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Original article

Synthesis, antimicrobial and anti-inflammatory activities of some 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles and 1,2,4-triazolo [3,4-*b*][1,3,4]thiadiazines bearing trichlorophenyl moiety

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Abstract

The reaction of 2,3,5-trichlorobenzoic acid hydrazide with carbon disulfide and potassium hydroxide followed by treatment with hydrazine hydrate afforded 3-(2,3,5-trichlorophenyl)-4-amino-1,2,4-triazole-5-thione (**6**). Alternatively, this triazole was also synthesized by fusing 2,3,5-trichlorobenzoic acid with thiocarbohydrazide. Condensation of (**6**) with various aromatic carboxylic acids in the presence of phosphorous oxychloride or with phenacyl bromides afforded two series of fused heterocycles namely 6-(substituted aryl)-3-(2,3,5-trichlorophenyl)-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadizoles (**7**) and 6-(substituted aryl)-3-(2,3,5-trichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**8**), respectively. The structures of these newly synthesized compounds are characterised by elemental analysis, IR, ^{1}H NMR and mass spectroscopic studies. All the synthesized compounds were screened for their antimicrobial and anti-inflammatory activities. Some of the compounds exhibited promising antimicrobial and anti-inflammatory activities.

Keywords: Trichlorophenyl moiety; Triazolothiadiazoles; Triazolothiadiazines; Anti-inflammatory; Antimicrobial screening

1. Introduction

In the last few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. 1,2,4-Triazole moieties have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatories, CNS stimulants, sedatives, anti-anxiety compounds, antimicrobial agents [1–3] and antimycotic ones such as fluconazole, intraconazole, voriconazole [4,5]. There are marketed drugs containing the 1,2,4-triazole group, e.g., Triazolam [6], Alprazolam [7], Etizolam [8], and Furacylin [9]. Moreover, sulphur containing heterocycles represent an important group of sulphur compounds that are promising for use in practical applications.

Among these heterocycles, the mercapto- and thione-substituted 1,2,4-triazole ring systems (A and B, Fig. 1) have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives, such as antibacterial [10–15], antifungal [16,17], antitubercular [18], antimycobacterial [19], anticancer [20,21], diuretic [22,23], and hypoglycemic [24] properties. In addition to these important biological applications, mercapto-1,2,4-triazoles are also of great utility in preparative organic chemistry, for example, in the presence of various reagents, undergo different types of reactions to yield other heterocyclic compounds, e.g., thiazolotriazoles, triazolothiadiazoles, triazolothiazines, triazolothiazepines and triazolothiadiazines. In this paper the most common and useful procedures for the

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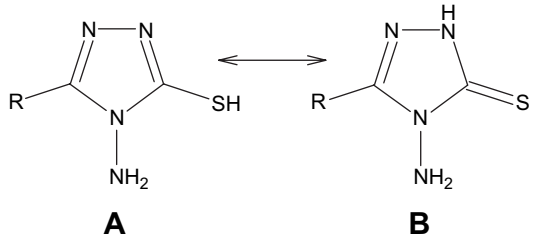


