Full Paper

Synthesis of New Thiazolylthiazolidinylbenzothiazoles and Thiazolylazetidinylbenzothiazoles as Potential Insecticidal, Antifungal, and Antibacterial Agents

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A series of 2-{[2′-(3″-chloro-2″-oxo-4″-substitutedaryl-1″-azetidinyl)-1′,3′-thiazol-4′-yl] thio}benzothiazoles (**4a – 4e**) and 2-{[(2′-(2″-substitutedaryl-4″-thiazolidinon-3″-yl)-1′,3′-thiazol-4′-yl]thio}benzothiazoles (**5a – 5e**) have been synthesized from 2-[(2′-substitutedarylidenylimino-1′,3′-thiazol-4′-yl)thio]benzothiazoles (**3a – 3e**). The structure of these compounds has been elucidated by elemental (C, H, N) and spectral (IR, ^{1}H-NMR, Mass) analysis. Furthermore, compounds **3a – 3e**, **4a – 4e**, and **5a – 5e** were screened for insecticidal activity against *Periplaneta americana* and antifungal, antibacterial activities *in vitro* against different strains of fungi and bacteria. Out of the fifteen compounds tested, compound **5b**, 2-{[2′-(2″-*p*-hydroxy-*m*-methoxyphenyl)-4″-thiazolidinon-3″-yl)-1′,3′-thiazol-4′-yl]thio}benzothiazole, was found to possess most prominent insecticidal activity.

Keywords: Antibacterial activity / Antifungal activity / Insecticidal activity / Thiazolylarylidinylbenzothiozoles

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Introduction

Insects have been a great menace to human beings posing immense harm to agriculture products and food stores. From time to time, various insecticidal agents have been synthesized and used to combat this menace. Moreover and unfortunately, these insecticides have shown significant toxicity against the human beings. Fenthiaprop, flurazole, and fosthiazate derivatives of benzothiazole, thiazole, and thiazolidinone, respectively, have been used as pesticidal agents [1]. It has been found in the current literature that the benzothiazole moiety is associated with a wide spectrum of biological activities such as insecticidal [2], antimicrobial [3], herbicidal [4], etc. Furthermore, different congeners of thiazole, thiazolidinone and azetidinone have also been reported to exhibit potential biological profiles like pesticidal and insecticidal [5 – 7], antimicrobial [8 – 10]. In view of these interesting biological properties associated with above heterocyclic nuclei, we undertook studies on the synthesis of benzothiazole derivatives substituted with a thiazole ring followed by thiazolidinone and azetidinone moieties. These newly synthesized compounds have been examined for insecticidal, antifungal, and antibacterial activities.

Results and discussion

Chemistry

The synthetic routes leading to the formation of titled compounds is outlined in Scheme 1. The reaction of 2-mercaptobenzothiazole with chloroacetyl chloride yielded the desired 2-[(chloroacetyl)thio]benzothiazole **1**, which, on treatment with thiourea, afforded 2-[(2′-amino-1′,3′-thiazol-4′-yl)thio]benzothiazole **2**. Compound **2** was condensed with different aromatic aldehydes to give 2-[(2′-substitutedarylidenylimino-1′,3′-thiazol-4′-yl)thio]ben-

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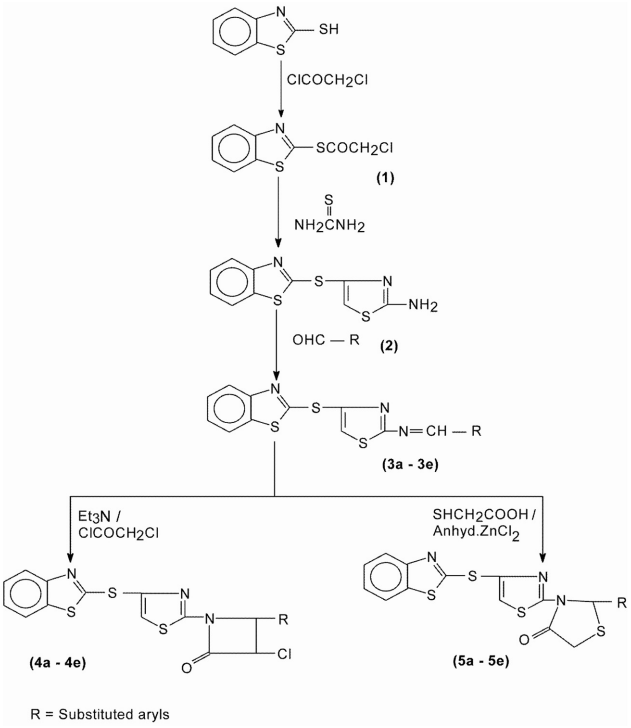

