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Original article

Synthesis of some novel 2,4-disubstituted thiazoles as possible antimicrobial agents

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Abstract

A series of novel 4-aryl/chloroalkyl-2-(2,3,5-trichlorophenyl)-1,3-thiazoles (**5a–g** and **7a–e**) were synthesized by condensing 2,3,5-trichlorobenzenecarbothioamide with phenacyl bromide/dichloroacetone. 2,3,5-Trichlorobenzaldehyde thiosemicarbazone on treatment with phenacyl bromide afforded 4-aryl-2-(2,3,5-trichlorophenylidenehydrazino)-1,3-thiazoles (**10a–g**) in good yield. The newly synthesized compounds are characterized by IR, ^{1}H NMR and mass spectral studies. These compounds were also screened for their antibacterial and antifungal activities. Preliminary results reveal that some of the synthesized compounds are showing promising antimicrobial activity.

Keywords: 2,3,5-Trichlorobenzenecarbothioamide; 2,4-Disubstitued thiazole; Antibacterial; Antifungal activity

1. Introduction

Thiazoles and their derivatives are found to be associated with various biological activities such as antibacterial, antifungal and anti-inflammatory activities [1–3]. Different thiazole bearing compounds possess anti-inflammatory activities [4] and some are known to be used as pharmaceutical as well as agrochemical products [5–10]. Among them ritonavir is a well known anti-HIV drug and imidacloprid is an important insecticide.

Tripathy and Pradhan [11] have reported the preparation of *N*-thiazolyl and halothiazolyl amides of halogenated and non-halogenated salicylic and naphthoic acids and these compounds were tested for their antifungal activity against three phytopathogenic fungi of rice. One of the tested compound *N*-[4-(4-chlorophenyl)-2-thiazolyl]salicylamide effectively exhibited inhibitory activities on *Helminthosporium oryza* and *Piriicularia oryza* on rice. Prompted by these investigations and in continuation of our search for bioactive molecules [12,13], we considered the synthesis of novel 4-substituted-2-(2,3,5-trichlorophenyl)-1,3-thiazoles starting from 2,3,5-trichlorobenzenecarbothioamide, 1,3-dichloroacetone and various substituted phenacyl bromides and tested them for their antibacterial and antifungal properties.

2. Results and discussion

2.1. Chemistry

The target molecules, 4-aryl-2-(2,3,5-trichlorophenyl)-1,3-thiazoles (**5a–g**) were synthesized in good yield by the

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reaction of 2,3,5-trichlorobenzenecarbothioamide (**4**) with various phenacyl bromides in refluxing ethanol. Compound **4** was prepared by the reaction of 2,3,5-trichlorobenzamide (**3**) with phosphorous penta disulfide in tetrahydrofuran at 50 °C. Compound **3** was prepared by the reaction of 2,3,5-trichlorobenzoyl chloride with aqueous ammonia. The 4-(chloromethyl)-2-(2,3,5-trichlorophenyl)-1,3-thiazole (**6**) was synthesized in moderate yield by the condensation of 2,3,5-trichlorobenzenecarbothioamide with 1,3-dichloroacetone in refluxing methyl isobutyl ketone. The target molecules 4-substituted-2-(2,3,5-trichlorophenyl)-1,3-thiazoles (**7a–e**) were prepared by reaction of 4-(chloromethyl)-2-(2,3,5-trichlorophenyl)-1,3-thiazole (**6**) with various amines and mercaptans in the presence of a base. 4-Aryl-2-(2,3,5-trichlorophenylidenehydrazino)-1,3-thiazoles (**10a–g**) were synthesized in excellent yields by the reaction of 2,3,5-trichlorobenzaldehyde thiosemicarbazone (**9**) with various phenacyl bromides in ethanol (Table 1). The starting material **9** was synthesized by condensing 2,3,5-trichlorobenzaldehyde (**1**) with thiosemicarbazide (**8**) in the presence of concentrated sulphuric acid in ethanol.

2.2. Biological activity

2.2.1. Antibacterial activity

The investigation of antibacterial screening (Table 2) revealed that all the newly synthesized compounds showed moderate to good inhibition at 1.56–25 μg/ml in DMSO. Compounds **5b**, **5e**, **7b**, **7e**, **10a** and **10f** exhibited comparatively good activity against the four tested bacterial stains. Compounds **5f**, **5g**, and **7d** showed good activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Compounds **7c**, **10c** and **10d** showed good activity against *Staphylococcus aureus* and *Bacillus subtilis*.