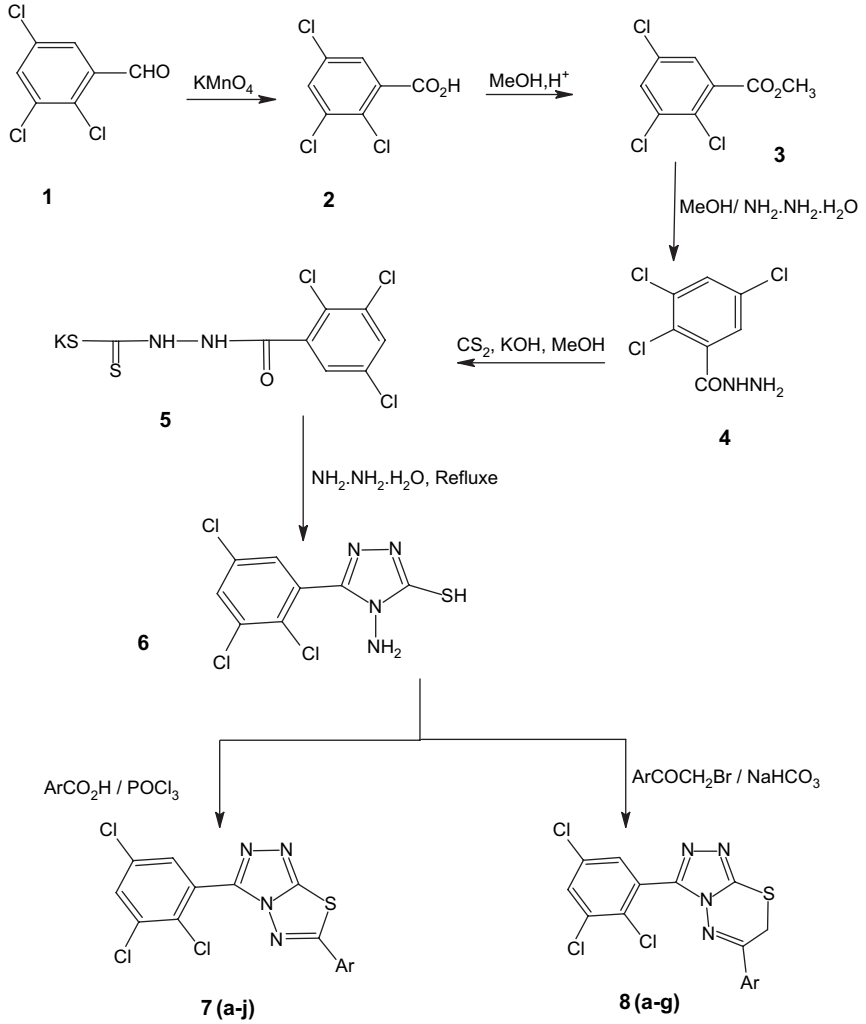
Fig. 1.

preparation of the 4-amino-5-substituted-4*H*-[1,2,4]triazole-3-thiol and their utility for the synthesis of well known heterocyclic ring systems namely triazolothiadiazoles and triazolothiazines are compiled and evaluation of their anti-microbial and anti-inflammatory activities are discussed.

A recent literature survey reveals that many of the halogen containing heterocycles have attracted attention due to the ability of halogen to act as polar hydrogen or hydroxy mimic. Substitution of hydrogen by halogen has been a strategy in designing molecules for biological studies [25]. Therefore it was envisaged that chemical entities with both 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles and 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines containing 2,3,5-trichlorophenyl moiety would result in compounds with enhanced biological activities. Prompted by these investigations and in continuation of our research work on the synthesis of novel heterocyclic compounds exhibiting biological activity, it was thought to be interesting to synthesize compounds containing the features namely, having 1,2,4-triazole moiety fused with the 1,3,4-thiadiazole/1,3,4-thiadiazine rings.

2. Chemistry

Reaction sequence employed for the synthesis of title compounds is shown in Scheme 1. The starting material 2,3,5-trichlorobenzaldehyde (**1**) was converted into 2,3,5-trichlorobenzoic acid (**2**) by oxidation with $KMnO_4$ and then it is esterified with methanol in the presence of acid to get methyl-2,3,5-trichlorobenzoate (**3**). 2,3,5-Trichlorophenyl carbohydrazide (**4**) was obtained by refluxing (**3**) with hydrazine hydrate in methanol. The compound (**4**) on reaction with carbon disulphide in methanolic potassium hydroxide yielded



Scheme 1. Synthesis of substituted Triazolothiadiazoles and Triazolothiadiazines.

corresponding dithiocarbazinate (**5**) in good yield and was directly used for the next step without further purification. The triazole (**6**) was synthesized by refluxing (**5**) with hydrazine hydrate. Condensation of (**6**) with various aromatic carboxylic acids in phosphorous oxychloride under reflux condition yielded, 6-(substituted aryl)-3-(2,3,5-trichlorophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**7a–j**) and with various phenacyl bromides in ethanol under reflux condition gave 6-(substituted aryl)-3-(2,3,5-trichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**8a–g**) in good yield. Assignment of new compounds was based on their elemental analysis and spectroscopic data. Characterization data of all new compounds are summarized in Table 1.