Scheme 1. Synthetic routes of title compounds.

zothiazoles, **3a–3e**. Finally, compounds **3a – 3e** were converted into their corresponding azetidinone and thiazolidinone congeners i.e. 2-{[2′-(3″-chloro-2″-oxo-4″-substitutedaryl-1″-azetidinyl)-1′,3′-thiazol-4′-yl]thio}benzothiazoles **4a – 4e** and 2-{[(2′-(2″-substitutedaryl-4″-thiazolidinon-3″-yl)-1′,3′-thiazol-4′-yl]thio}benzothiazoles **5a – 5e**, respectively, on treatment with triethylamine/chloroacetylchloride and thioglycolic acid/anhydrous $ZnCl_2$, respectively.

Biological studies

Fifteen compounds **3a – 3e**, **4a – 4e**, **5a – 5e** and the reference drug parathion, were screened for insecticidal activity against *Periplaneta americana* at a concentration of 5 g/L. The majority of compounds showed statistically significant insecticidal activity (Table 1). Compound **5b** was found to possess more potent insecticidal activity in comparison to standard drug (Table 1). Further, this compound and reference drug were also subjected to screening at two more concentrations, *i.e.* 10 g/L and 20 g/L, and this compound displayed better activity at all the tested doses than the reference used (Table 1).

In another set of experiments, the above mentioned fifteen compounds **3a – 3e, 4a – 4e**, **5a – 5e** were also examined *in vitro* for antifungal and antibacterial activities against different strains of fungi and bacteria (Table 2). Fluconazole and chloroamphenicol were used as standard drugs for the comparison of results of antifungal and antibacterial activities, respectively. The results given in Table 2 indicated that among fifteen compounds tested, only compounds **3a, 3b, 3c**, **5c**, and **5e** showed good antifungal activity (Table 2). None of the compounds possessed antifungal activity against *C. Krusei G03*. Compounds **4a – 4e** containing the azetidinone moiety displayed antibacterial activity against Gram-positive bacteria *S. aureus 209 p* and Gram-negative bacteria *E. coli ESS 2231* (Table 2).

The characteristic feature of compounds **3a – 3e** is the presence of a substituted arylidene moiety as side chain, while the characteristic feature of compounds **4a – 4e** and **5a – 5e** is the presence of azetidinone and thiazolidinone rings, respectively. Table 3 gives the physical data of compounds **3a – 3e**, **4a – 4e**, and **5a – 5e**.

Compounds **3a – 3e** exhibited mild to moderate insecticidal activity (284, 221, 415, 270.4, 343 min, respectively); cyclization of these compounds into their corresponding thiazolidinone congeners **5a – 5e** enhanced the activity (207, 147, 271, 183, 255 minutes, respectively). On the

Table 1. Insecticidal activity of compounds **3a – 3e**, **4a – 4e**, and **5a – 5e** against cockroaches (*periplaneta americana*).