2.2.2. Antifungal activity

The investigation of antifungal screening (Table 3) revealed that all the newly synthesized compounds showed moderate to good inhibition at 1.56–25 μg/ml in DMSO. Compounds **5c**, **5d**, **7d**, **10b** and **10c** exhibited good activity against all the four fungal strains. Compounds **5e**, **7e** and **7b** showed good activity against *Aspergillus flavus* and *Penicillium marneffei*. Compounds **5g**, **7b** and **10f** showed good activity against *Trichophyton mentagrophytes* and *Aspergillus fumigatus*.

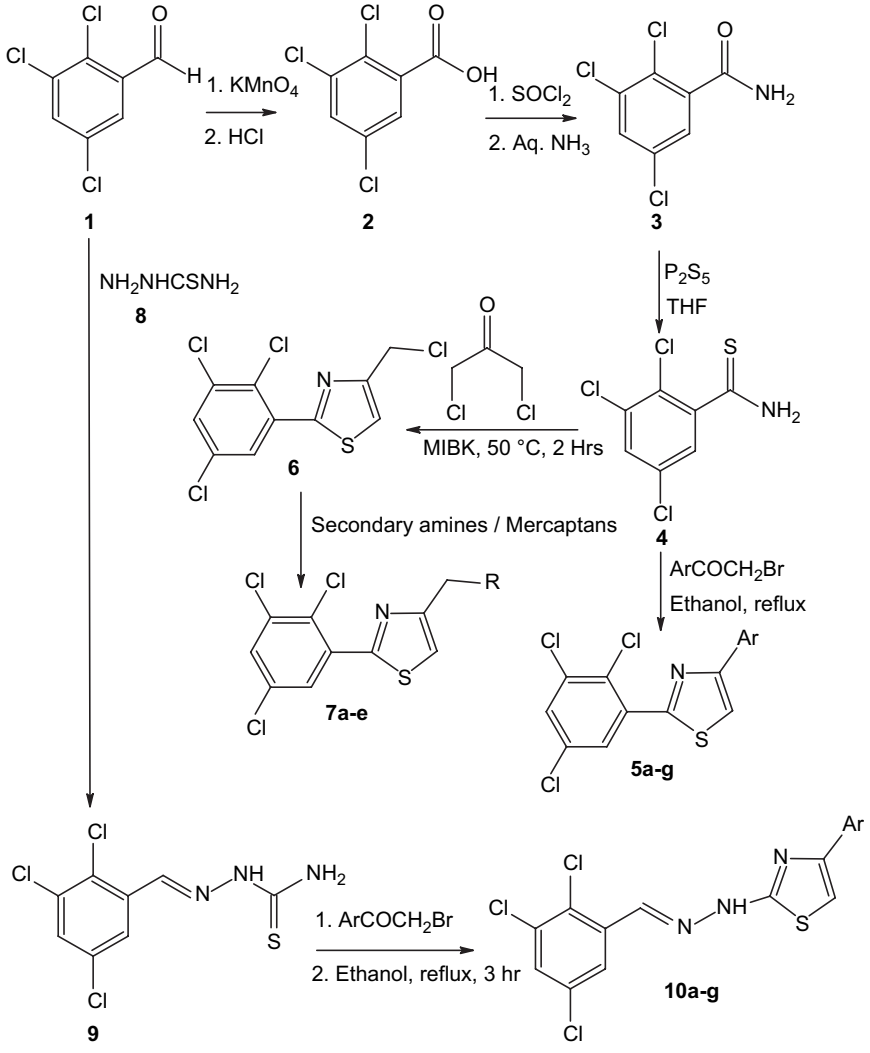

Table 1
Characterization data of 2-(2,3,5-trichloropheyl)-4-aryl-1,3-thiazoles (**5a–g**), (**7a–e**) and (**10a–g**)