3. Biological activities

3.1. Antibacterial

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (recultured) bacterial strains by serial plate dilution method [26,27]. Serial dilutions of the drug in Muller–Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. 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 observing 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. Agar media were poured into each Petri dish. Excess of suspension was decanted and placing in incubator at 37 °C for 1 h dried the plates. 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 labelled well. A control was also prepared for the plates in the same way using solvent 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 [28,29]. Zone of inhibition was determined for (**7a–j**) and (**8a–g**) and the results are summarized in Table 2.

3.2. Antifungal

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* [NCIM No.524], *Aspergillus fumigatus* [NCIM No. 902], *Penicillium marneffei* [recultured] and *Trichophyton mentagrophytes* [recultured] in DMSO by serial plate dilution method [30,31]. Agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Agar media of 20 mL were poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch each labelled well were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labelled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dish 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 standard. Zones of inhibition were determined for (**7a–j**) and (**8a–g**) and the results are summarized in Table 3.

Table 1
Characterization data of substituted-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadizoles (**7a–j**) and substituted-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**8a–g**)

Compd.	Ar	Molecular formula (MW)	MP (°C)	Yield (%)	% Analysis of C, H, N found (calculated)		
					C	H	N
7a	4-Methylphenyl	$C_{16}H_9Cl_3N_4S$ (394)	182–184	80	48.70 (48.73)	2.24 (2.28)	14.20 (14.21)
7b	4-Methoxyphenyl	$C_{16}H_9Cl_3N_4OS$ (410)	180–182	85	46.71 (46.82)	2.14 (2.19)	13.15 (13.65)
7c	3,5-dichlorophenyl	$C_{15}H_5Cl_5N_4S$ (448)	232–233	84	40.13 (40.17)	1.09 (1.11)	12.47 (12.50)
7d	3,5-Dimethylphenyl	$C_{17}H_{11}Cl_3N_4S$ (408)	243–245	86	49.96 (50.00)	2.62 (2.69)	13.68 (13.72)
7e	Phenoxymethyl	$C_{16}H_9Cl_3N_4OS$ (410)	220–222	82	46.12 (46.82)	2.10 (2.19)	13.50 (13.65)
7f	5-Quinolyl	$C_{18}H_8Cl_3N_5S$ (431)	236–238	85	50.00 (50.11)	1.80 (1.85)	16.21 (16.29)
7g	Phenyl	$C_{15}H_7Cl_3N_4S$ (380)	255–257	86	47.26 (47.36)	1.82 (1.84)	14.62 (14.73)
7h	Pyridyl	$C_{14}H_6Cl_3N_5S$ (381)	267–269	81	44.00 (44.09)	2.28 (2.26)	14.85 (14.96)
7i	5-Isoquinolyl	$C_{18}H_8Cl_3N_5S$ (431)	280–282	85	50.09 (50.11)	1.82 (1.85)	16.23 (16.24)
7j	2-Bromopyridyl	$C_{14}H_5BrCl_3N_5S$ (460)	256–258	84	36.11 (36.52)	1.04 (1.08)	15.08 (15.21)
8a	Biphenyl	$C_{22}H_{13}Cl_3N_4S$ (470)	220–222	83	56.10 (56.17)	2.71 (2.76)	11.08 (11.91)
8b	4-Hydroxy-3-benzamide-1-yl	$C_{17}H_{10}Cl_3N_5O_2S$ (453)	220–222	85	45.00 (45.03)	2.01 (2.20)	15.40 (15.45)
8c	4-Chlorophenyl	$C_{16}H_8Cl_4N_4S$ (428)	242–244	86	44.75 (44.85)	1.80 (1.86)	13.02 (13.08)
8d	4-Methylthiophenyl	$C_{17}H_{11}Cl_3N_4S_2$ (440)	248–250	84	46.25 (46.36)	2.4 (2.5)	12.64 (12.72)
8e	Phenyl	$C_{16}H_9Cl_3N_4S$ (394)	256–258	86	48.73 (48.73)	2.20 (2.28)	14.28 (14.21)
8f	4-Methoxyphenyl	$C_{17}H_{11}Cl_3N_4S$ (424)	252–254	85	48.11 (48.00)	2.59 (2.51)	14.28 (14.26)
8g	4-Methylphenyl	$C_{17}H_{11}Cl_3N_4S$ (408)	240–242	82	49.12 (50.00)	2.50 (2.61)	13.60 (13.72)