Compounds	R	Concentration (g/L)	Mean killing time (min) ± S.E.M.
Control[a]		0.02 mL	720 ± 10.29
Parathion		5 g/L	280 ± 11.74[b]
		10 g/L	247 ± 9.29[b]
		20 g/L	231 ± 13.75[b]
3a	$-C_6H_5$	5 g/L	284 ± 4.20
3b	$-C_6H_3$ (m-OH, p-OCH_3)	5 g/L	221 ± 4.29[d]
3c	$-C_6H_4$(o-OH)	5 g/L	415 ± 14.57[e]
3d	$-C_6H_4$ (p-OCH_3)	5 g/L	270.4 ± 5.11
3e	$-C_6H_4$(p-OH)	5 g/L	343 ± 7.51[d]
4a	$-C_6H_5$	5 g/L	388.8 ± 6.32[e]
4b	$-C_6H_3$(m-OH, p-OCH_3)	5 g/L	312.2 ± 7.47[c]
4c	$-C_6H_4$(o-OH)	5 g/L	441 ± 7.17[d]
4d	$-C_6H_4$ (p-OCH_3)	5 g/L	402 ± 9.02[d]
4e	$-C_6H_4$(p-OH)	5 g/L	448.2 ± 12.41[d]
5a	$-C_6H_5$	5 g/L	207 ± 6.41[e]
5b	$-C_6H_3$(m-OH, p-OCH_3)	5 g/L	147 ± 9.04[e]
		10 g/L	135 ± 5.70[e]
		20 g/L	104 ± 11.78[e]
5c	$-C_6H_4$(o-OH)	5 g/L	271 ± 4.33
5d	$-C_6H_4$ (p-OCH_3)	5 g/L	183 ± 5.61[e]
5e	$-C_6H_4$(p-OH)	5 g/L	255 ± 2.51[c]

n = 5 in each group.
[a] acetone.
[b] $P < 0.001$ in comparison to control.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] $P < 0.001$ in comparison to standard.

Table 2. Antifungal and antibacterial activities of compounds **3a–3e**, **4a–4e**, and **5a–5e** by agar disc diffusion and filter paper disc methods, respectively.

Compounds	Antifungal activity[a] [Diameter of the inhibition zone (mm)]					Antibacterial activity[a] [Diameter of the inhibition zone (mm)]	
	Aspergillus fumigatus	*Candida albicans ATCC 2091*	*Candida albicans ATCC 10231*	*Candida krusei G03*	*Candida glabrata H05*	*Staphylococcus aureus 209p*	*Escherichia coli ESS 2231*
Control[b]	0	0	0	0	0	0	0
Fluconazole	0	29	25	19	15	–	–
Chloramphenicol	–	–	–	–	–	20	20
3a	17	13	13	0	10	0	0
3b	18	12	15	0	10	0	0
3c	16	13	12	0	11	0	0
3d	0	0	0	0	0	0	0
3e	17	12	11	0	10	0	0
4a	0	0	0	0	0	12	11
4b	0	0	0	0	0	15	16
4c	0	0	0	0	0	08	0
4d	0	0	0	0	0	08	10
4e	0	0	0	0	0	13	11
5a	0	0	0	0	10	0	0
5b	0	0	0	0	0	0	0
5c	16	12	11	0	10	0	0
5d	0	0	0	0	0	0	0
5e	15	11	12	0	0	0	0

[a] Concentration was 250 μg/mL.
[b] 10% DMSO in methanol; – No activity done; 0 No inhibition zone.

Table 3. Physical data of compounds **3a–3e, 4a–4e**, and **5a–5e.**

Compound N°	R	M.p. (°C)	Yield (%)	Recrystallization solvent[a]	Molecular formula
3a	$-C_6H_5$	120–122	55	C	$C_{17}H_{11}N_3S_3$
3b	$-C_6H_3$ (m-OH, p-OCH_3)	137–138	60	D	$C_{18}H_{13}N_3S_3O_2$
3c	$-C_6H_4$(o-OH)	164–165	52	B	$C_{17}H_{11}N_3S_3O$
3d	$-C_6H_4$ (p-OCH_3)	154–155	50	A	$C_{18}H_{13}N_3S_3O$
3e	$-C_6H_4$(p-OH)	145–146	45	D	$C_{17}H_{11}N_3S_3O$
4a	$-C_6H_5$	55–56	40	A	$C_{19}H_{12}N_3S_3OCl$
4b	$-C_6H_3$ (m-OH, p-OCH_3)	131–132	45	D	$C_{20}H_{14}N_3S_3O_3Cl$
4c	$-C_6H_4$(o-OH)	100–102	42	C–W	$C_{19}H_{12}N_3S_3O_2Cl$
4d	$-C_6H_4$ (p-OCH_3)	183–184	40	F	$C_{20}H_{14}N_3S_3O_2Cl$
4e	$-C_6H_4$(p-OH)	124–125	50	C–W	$C_{19}H_{12}N_3S_3O_2Cl$
5a	$-C_6H_5$	104–105	50	D	$C_{19}H_{13}N_3S_4O$
5b	$-C_6H_3$ (m-OH, p-OCH_3)	125–126	62	C	$C_{20}H_{15}N_3S_4O_3$
5c	$-C_6H_4$(o-OH)	119–120	55	C–W	$C_{19}H_{13}N_3S_4O_2$
5d	$-C_6H_4$ (p-OCH_3)	199–200	52	F	$C_{20}H_{15}N_3S_4O_2$
5e	$-C_6H_4$(p-OH)	65–66	50	D	$C_{19}H_{13}N_3S_4O_2$