Compounds	Ar/R	Molecular formula	MW	M.p. (°C)	Yield (%)	% Analysis of C, H, N found (calculated)		
						C	H	N
5a	4-OCH_3–C_6H_4	$C_{16}H_{10}Cl_3NOS$	369	95–97	80	52.00 (52.03)	2.73 (2.71)	3.80 (3.79)
5b	4-SCH_3–C_6H_4	$C_{16}H_{10}Cl_3NS_2$	385	119–121	85	49.84 (49.87)	2.58 (2.59)	3.65 (3.63)
5c	3-Pyridyl	$C_{14}H_{10}Cl_3N_2S$	344	199–200	84	48.80 (48.83)	2.93 (2.90)	8.15 (8.13)
5d	Biphenyl	$C_{21}H_{12}Cl_3NS$	415	162–164	86	60.45 (60.72)	2.86 (2.89)	3.34 (3.37)
5e	4-OH-3-$CONH_2$–C_6H_3	$C_{16}H_9Cl_3N_2O_2S$	398	210–212	82	48.04 (48.24)	2.27 (2.26)	7.05 (7.03)
5f	4-NO_2–C_6H_4	$C_{15}H_7Cl_3N_2O_2S$	384	190–192	85	46.85 (46.87)	1.80 (1.82)	7.26 (7.29)
5g	4-Cl–C_6H_4	$C_{15}H_7Cl_4NS$	373	162–164	86	48.20 (48.25)	1.85 (1.87)	3.73 (3.75)
7a	Morpholino	$C_{14}H_{13}Cl_3N_2OS$	362	104–106	81	46.42 (46.40)	3.56 (3.59)	7.71 (7.73)
7b	*N*-Methylpiperazino	$C_{15}H_{16}Cl_3N_3S$	375	280–282	85	48.03 (48.00)	3.56 (3.59)	7.71 (7.73)
7c	Piperidino	$C_{15}H_{15}Cl_3N_2S$	360	256–258	84	50.03 (50.00)	4.22 (4.26)	11.275 (11.20)
7d	4-Mercaptopyrazolopyrimidine	$C_{17}H_{10}Cl_3N_5S_2$	455	220–222	83	44.80 (44.83)	4.14 (4.16)	7.73 (7.77)
7e	4,6-$(CH_3)_2$-2-Mercaptopyrimidine	$C_{16}H_{12}Cl_3N_3S_2$	415	242–244	83	46.22 (46.26)	2.22 (2.19)	15.35 (15.38)
10a	4-OH-3-$CONH_2$–C_6H_3	$C_{17}H_{14}Cl_3N_4O_2S$	444	256–258	85	46.19 (45.94)	3.16 (3.15)	12.63 (12.61)
10b	3-Pyridyl	$C_{15}H_9Cl_3N_4S$	382	208–210	86	47.10 (47.12)	2.38 (2.35)	14.63 (14.65)
10d	4-NO_2–C_6H_4	$C_{16}H_9Cl_3N_4O_2S$	426	256–258	86	45.00 (45.02)	2.13 (2.11)	13.00 (13.14)
10e	4-NO_2–C_6H_4	$C_{16}H_9Cl_4N_3$	415	238–40	85	46.22 (46.26)	2.14 (2.16)	10.15 (10.12)
10f	4-SCH_3–C_6H_4	$C_{17}H_{12}Cl_3N_3S_2$	427	134–135	82	47.54 (47.77)	2.85 (2.81)	9.80 (9.83)
10g	4-OCH_3–C_6H_4	$C_{17}H_{12}Cl_3N_3OS$	411	228–230	84	49.60 (49.63)	2.95 (2.91)	10.19 (10.2)

3. Conclusion

We have synthesized several thiazole derivatives containing 2,3,5-trichlorophenyl moiety and studied their antibacterial and antifungal properties. In particular the thiazoles **5b** and **10f** carrying 4-(methylthio)phenyl, **5e** and **10a** carrying salicylamide, **7b** carrying *N*-methylpiparazino and **7e** carrying 4,6-dimethyl-2-mercaptopyrimidine substituents appear to exhibit the highest antibacterial activity. Also thiazoles **5c**, **10b** carrying 3-pyridyl, **5d**, **10c** carrying biphenyl and **7d** carrying 4-mercaptopyrazolopyrimidine substituents appear to exhibit the highest antifungal activity. Compounds **5e** and **10a** structurally resemble a known antifungal agent *N*-[4-(4-chlorophenyl)-2-thiazolyl]salicylamide [11].

