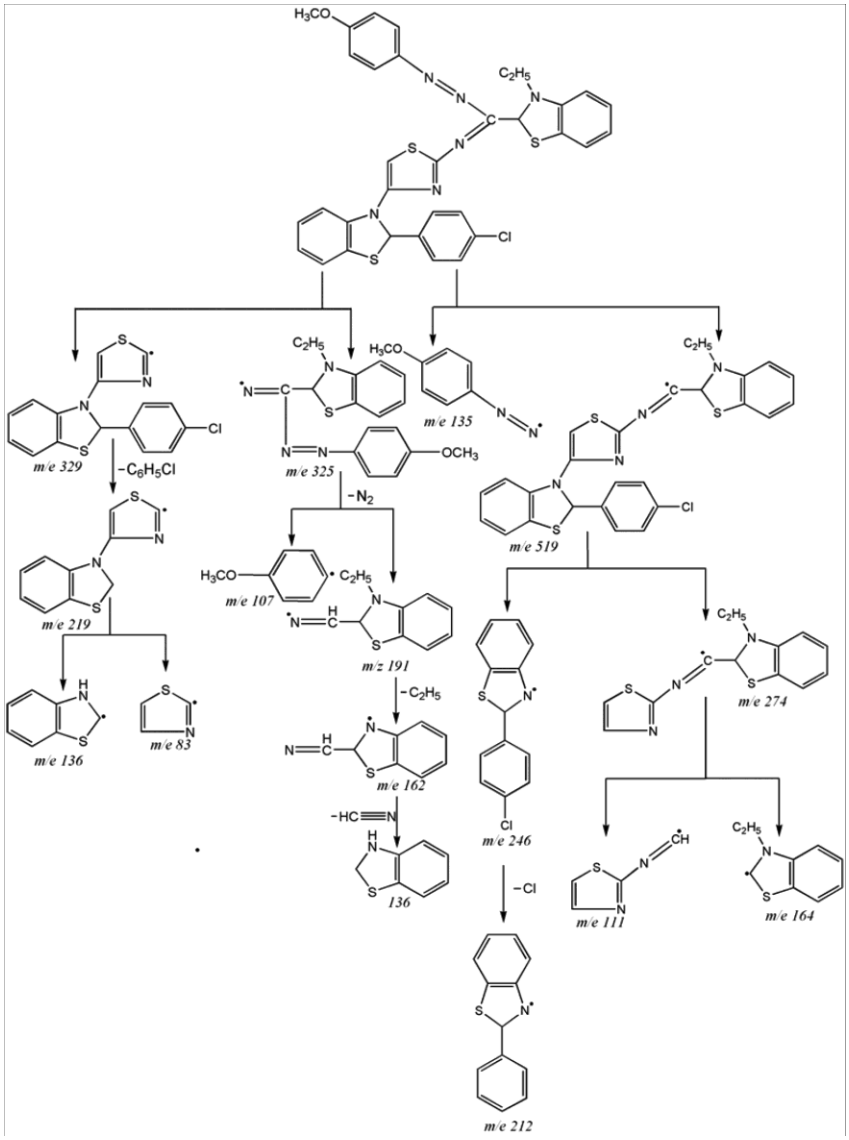


Figure 1 — The pathway fragmentation pattern of the mass spectrum of compound (**4f'**)

126.1, 126.0, 124.3, 121.0, 119.1, 119.0, 118.5, 118.3, 117.4, 114.3, 113.7, 66.5, 57.3, 55.9. FAB mass peaks [M^+] m/e 702.29, 667.84, 637.81, 591.74, 568.15, 520.06, 492.05, 456.56, 425.52, 373.45, 350.43, 329.84, 274.32, 267.32, 246.74, 219.29, 191.21, 163.20, 136.20, 111.55, 84.12, 77.05, 31.03.