Table 2
Antibacterial activity of compounds (**7a–j**) and (**8a–g**)

Compd.	MIC in μg mL^{-1} and zone of inhibition (mm)			
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
7a	12 (11–15)	6.25 (16–20)	6.25 (16–20)	12 (11–15)
7b	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7c	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7d	6.25 (16–20)	12 (11–15)	6.25 (16–20)	12 (11–15)
7e	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7f	6.25 (16–20)	6.25 (16–20)	12 (11–15))	6.25 (16–20)
7g	6.25 (16–20)	12 (11–15))	6.25 (16–20)	6.25 (16–20)
7h	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7i	12 (11–15)	25 (<10)	25 (<10)	12 (11–15)
7j	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8a	12 (11–15)	6.25 (16–20)	12 (11–15)	25 (<10)
8b	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8c	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8d	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8e	12 (11–15)	6.25 (16–20)	6.25 (16–20)	12 (11–15)
8f	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8g	6.25 (16–20)	12 (11–15)	12 (11–15)	6.25 (16–20)
Ciprofloxacin	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	1.56 (22–30)

Note: the MIC values were evaluated at concentration range, 1.56–25 μg mL^{-1}.

3.3. Anti-inflammatory activity

The 6-substituted-3-(2,3,5-trichlorophenyl)-[1,2,4]triazolo [3,4-*b*][1,3,4]thiadizoles and 6-substituted-3-(2,3,5-trichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines were also screened for their anti-inflammatory activity. The screening was conducted in acute inflammatory model. Carrageenan induced rat paw oedema method [32] was used. Carrageenan (an irritant) at a concentration of 1 mg mL^{-1} was injected subcutaneously into the hind paw of the rat to produce oedema. Different groups of animals were administered with standard drug Indomethacin, the test samples and the vehicle used for the preparation of samples. The increase in the paw volume was measured before and after 3 h of administration and the results were compared. Fifty-four healthy albino rats of body weight 180–250 g were selected and made into nine groups of six animals each. All the animals were kept on fasting for 18 h. One group of animals was kept as control, which received 2% w/v acacia mucilage, which was used to suspend the sample. Another group received the standard drug Indomethacin 1.5 mg kg^{-1} body weight intraperitonially. Remaining seven groups of animals received seven different test compounds (1.5 mg kg^{-1} body weight) intraperitonially. After 30 min, 0.1 mL of w/v carrageenan was injected subcutaneously into the right hind paw of the rats. A mark was made at the right hind paw, which was dipped in the plethismograph up to the

Table 3
Antifungal activity data of compounds (**7a–j**) and (**8a–g**)

Compd.	MIC in μg mL^{-1} and zone of inhibition (mm)			
	P. marneffei	*T. mentagrophytes*	*A. flavus*	*A. Fumigatus*
7a	6.25 (16–20)	12 (11–15)	12 (11–15)	6.25 (16–20)
7b	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.5 (16–20)
7c	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7d	12 (11–15)	6.25 (16–20)	6.25 (16–20)	12 (11–15)
7e	6.25 (16–20)	12 (11–15)	12 (11–15)	6.25 (16–20)
7f	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7g	12 (11–15)	6.25 (16–20)	6.25 (16–20)	12 (11–15)
7h	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
7i	6.25 (16–20)	25 (<10)	25 (<10)	6.25 (16–20)
7j	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8a	12 (11–15)	6.25 (16–20)	12 (11–15)	12 (11–15)
8b	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8c	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8d	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8e	12 (11–15)	12 (11–15)	12 (11–15)	6.25 (16–20)
8f	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
8g	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
Ciclopiroxolamine	6.25 (16–20)	3.125 (27–33)	3.125 (25–30)	6.25 (16–20)

Note: the MIC values were evaluated at concentration range, 1.56–25 μg mL^{-1}.

mark and the volume was measured immediately and after 3 h. The change in paw volume was compared with that in the vehicle treated control animals. The percentage inhibition of oedema was calculated using the formula,

$$\%\ \text{Oedema inhibition} = 100 - (V_{\text{test}}/V_{\text{control}}) \times 100.$$

The percentage of inhibition was compared with that of the standard drug Indomethacin. Interestingly five compounds **7a**, **7b**, **7i** and **8c** carrying 4-methylphenyl, 4-methoxyphenyl, isoquinolyl and 4-chlorophenyl substituents exhibited good anti-inflammatory activity against Indomethacin (Table 4).