4. Experimental

Melting points were determined by the open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a NICOLET AVATAR 330-FTIR spectrometer. PMR spectra were recorded ($CDCl_3$/DMSO-d_6) on a Bruker-AC 300F (300 MHz) NMR spectrometer using TMS as an internal standard. Chemical shift values are given in δ scales. The FAB mass spectra were recorded on a VG

Table 2
Antibacterial activity data of prepared compounds (**5a–g**), (**7a–e**) and (**10a–g**)

Compounds	MIC in μg/ml (zone of inhibition in mm)			
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
5a	6.25 (16–20)	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)
5b	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
5c	12.5 (11–15)	12.5 (11–15)	25 (<10)	12.5 (11–15)
5d	25 (<10)	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)
5e	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
5f	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	25 (<10)
5g	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	25 (<10)
7a	12.5 (11–15)	12.5 (11–15)	25 (<10)	12.5 (11–15)
7b	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7c	25 (<10)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
7d	6.25 (16–20)	25 (<10)	6.25 (16–20)	25 (<10)
7e	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
10a	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
10b	12.5 (11–15)	25 (<10)	12.5 (10–15)	12.5 (10–15)
10c	25 (<10)	6.25 (16–20)	25 (<10)	6.25 (16–20)
10d	25 (<10)	6.25 (16–20)	25 (<10)	6.25 (16–20)
10e	12.5 (11–15)	25 (<10)	25 (<10)	25 (<10)
10f	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
10g	25 (<10)	25 (<10)	25 (<10)	25 (<10)
Ciprofloxacin	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	1.56 (21)

Note: the MIC values were evaluated at concentration range 1.56–25 μg/ml. The values in the table show the MIC values and the corresponding zone of inhibition (in mm).

Table 3
Antifungal activity data of prepared compounds (**5a–g**), (**7a–e**) and (**10a–g**)

Compounds	MIC in μg/ml (zone of inhibition in mm)			
	Aspergillus flavus	*Trichophyton mentagrophytes*	*Aspergillus fumigatus*	*Penicillium marneffei*
5a	25 (<10)	25 (<10)	25 (<10)	25 (<10)
5b	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)	25 (<10)
5c	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
5d	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
5e	6.25 (16–20)	25 (<10)	25 (<10)	6.25 (16–20)
5f	12.5 (11–15)	25 (<10)	12.5 (11–15)	25 (<10)
5g	25 (<10)	6.25 (16–20)	6.25 (16–20)	25 (<10)
7a	25 (<10)	25 (<10)	25 (<10)	12.5 (11–15)
7b	25 (<10)	6.25 (16–20)	6.25 (16–20)	25 (<10)
7c	25 (<10)	25 (<10)	25 (<10)	25 (<10)
7d	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7e	6.25 (16–20)	25 (<10)	25 (<10)	6.25 (16–20)
10a	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)
10b	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
10c	6.25(16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
10d	25 (<10)	25 (<10)	25 (<10)	25 (<10)
10e	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)
10f	25 (<10)	6.25 (16–20)	6.25 (16–20)	25 (<10)
10g	25 (<10)	12.5 (11–15)	25 (<10)	25 (<10)
Ciclopiroxolamine	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)

Note: the MIC values were evaluated at concentration range 1.56–25 μg/ml. The values in the table show the MIC values and the corresponding zone of inhibition (in mm).

Micro mass spectrometer operating at 70 eV. Elemental analysis was carried out using Flash EA 1112 elementar analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F_{254}) using a mixture of ethylacetate and hexane (1:4 v/v). Commercial grade solvents and reagents were used without further purification.

*4.1. Procedure for the synthesis of 2,3,5-trichlorobenzoic acid (**2**)*

To a solution of 2,3,5-trichlorobenzaldehyde (37 g, 0.176 mol) in 30 ml water a solution of potassium permanganate (30 g, 0.191 mol) in 60 ml water was added at 70–80 °C over a period of 2 h. The reaction mass was stirred at the same temperature for 2 h. After filtration of reaction mass, the filtrate was acidified using conc. hydrochloric acid at 0–5 °C. The product obtained was filtered, washed with water and dried. The crude product was recrystallized from methanol. Yield 75%, m.p. 167–168 °C [20].

*4.2. Procedure for the synthesis of 2,3,5-trichlorobenzamide (**3**)*

In a four-necked round-bottomed flask mixture of 2,3,5-trichlorobenzoic acid (30 g, 0.133 mol) and thionyl chloride (100 ml) were refluxed for 2 h. After completion of the reaction excess thionyl chloride was distilled off under reduced pressure. To the cooled residue in acetone (50 ml) a solution of aqueous ammonia (200 ml) in acetone (50 ml) was added at 0–5 °C. The reaction temperature was maintained at 10–15 °C for 30 min, the resulting solid mass was filtered, washed with water and dried. The crude product was recrystallized from methanol. Yield 73%, m.p. 198–200 °C.