[a] A: acetone; B: benzene; C: methanol; D: ethanol; W: water; F: acetic acid.

other hand, conversion of compounds **3a–3e** into compounds **4a–4e**, containing azetidinyl moiety, has not potentiated the insecticidal activity (388.8, 414.2, 312.2, 402, 442 min, respectively). Hence, the higher insecticidal activity associated with compounds **5a–5e** is just because of the presence of thiazolidinone moiety.

It is significant to note that for compounds **3e**, **4e**, and **5e**, having *p*-hydroxyphenyl as a substituent, they showed least insecticidal activity (343, 441, 255 min, respectively), whereas substitution with *p*-hydroxy-*m*-methoxyphenyl group as seen in compounds **3b**, **4b**, and **5b** exhibited maximum insecticidal activity (221, 312.2, 147 min, respectively).

Out of five thiazolidinone congeners **5a–5e**, compound **5b** was found to be the most potent compound of the series. This compound showed mortality of cock-

Table 4. Analytical data of compounds **1, 2, 3a – 3e, 4a – 4e**, and **5a – 5e.**

N°	R	Elemental analysis (%)[a]					
		C		H		N	
		Calcd.	Found	Calcd.	Found	Calcd.	Found
1	–	44.35	44.14	2.46	2.75	5.75	5.98
2	–	45.28	45.50	2.64	2.38	15.85	15.52
3a	$-C_6H_5$	57.79	57.48	3.12	3.50	11.90	11.69
3b	$-C_6H_3$ (m-OH, p-OCH_3)	54.14	54.44	3.26	3.10	10.53	10.82
3c	$-C_6H_4$(o-OH)	55.28	55.56	2.98	2.66	11.38	11.10
3d	$-C_6H_4$(p-OCH_3)	56.40	56.66	3.39	3.45	10.97	11.15
3e	$-C_6H_4$(p-OH)	55.28	55.05	2.98	3.20	11.38	11.12
4a	$-C_6H_5$	53.08	53.29	2.79	3.10	9.78	9.46
4b	$-C_6H_3$ (m-OH, p-OCH_3)	50.47	50.68	2.94	3.12	8.83	8.55
4c	$-C_6H_4$(o-OH)	51.18	51.29	2.69	2.40	9.43	9.10
4d	$-C_6H_4$(p-OCH_3)	52.23	52.36	3.05	2.89	9.14	9.35
4e	$-C_6H_4$(p-OH)	51.18	51.35	2.69	2.80	9.43	9.50
5a	$-C_6H_5$	53.40	53.48	3.04	3.22	9.84	9.60
5b	$-C_6H_3$ (m-OH, p-OCH_3)	50.74	50.49	3.17	3.35	8.88	8.49
5c	$-C_6H_4$(o-OH)	51.47	51.18	2.93	3.13	9.48	9.72
5d	$-C_6H_4$(p-OCH_3)	52.52	52.25	3.28	3.50	9.19	9.02
5e	$-C_6H_4$(p-OH)	51.47	51.25	2.93	2.75	9.48	9.62

[a] C, H, N were found within ±0.4% of theoretical values.

roaches at 147 min at 5 g/L concentration, while the standard drug showed at 280 min at same concentration.

Hence, it is concluded that (a) substituted arylidene congeners **3a – 3e** exhibited mild insecticidal activity but possessed good antifungal activity in comparison to rest of the compounds; (b) conversion of compounds **3a – 3e** into their corresponding azetidinone congeners **4a – 4e** does not seem to be beneficial for insecticidal activity; (c) conversion of compounds **3a – 3e** into their corresponding thiazolidinones **5a – 5e** enhanced the insecticidal activity; introduction of *p*-hydroxy-*m*-methoxyphenyl group increases the insecticidal activity in all the derivatives.