4. Results and discussion

The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **7a–j** and **8a–g** showed moderate to good inhibition at 1.56–25 μg mL^{-1} in DMSO. The compounds **7b**, **7c**, **7e**, **7h**, **7j**, **8b**, **8c**, **8d**, **8f** and **8g** showed comparatively good activity against all the bacterial strains. The good activity is attributed to the presence of pharmacologically active $-OCH_3$, 2,3-dichloro, 4-hydoxy-3-amide, 4-chloro, SCH_3 groups attached to phenyl ring, pyridyl, bromopyridyl and aryloxy groups of the thiadiazole and thiadiazine ring. The compounds **7b**, **7c**, **7f**, **7h**, **7j**, **8a**, **8b**, **8c**, **8d** and **8f** showed comparatively good activity against all the fungal strains. The good activity is attributed to the presence of pharmacologically active $-CH_3$, OCH_3, 2,3-dichloro, 4-hydoxy-3-amide, 4-chloro, SCH_3, phenyl groups attached to phenyl ring, pyridyl, and bromopyridyl groups of the thiadiazole and thiadiazine ring. It has been observed that the thiadiazole derivatives are found to be more active than thiadiazines.

5. Conclusions

The research study reports the successful synthesis and antimicrobial activity of new 1,2,4-triazolothiadiazoles and 1,2,4-triazolothiadiazines bearing 2,3,5-trichlorophenyl moiety. The antimicrobial activity study revealed that all the compounds tested showed moderate to good antibacterial and antifungal activities against pathogenic strains. Structure and biological activity relationship of title compounds showed that presence 2,3,5-trichloro groups and biologically active groups like $-OCH_3$, 2,3-dichloro, 4-hydoxy-3-amido, 4-chloro, SCH_3 groups attached to phenyl ring, pyridyl, and bromopyridyl groups attached to the thiadiazole and thiadiazine ring of the title compounds are responsible for good antimicrobial activity. In case of anti-inflammatory activity, compound **8c** (6-(4-chlorophenyl)-3-(2,3,5-trichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine) showed good activity among the tested compounds. Compound **8c** structurally resembles a known anti-inflammatory agent sodium dichlofenic [33] and these tested compounds showed moderate anti-inflammatory activity.

Table 4

Compd.	Paw oedema volume Mean + S.E. (mL)	Percentage inhibition of paw oedema	Dose (mg kg^{-1} p.o)
2% Gumacacia (control)	0.62 ± 0.029		10 mL kg^{-1}
Indomethacin	0.25 ± 0.012	59.67	1.5
7a	0.48 ± 0.012	22.58	50
7b	0.46 ± 0.19	25.8	50
7f	0.52 ± 0.178	9.67	50
7g	0.55 ± 0.015	16.12	50
7h	0.55 ± 0.013	11.29	50
7i	0.48 ± 0.013	22.58	50
7j	0.50 ± 0.019	19.35	50
8c	0.56 ± 0.019	30.64	50
8d	0.56 ± 0.020	9.67	50
8f	0.50 ± 0.019	19.35	50
8g	0.50 ± 0.018	19.35	50

6. Experimental protocols

Melting points were determined in open capillaries and are uncorrected [melting point apparatus: SERWELL Instruments Inc., India]. Purity of the compounds was checked by thin layer chromatography (TLC) on a silica coated aluminum sheet (silica gel $60F_{254}$) using chloroform and methanol (9:1, v/v). IR spectra were recorded on NICOLET AVATAR 330-FTIR Spectrometer. ^{1}H NMR spectra recorded on a Varian 300 MHz NMR spectrometer using TMS as an internal standard. Chemical shifts are reported in parts per million (δ) and signals are described as singlet (s), doublet (d), triplet (t), quartet (q), broad (br) and multiplet (m). The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 spectrophotometer/Data system using argon/xenon (6 kV, 10 mA) FAB gas, at 70 eV. Elemental analysis was carried out using Flash EA 1112 Series, CHNSO Analyzer (Thermo). Solvents and reagents were purchased from the commercial venders in the appropriate grade and were used without purification.