^{1}H NMR (DMSO-d_6): δ 7.54 (d, 1H, $J = 2.1$ Hz, 2,3,5-trichlorophenyl), 7.81 (s, 1H, $CONH_2$), 7.91 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 8.04 (s, 1H, $CONH_2$); FAB MS (*m/z*, %): 231 (2, M + 6), 229 (5, M + 4), 227 (4, M + 2), 225 (90, M^+), 207 (5), 165 (7), 120 (10), 107 (35), 89 (20).

*4.3. Procedure for the synthesis of 2,3,5-trichlorobenzenecarbothioamide (**4**)*

To a solution of 2,3,5-trichlorobenzamide (20 g, 0.089 mol) in tetrahydrofuran (50 ml) phosphorous penta disulfide (40 g, 0.18 mol) was slowly added at 50 °C over a period of 2 h. The resulting mass was stirred at 50–55 °C for 2 h and the reaction mass was poured into ice-cold water. The precipitated solid was filtered, washed with water and dried. The product was recrystallized from ethylacetate. Yield 74%, m.p. 218–220 °C.

^{1}H NMR (DMSO-d_6): 6.99 (s, 1H, $CSNH_2$), 7.01 (d, 1H, $J = 2.1$ Hz, 2,3,5-trichloro phenyl), 7.52 (d, 1H, $J = 2.1$ Hz, 2,3,5-trichlorophenyl), 7.72(s, 1H, $CSNH_2$); FAB MS (*m/z*, %): 425 (6, M + 6), 245 (10, M + 4), 243 (60, M + 2), 241 (95, M^+), 224 (70), 206 (30), 167 (20), 107 (35), 89 (20).

*4.4. General procedure for the synthesis of 4-substituted-2-(2,3,5-trichlorophenyl)thiazoles (**5a–g**)*

An equimolar mixture of the appropriate 2,3,5-trichlorobenzenecarbothioamide (**4**) (0.01 mol) and substituted phenacyl

bromide (0.01 mol) in methanol was refluxed for 4 h, after completion of the reaction. The mass was cooled to room temperature. The solid obtained was filtered and recrystallized from chloroform.

4.4.1. 4-(4-Methoxyphenyl)-2-(2,3,5-trichlorophenyl)-1,3-thiazole (**5a**)

IR (KBr, ν_{max} cm^{-1}): 1529 (C=C), 1598 (C=N), 1078 (C=S), 708, 830, 990 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 7.00 (d, 2H, $J = 8.4$ Hz, *p*-anisyl), 7.55 (d, 1H, $J = 2$ Hz, 2,3,5-trichlorophenyl), 7.57 (s, 1H, thiazole H), 7.92 (d, 2H, $J = 8.4$ Hz, *p*-anisyl), 8.36 (d, 1H, $J = 2$ Hz, 2,3,5-trichlorophenyl); FAB MS (*m/z*, %): 375 (5, M + 6), 373 (30, M + 4), 371 (75, M + 2), 369 (80, M^+), 273 (5), 245 (5), 165 (5), 120 (10), 107 (25), 88 (20).

4.4.2. 4-[4-(Methylthio)phenyl]-2-(2,3,5-trichlorophenyl)-1,3-thiazole (**5b**)

IR (KBr, ν_{max} cm^{-1}): 1540 (C=C), 1580 (C=N), 1068 (C=S), 707, 820, 1005 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.52 (s 3H, SCH_3), 7.12 (d, 1H, $J = 8.7$ Hz, *p*-thioanisyl), 7.52 (s, 1H, thiazole H), 7.75 (d, 1H, $J = 2$ Hz, 2,3,5-trichlorophenyl), 7.91 (d, 2H, $J = 4.8$ Hz, *p*-thioanisyl), 8.38 (d, 2H, $J = 4.8$ Hz, 2,3,5-trichlorophenyl).

4.4.3. 4-(1,1′-Biphenyl-4-yl)-2-(2,3,5-trichlorophenyl)-1,3-thiazole (**5d**)

FAB MS (*m/z*, %): 422 (10, M + 6), 420 (30, M + 4), 418 (75, M + 2), 416 (70, M^+), 273 (10), 242 (5), 210 (10), 178 (10), 165 (15), 107 (20), 89 (10), 77 (10).