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Experimental

General Chemistry

All reagents and solvents were generally used as received from the commercial supplier. Reactions were routinely performed in oven-dried glassware. Melting points were determined with an electrothermal melting point apparatus, and are uncorrected. The homogeneity of all newly synthesized compounds was checked by thin layer chromatography (TLC) on silica gel-G coated plates. The eluent was a mixture of different solvents in different proportions, and spots were visualized under iodine chamber. Elemental analyses (C, H, N) of all the compounds were performed on Carlo Erba-1108 elemental analyzer (Carlo Erba, Milan, Italy), and results were found within the ±0.4% of theoretical values (Table 4). Infrared (IR) spectra (KBr) were recorded on Brucker-IFS-66 FT-IR instrument (Bruker) and wave number (ν) was recorded in cm^{-1}. ^{1}H-NMR spectra were recorded JEOL, GSX-400 FT-NMR instrument (Jeol, Tokyo, Japan) at 400 MHz in $CDCl_3$ or DMSO-d_6 unless otherwise specified, and chemical shifts (δ) are reported in ppm relative to tetramethylsilane as an internal standard. Mass spectra were determined from Mass Finnigan-Mat 8230 MS (ThermQuest).

Chemistry

*2-[(Chloroacetyl)thio]benzothiazole **1***

To a mixture of 2-mercaptobenzothiazole (0.01 mol) in methanol (60 mL), chloroacetyl chloride (0.02 mol) was added dropwise with occasional shaking for 1 h. This reaction mixture was heated at reflux for 2 h, cooled and evaporated before quenching with ice water, filtered,and finally recrystallized from ethanol to obtain compound **1**: m.p. 120 – 122°C; yield 75%; molecular formula $C_9H_6NS_2OCl$. IR (KBr) ν in cm^{-1}: 3025 (C – H aromatic), 2950 (C – H aliphatic), 1710 (C=O), 1621 (C=N), 1580 (C···C of aromatic ring), 772 (C-Cl), 675 (C-S-C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 7.50 – 7.15 (m, 4H, Ar-*H*), 4.50 (s, 2H, C*H*$_2$). MS m/z: 243 $[M]^+$.

*2-[(2′-Amino-1′,3′-thiazol-4′-yl)thio]benzothiazole **2***

A mixture of thiourea (0.02 mol) and 2-[(chloroacetyl)thio]benzothiazole (**1**, 0.02 mol) in acetone (80 mL) was refluxed for 12 h. The excess of solvent was removed by evaporation, and the resultant solid was poured in cold water, filtered, and then recrystallized from methanol. The solid obtained was washed with 2% sodium carbonate solution and then with water to liberate the base completely, dried, and recrystallized from ethanol/water to give compound **2**: m.p. 105°C; yield 68%; molecular formula

$C_{10}H_7N_3S_3$. IR (KBr) ν in cm^{-1}: 3260 (N–H), 3028 (C–H aromatic), 1615 (C=N), 1570 (C···C of aromatic ring), 1150 (C–N), 676 (C–S–C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 7.45–7.10 (m, 5H, Ar-*H*), 6.10 (s, 2H, NH_2, exchangeable with D_2O). MS m/z: 265 $[M]^+$.

2-[(2′-Substitutedarylidenylimino-1′,3′-thiazol-4′-yl)thio]benzothiazoles ***3a–3e***