Pharmacology

Antimicrobial activity

Antimicrobial screening of the newly synthesized compound were evaluated using agar well diffusion method[28]. The biological activity of the compounds and standard drug (antibacterial lmipenem and antifungal miconazol) were studied against the *Staphylococcus aureus, Bacillus subtilis* (as gram positive bacteria) and *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* (as gram negative bacteria) and fungi *Rizoctonia sp., Aspergillus sp., Penicillium sp*. All strains were obtained from Microbial Type Collection and Gene Bank, Institute of Microbial Technology (IMTECH) Chandigarh, India. The solution of different concentration 1, 1.5 and 2 mg/mL of each compound including standard drug lmipenem and miconazol in DMSO was prepared for testing against spore germination of fungi and bacteria. Centrifuged pellets of microorganism from a 24 h old culture containing approximately 10^4 CFU (colony forming unit) per mL were spread on the surface of Muller Hinton Agar media plates. Wells with 6 mm diameter made, and

then solution of test compound was filled to the wells. The plates were incubated at 30°C for 24h. The activity of the compounds was determined by measuring diameter of the inhibition zone (in mm) each test was carried in triplicate[29,30]

Statistical Analysis

One way ANOVA analysis is done with a suitable transformation to know the significance difference in the mean biological action. The interaction between the three factors temperature, concentration and zone of inhibition, in which temperature was fixed and two factors concentration and zone of inhibition were variable, in Table VIII the investigation was response in term of zone of inhibition (mm) at different experimental condition. The mode F-value 14.2 and mode P-value <.0001 implied the mode is significant. In Table IX, the mode F-value and P-value were

Table VIII — One way ANOVA analysis is done, the zone of inhibition fungal strain using newly synthesized compounds and standard drug Miconazole Results were obtained using the link, http://vassarstats.net/anova1u.html

ANOVA Summary Correlated Samples k=4

Source	**SS**	**df**	**MS**	**F**	**P**
Treatment [between groups]	2838.7969	3	946.2656	14.2	<.0001
Error	3998.4375	60	66.6406		
Ss/Bl					
Total	6837.2344	63			

Ss/BI = Subjects or Blocks depending on the design.
Applicable only to correlate samples ANOVA.

Tukey HSD Test.

HSD[.05]=7.65; HSD[.01]=9.4
M1 vs M2 P<.05
M1 vs M3 P<.01
M1 vs M4 nonsignificant
M2 vs M3 P<.01
M2 vs M4 nonsignificant
M3 vs M4 P<.01

M1 = mean of Sample 1
M2 = mean of Sample 2
and so forth.
HSD = the absolute [unsigned] difference between any two sample means required for significance at the designated level. HSD[.05] for the .05 level;
HSD[.01] for the .01 level.

The interaction between the three factors temperature, concentration and zone of inhibition, in which temperature was fixed and two factors concentration and zone of inhibition were variable.

Table IX — One way ANOVA analysis is done, the zone of inhibition of bacterial strain using newly synthesized compounds and standard drug Imipenem Results were obtained using the link, http://vassarstats.net/anova1u.html

ANOVA Summary Correlated Samples k=5

Source	SS	df	MS	F	P
Treatment [between groups]	12980.2	4	3245.05	51.89	<.0001
Error	4690.1875	75	62.5358		
Ss/Bl					
Total	17670.3875	79			

Ss/BI = Subjects or Blocks depending on the design.
Applicable only to correlate samples ANOVA.

Tukey HSD Test.

HSD[.05]=7.83; HSD[.01]=9.45
M1 vs M2 nonsignificant
M1 vs M3 P<.01
M1 vs M4 P<.01
M1 vs M5 P<.01
M2 vs M3 P<.01
M2 vs M4 P<.01
M2 vs M5 P<.01
M3 vs M4 nonsignificant
M3 vs M5 P<.05
M4 vs M5 nonsignificant

M1 = mean of Sample 1
M2 = mean of Sample 2
and so forth.
HSD = the absolute [unsigned] difference between any two sample means required for significance at the designated level. HSD[.05] for the .05 level;
HSD[.01] for the .01 level.

The interaction between the three factors temperature, concentration and zone of inhibition, in which temperature was fixed and two factors concentration and zone of inhibition were variable.

significant 51.89 and <.0001, respectively. Results were obtained using the link, http://vassarstats.net/anova1u.html[31]

Conclusion

The newly synthesized compounds having **R** ($–C_2H_5$) substituted group at the third position of benzothiazole were shown to be more biologically active as compare to other compounds. Compound **4e'** {N'-(4-(2-(4-chlorophenyl)benzo[d]thiazol-3(2H)-yl)thiazol-2-yl)-N-(4-chlorophenylimino)-3-ethyl-2,3-dihydrobenzo[d]thiazole-2-carboxamidine} was the most active compound as compare to reference drug..

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