4.4.4. 2-[Amino(hydroxy)methyl]-4-[2-(2,3,5-trichlorophenyl)-1,3-thiazol-4-yl]phenol (**5e**)

IR (KBr, ν_{max} cm^{-1}): 1530 (C=C), 1600 (C=N), 70 (C=S), 710, 815,1010 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 7.02 (d, 1H, $J = 8.7$ Hz, salicylamide), 8.14 (d, 1H, $J = 8.7$ Hz, salicylamide), 8.25 (s, 1H, thiazole H), 8.00 (d, 1H, $J = 2.1$ Hz, 2,3,5-trichlorophenyl), 8.03 (s, 1H, NH amide), 8.47 (s, 1H, NH amide), 8.56 (d, 1H, $J = 2.1$ Hz, 2,3,5-trichlorophenyl), 8.62 (s, 1H, $J = 8.7$, salicylamide), 13.2 (s, 1H, OH); FAB MS (*m/z*, %): 405 (10, M + 6), 403 (30, M + 4), 401 (70, M + 2), 399 (75, M^+), 391 (60), 383 (30), 329 (5), 215 (50), 176 (10), 120 (20), 107 (40), 95 (20).

4.4.5. 4-(4-Nitrophenyl)-2-(2,3,5-trichlorophenyl)-1,3-thiazole (**5f**)

FAB MS (*m/z*, %): 401 (5, M + 6), 389 (10, M + 4), 387 (40, M + 2), 385 (25, M^+), 371 (5), 273 (5), 197 (10), 165 (15), 120 (20), 107 (30), 89 (20), 77 (10).

4.4.6. 4-(4-Chlorophenyl)-2-(2,3,5-trichlorophenyl)-1,3-thiazole (**5g**)

^{1}H NMR (DMSO-d_6): δ 7.5 (s, 1H, thiazole H), 7.67 (d, 1H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 7.34 (d, 2H, $J = 4.8$ Hz, *p*-chlorophenyl), 7.36 (d, 1H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 7.83 (d, 2H, $J = 4.8$ Hz, *p*-chlorophenyl).

4.5. Procedure for the synthesis of 4-(chloromethyl)-2-(2,3,5-trichlorophenyl)-1,3-thiazole (**6**)

An equimolar mixture of the appropriate 2,3,5-trichlorobenzenecarbothioamide (**4**) (16 g, 0.066 mol) and 1,3-dichloroacetone (12.5 g, 0.099 mol) in methylisobutylketone (MIBK) was refluxed for 3 h. The reaction mass was poured into cold water, the separated organic layer was washed with water, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The obtained product was recystallized using chloroform. Yield 76%, m.p. 118–120 °C.

^{1}H NMR ($CDCl_3$): δ 4.76 (s, 2H, CH_2), 7.55 (s, 1H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 7.59 (s, 1H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 8.22 (s, 1H, thiazole H); FAB MS (*m/z*, %): 319 (4, M + 6), 317 (10, M + 4), 315 (15, M + 2), 313 (20, M^+), 281 (20), 267 (10), 224 (15), 207 (20), 167 (10), 120 (20), 107 (30).

4.6. General procedure for the synthesis of 4-substituted-2-(2,3,5-trichlorophenyl)thiazoles (**7a–e**)

An equimolar mixture of **6** (0.02 mol) and amines/mercaptans (0.02 mol) was refluxed in methylisobutylketone (MIBK) (25 ml) in presence of potassium carbonate/sodium hydroxide for 2–3 h. After completion of the reaction, the mass was poured into water; the organic layer was separated, washed with water and dried over anhydrous sodium sulphate. The organic layer was concentrated under reduced pressure and the solid obtained after cooling was filtered and recrystallized from chloroform.

4.6.1. 4-{[2-(2,3,5-Trichlorophenyl)-1,3-thiazol-4-yl]-methyl}morpholine (**7a**)

IR (KBr, ν_{max} cm^{-1}): 3260 (NH), 1525 (C=C), 1598 (C=N), 1068 (C=S), 990, 830 and 708 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.48–2.45 (t, 4H, morpholine ring protons), 3.609–3.579 (t, 4H, morpholine ring protons), 3.7 (s, 2H, CH2), 7.81 (s, 1H, thiazole H), 8.00 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 8.16 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl); FAB MS (*m/z*, %): 368 (10, M + 6), 366 (30, M + 4), 364 (80, M + 2), 362 (90, M^+), 277 (20), 155 (40), 120 (10), 107 (20).