To a solution of compound **2** (0.01 mol) in methanol (100 mL), proper aromatic aldehyde (0.01 mol) along with few drops of glacial acetic acid were added. This resulting mixture was refluxed for 10 h, while progress and completion of the reaction was monitored by TLC. The reaction mixture was distilled off, cooled, and then poured onto crushed ice and filtered. The solid thus separated out was recrystallized from appropriate solvent giving compound **3**. By this procedure, compounds **3a–3e** were obtained starting from benzaldehyde, *o*-hydroxy-*m*-methoxybenzaldehyde, *o*-hydroxybenzaldehyde, *p*-methoxybenzaldehyde, and *p*-hydroxybenzaldehyde, respectively. Their physical data are given in Table 3. Compound **3b**: IR (KBr) ν in cm^{-1}: 3530 (O–H), 3014 (C-H aromatic), 2966 (C–H aliphatic), 1623 (C=N), 1588 (C···C of aromatic ring), 1161 (C–N), 1093 (C–O–C), 678 (C–S–C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 11.20 (s, 1H, O*H*, exchangeable with D_2O), 7.40–6.95 (m, 8H, Ar-*H*), 8.54 (s, 1H, C*H*-Ar), 3.48 (s, 3H, OCH_3). MS m/z: 399 $[M]^+$.

2-{[2′-(3″-Chloro-2″-oxo-4″-substitutedaryl-1″-azetidinyl)-1′,3′-thiazol-4′-yl]thio}benzothiazoles ***4a–4e***

To a solution of the proper compound **3** (0.01 mol) in absolute ethanol (70 mL), chloroacetyl chloride (0.02 mol) and triethylamine (0.02 mol) were added with constant stirring. This reaction mixture was refluxed for 8 h and excess of solvent was distilled off. The precipitated product was cooled, poured in ice water, then filtered and recrystallized from appropriate solvent to give compound **4**. By this procedure compounds **4a-4e** were obtained starting from **3a–3e**, respectively. The physical data of compounds **4a–4e** are depicted in Table 3. Compound **4b**: IR (KBr) ν in cm^{-1}: 3534 (O–H), 3033 (C–H aromatic), 2930 (C–H aliphatic), 1735 (C=O), 1600 (C=N), 1575 (C···C *of aromatic ring), 1123 (C–N), 1080 (C–O–C), 670 (C–S–C).* ^{1}*H-NMR (*$CDCl_3$*)* δ *in ppm: 11.15 (s, 1H, OH, exchangeable with* D_2O*), 7.34–6.82 (m, 8H, Ar-H), 5.0 (d, J = 8.5 Hz, 1H, CH-Cl), 4.75 (d, J = 5.6 Hz, 1H, CH-Ar), 3.37 (s, 3H,* OCH_3*). MS m/z: 475* $[M]^+$.

2-{[(2′-(2″-Substitutedaryl-4″-thiazolidinon-3″-yl)-1′,3′-thiazol-4′-yl]thio}benzothiazoles ***5a–5e***

A solution of proper compound **3** (0.01 mol) and thioglycolic acid (0.01 mol) in absolute ethanol (50 mL) in the presence of anhydrous $ZnCl_2$ (2 g) was refluxed for 10 h. Excess of solvent was removed through distillation, the solid thus obtained was poured onto crushed ice, filtered, dried, and recrystallized from the appropriate solvent to yield compound **5**. By this procedure, compounds **5a–5e** were synthesized starting from **3a–3e**, respectively. Their physical data are shown in Table 3. Compound **5b**: IR (KBr) ν in cm^{-1}: 3510 (OH), 3030 (C–H aromatic), 2928 (C–H aliphatic), 1688 (C=O), 1603 (C=N), 1580 (C···C *of aromatic ring), 1120 (C–N), 676 (C–S–C).* ^{1}*H-NMR (*$CDCl_3$*)* δ *in ppm: 11.20 (ss, 1H, OH, exchangeable with* D_2O*), 7.40–6.80 (m, 8H, Ar-H), 4.85 (s, 1H, CH-Ar), 4.10 (s, 2H,* CH_2 *of thiazolidinone ring), 3.45 (s, 3H,* OCH_3*).*

The proposed mass spectral fragmentation of compound **5b** is illustrated in Scheme 2. On electron impact, compound **5b** gave the molecular ion peak $[M]^+$ at m/z 473, which was not a base peak. The molecular ion underwent disintegration via different pathways, *i.e.* I, II, and III.

Pathway I involved the fission through benzothiazole ring to give ion $[a]^+$ at m/z 108 and pathway II exhibited cleavage between the benzothiazole and thiazole rings to yield the ion $[b]^+$ with m/z 134. A similar type of fragmentation pattern of a benzothiazole ring has been reported by [11] and [12].