4.6.2. 1-Methyl-4-{[2-(2,3,5-trichlorophenyl)-1,3-thiazol-4-yl]-methyl}piperazine (**7b**)

IR (KBr, ν_{max} cm^{-1}): 3360 (NH), 1527 (C=C), 1590 (C=N), 1053 (C=S), 998, 840 and 718 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 3.5 (s, 3H, N–CH_3), 2.30–2.33 (t, 4H, *N*-methylpiperidine protons), 3.63–3.60 (t, 4H, *N*-methylpiperidine protons), 3.72 (s, 2H, CH_2), 7.85 (s, 1H, thiazole H), 8.1 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 8.26 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl).

4.6.3. 1-{[2-(2,3,5-Trichlorophenyl)-1,3-thiazol-4-yl] methyl}piperidine (**7c**)

^{1}H NMR (DMSO-d_6): δ 1.80–2.43 (m, 6H, piperidine protons), 3.53–3.50 (t, 4H, piperidine protons), 3.62 (s, 2H, CH2),

7.8 (s, 1H, thiazole H), 8.0 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 8.25 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl).

4.6.4. 4,6-Dimethyl-2-({[2-(2,3,5-trichlorophenyl)-1,3-thiazol-4-yl]methyl}thio)pyrimidine (**7e**)

^{1}H NMR ($CDCl_3$): δ 2.42 (s, 6H, dimethylpyrimidine), 4.62 (s, 2H, CH_2), 6.72 (s, 1H, dimethylpyrimidine ring proton), 7.48 (s, 1H, thiazole H), 7.52 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 8.21 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl); FAB MS (*m/z*, %): 421 (3, M + 6), 419 (10, M + 4), 417 (30, M + 2), 415 (40, M^+), 401 (20), 355 (5), 327 (20), 278 (50), 267 (30), 221 (20), 207 (25) 147 (60), 107 (10), 95(10).

4.7. Procedure for the synthesis of 2,3,5-trichlorobezaldehyde thiasemicarbazone (**9**)

An equimolar mixture of 2,3,5-trichlorobenzaldehyde (30 g, 0.143 mol) and thiosemicarbazide (15 g, 0.164 mol) in methanol (25 ml) and catalytic amount of concentrated sulphuric acid was refluxed for 3 h. The solid separated was filtered, dried and recrystallized using methanol/DMF mixture. Yield 77%, m.p. 260–262 °C.

FAB MS (*m/z*, %): 279 (6, M + 6), 275 (12, M + 4), 273 (30, M + 2), 271 (5, M^+), 242 (15), 209 (20), 176 (10), 165 (40), 120 (70), 107 (90), 89 (75).

4.8. General procedure for the synthesis of 4-substituted-2-(2,3,5-trichlorophenyl)thiazoles (**10a–g**)

An equimolar mixture of 2,3,5-trichlorobezaldehyde thiasemicarbazone (**9**) (0.01 mol) and substituted phenacyl bromide (0.01 mol) in methanol was refluxed for 4 h. After completion of the reaction, the resulting reaction mixture was allowed to cool. The solid thus separated was collected by filtration and recrystallized using chloroform.

4.8.1. 2-[Amino(hydroxy)methyl]-4-{2-[(*E*)-(2,3,5-trichlorobenzyl)diazenyl]-1,3-thiazol-4-yl}phenol (**10a**)

IR (KBr, ν_{max} cm^{-1}): 1529 (C=C), 1598 (C=N), 1078 (C=S), 708.830.990 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 7.82 (d, 1H, $J = 8.3$ Hz, salicylamide), 7.84 (s, 1H, thiazole H), 7.86 (d, 1H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 7.87 (s, 1H, salicylamide), 7.92 (d, 1H, $J = 8.7$ Hz, salicylamide), 7.95 (s, 1H, NH amide), 8.34 (d, 1H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 8.36 (s, 1H, NH), 8.48 (s, 1H, NH amide), 12.62 (s, 1H, –N=CH), 12.95 (s, 1H, OH); FAB MS (*m/z*, %): 450 (40, M + 6), 448 (5, M + 4), 446 (20, M + 2), 444 (10, M^+), 400 (50), 391(30), 354 (20), 324 (30), 281 (50), 232 (20), 207 (30), 176 (60), 147 (80), 120 (60), 107 (80), 95 (40).