Pathway III possessed splitting across the thiazole ring giving the fragment $[c]^+$ at m/z 281 [13]. Fragment $[c]^+$ was further broken up via a- and b-routes (see Scheme 2) to yield $[d]^+$ and $[e]^+$ with m/z 210 and 57, respectively. Ion $[e]^+$ was found to be as base peak. Radical ion $[c]^+$ exhibited CH-S bond cleavage (β-cleavage) followed by a rearrangement through a 1,4-hydrogen shift leading to the formation of ion $[f]^+$ at m/z 208 (loss of SCHCO) [14]. By the cleavage of ion $[f]^+$ a radical ion $[g]^+$ has been observed at m/z 123. Fragment $[g]^+$ readily removed the OH radical to give the anisole radical ion $[h]^+$ at m/z 106. This radical ion, on expulsion of methyl radical (CH_3) and followed by rearrangement, gave ion $[i]^+$ at m/z 91, which was further rearranged into $[j]^+$ ion with the same m/z value. Finally, a carbonyl radical (CO) was ejected from fragment $[j]^+$ to give the cation $[k]^+$ at m/z 63 [15].

Biological evaluation

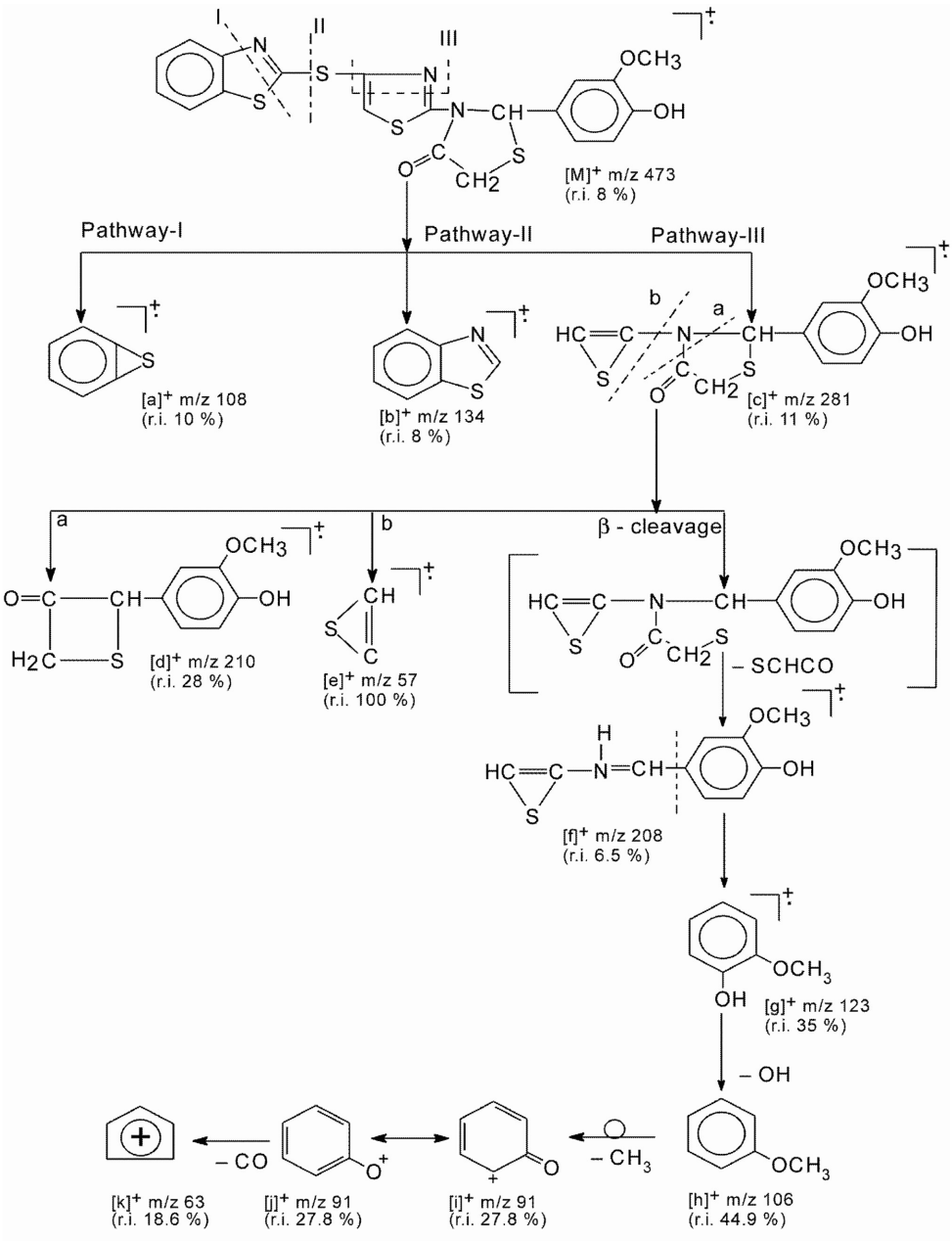
Various compounds **3a–3e**, **4a–4e**, and **5a–5e** have been evaluated for insecticidal activity against male or female cockroaches (*Periplaneta americana*). These compounds were also assayed *in vitro* for their antifungal and antibacterial activities.