4.8.2. 4-(4-Nitrophenyl)-2-[(*E*)-(2,3,5-trichlorobenzyl)diazenyl]-1,3-thiazole (**10d**)

FAB MS (*m/z*, %): 434 (2, M + 6), 432 (5, M + 4), 430 (10, M + 2), 428 (20, M^+), 317 (20), 397 (20), 377 (20), 366 (20), 355 (30), 307 (10), 279 (10), 197 (20), 176 (40), 165 (40), 115 (30), 107 (50), 97 (50), 77 (40), 75 (5).

4.8.3. 4-(4-Chlorophenyl)-2-[(*E*)-(2,3,5-trichlorobenzyl)diazenyl]-1,3-thiazole (**10e**)

^{1}H NMR (DMSO-d_6): δ 7.46 (d, 2H, $J = 4.8$ Hz, *p*-chlorophenyl), δ 7.58 (d, 2H, $J = 4.8$ Hz, *p*-chlorophenyl), 7.69 (d, 1H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 7.57 (s, 1H, thiazole H), 8.12 (d, 1H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 8.23 (s, 1H, NH), 11.88 (s, 1H, –N=CH); FAB MS (*m/z*, %): 421 (7, M + 6), 419 (15, M + 4), 417 (40, M + 2), 415 (60, M^+), 391 (40), 374 (10), 273 (10), 210 (25), 165 (10), 120 (20), 107 (40).

4.8.4. 4-[4-(Methylthio)phenyl]-2-[(*E*)-(2,3,5-trichlorobenzyl)-diazenyl]-1,3-thiazole (**10f**)

^{1}H NMR (DMSO-d_6): δ 2.52 (s, 3H, SCH_3), 7.23 (d, 1H, $J = 4.8$ Hz, *p*-thioanisyl), 7.71 (s, 1H, thiazole H), 7.61 (d, 1H, $J = 2.1$ Hz, 2,3,5-trichlorophenyl), 7.91 (d, 2H, $J = 4.8$ Hz, *p*-thioanisyl), 8.12 (d, 2H, $J = 2.2$ Hz, 2,3,5-trichlorophenyl), 7.95 (s, 1H, NH), 11.91 (s, 1H, –N=CH).

4.8.5. 4-(4-Methoxyphenyl)-2-[(*E*)-(2,3,5-trichlorobenzyl)-diazenyl]-1,3-thiazole (**10g**)

^{1}H NMR (DMSO-d_6): δ 3.86 (s, 3H, OCH_3), 7.02 (d, 2H, $J = 8.4$ Hz, *p*-anisyl), 7.58 (d, 1H, $J = 2.1$ Hz, 2,3,5-trichlorophenyl), 7.6 (s, 1H, thiazole H), 7.93 (d, 2H, $J = 8.4$ Hz, *p*-anisyl), 8.38 (d, 1H, $J = 2.1$ Hz, 2,3,5-trichlorophenyl), 7.95 (s, 1H, NH), 11.91 (s, 1H, –N=CH).

4.9. Experimental procedure for antibacterial testing

The newly synthesized compounds were screened for their antibacterial activity against *E. coli* (ATTC-25922), *S. aureus* (ATTC-25923), *P. aeruginosa* (ATTC-27853) and *B. subtilis* (recultured) bacterial stains by serial plate dilution method [14,15]. Serial dilutions of the drug in Muller–Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16–18 h at 37 °C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial discs are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and the dishes were placed in an incubator at 37 °C for 1 h. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each well labeled. A control was also prepared for the plates in the same way using DMSO. The petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard [18,19]. Zones of inhibition were determined for (**5a–g**), (**7a–e**) and (**10a–g**) and the results of such studies are summarized in Table 2.

4.10. Experimental procedure for antifungal testing

Newly synthesized compounds were screened for their antifungal activity against *A. flavus* (NCIM No.524), *T. mentagrophytes* (recultured), *A. fumigatus* (NCIM No.902) and *P. marneffei* (recultured) in DMSO by serial plate dilution method [16,17]. Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each well labeled. A control was also prepared in the same way using solvent DMSO. The petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with ciclopiroxolamine as the standard. Zones of inhibition were determined for (**5a–g**), (**7a–e**) and (**10a–g**) and the results are summarized in Table 3.

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