Insecticidal activity

The insecticidal activity was determined by the method of Joshi and Tholia [16]. The cockroaches of either sex were divided in groups having five cockroaches each. An acetone solution (0.02 mL of 5 g/L) of standard insecticide parathion and different test compounds were injected on the ventral side of the insect, between the fourth and fifth abdominal segments with the help of a micrometer syringe. Insects receiving 0.02 mL of acetone by the same route served as control. The treated cockroaches were kept under observation to record the time taken until 100% mortality. During this period, no food was given. In another set of experiments, most active compound of each series at two graded doses, *i.e.* 0.02 mL of 10 and 20 g/L were also injected into groups of insects with identical doses of parathion. The statistical significance of the difference between the data of standard and test compounds was calculated by employing student's t-test.

Antifungal activity

The standard agar disc diffusion method [17] was performed to evaluate the antifungal property of the test compounds and standard fluconazole. *Aspergillus fumigatus, Candida albicans ATCC 2091, Candida albicans ATCC 10231, Candida Krusei G03,* and *Candida glabrata HO5* were used in this study. All cultures were routinely maintained on SDA and incubated at 300°C. In order to prepare homogeneous suspensions of these fungi for disc assays, they were grown overnight in sabouraud broth, centrifuged to collect the pellet, and resuspended in sterile phosphate buffered saline. The fungal pellet was homogenized in a sterile hand-held homogenizer. This suspension was then plated onto SDA using a bacterial spreader to obtain an even growth field.



Scheme 2. Fragmentation pattern of compound **5b**.

Sterile 6 mm Whatman filter paper discs were impregnated with 250 μg/mL concentration of various test compounds and standard drug, fluconazole. These discs were then placed in the centre of each quadrant of an SDA plate. Each plate had one control disc impregnated with 10% DMSO in methanol. The plates were incubated at 30°C. After 48 h, the plates were removed and the radius of the zone of inhibition (in mm) was measured.

Antibacterial activity

The antibacterial activity of test compounds and standard chloramphenicol was done by filter paper disc method [18] against *Staphyloccous aureus 209 p* and *Escherichia coli ESS 2231*, at a concentration of 250 μg/mL. Media with 10% DMSO in methanol was set up as control. The presence of methanol caused no visible change in the bacterial growth. The Whatman filter paper discs of standard size (7 mm) were prepared. These discs were put into 1-oz screw capped wide-mouthed containers. These bottles are then sterilized in hot-air oven at 150°C. Then, solution was added to each bottle. Before use, the bottles should be shaken to distribute the discs around the walls of the container and this allows them to be picked up more easily with the forceps. The discs are transferred to the inoculated plates with a pair of fine pointed tweezers. The tweezers may be kept with their tips

immersed in 70% alcohol, which is flamed off before use to prevent contamination. The test organisms were grown on nutrient agar and before use, were subcultured in nutrient broth at 37°C for 18–20 h. Each disc was applied carefully to the surface of the agar without lateral movement once the surface had been touched; where necessary, they were flattened down with the points of the forceps. The plates were then incubated for 24 h at 37°C, and the resulting zones of inhibition (in mm) were measured.

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