*6.1. 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 same temperature for 2 h and filtered. 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%, mp 167–168 °C [34].

*6.2. Methyl-2,3,5-trichlorobenzoate (**3**)*

To a solution of 2,3,5-trichlorobenzoic acid (**2**) (30 g) in methanol (150 mL). Conc. sulphuric acid was added slowly at 0–5 °C over a period of 30 min and refluxed for 2 h. After quenching into cold water, precipitated solid was filtered, washed with water and dried. It has been judged to be pure (95–97%) by TLC. Yield 85%, mp 72–74 °C.

IR (KBr, cm^{-1}): 3038 (aromatic C–H), 1612 (aromatic C=C), 1736 ($-CO_2CH_3$), 878 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 3.82 (s, 3H, $-OCOCH_3$), 7.72 (d, 1H, $J = 2.4$ Hz,

2,3,5-trichlorophenyl), 8.22 (d, 1H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl).

6.3. 2,3,5-Trichlorophenylcarbohydrazide (**4**)

A mixture of methyl-2,3,5-trichlorobenzoate (**3**) (1 mmol) and hydrazine hydrate (2 mmol) in 10 mL of methanol was heated under reflux for 5–6 h and after cooling solid obtained was collected by filtration. It was washed with methanol, dried and recrystallized from methanol. Yield 90%, mp 196–198 °C.

IR (KBr, cm^{-1}): 3310, 3246 ($-NH_2$), 3038 (aromatic C–H), 1680 (>C=O), 1544 (>C=C<), 870 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 7.34 (d, 1H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl), 7.51 (d, 1H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl).

6.4. 4-Amino-5-(2,3,5-trichlorophenyl)-4H-[1,2,4]-triazole-3-thiol (**6**)

2,3,5-Trichlorophenylcarbohydrazide (**4**) (20 g, 0.083 mol) was treated with a solution of potassium hydroxide (6.4 g, 0.125 mol) dissolved in methanol (50 mL) at 0–5 °C under stirring. Then (9.6 g, 0.125 mol) carbon disulfide was added slowly and the reaction mixture was stirred overnight at room temperature. The solid product of potassium dithiocarbazinate (**5**) was filtered, washed with chilled methanol and dried. It was directly used for next step without purification. The above potassium dithiocarbazinate (**5**) was taken in water (8 mL) and hydrazine hydrate (2 mmol) and refluxed for 4–5 h. During progress of the reaction, the reaction mixture turned to green with evolution of hydrogen sulphide and finally it became homogeneous. It was then diluted with little cold water and acidified with conc. hydrochloric acid. The white precipitate was filtered, washed with cold water and recrystallized from aqueous methanol. Yield 85%, mp 200–201 °C (methanol).

IR (cm^{-1}): 3313 (NH_2), 2667 (SH), 1617 (C=N), 964 (N–C=S); ^{1}H NMR (DMSO-d_6): δ 7.78 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 8.07 (d, 1H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl), 14.11 (s, 1H, SH); FAB MS (*m/z*, %): 295 (M^+, 100), 296 (M + 1, 50), 297 (M + 2, 95), 299 (M + 4, 40), 301 (M + 6, 5), 289 (45), 263 (15), 20 (7), 166 (4), 107 (20).

6.5. General procedure for the preparation of 6-aryl-3-(2,3,5-trichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**7a–j**)

A mixture 4-amino-5-(2,3,5-trichlorophenyl)-4*H*-[1,2,4] triazole-3-thiol (**6**) (1 mmol) and substituted benzoic acid (1.1 mmol) in $POCl_3$ (5 mL) was refluxed for 6–7 h. The reaction mixture was slowly poured into crushed ice with stirring and neutralized with solid sodium bicarbonate. Solid material was filtered, washed with cold water, dried, and recrystallized from chloroform.

6.5.1. 6-(4-Methylphenyl)-3-(2,3,5-trichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (**7a**)

IR (KBr, cm^{-1}): 2873 (C–H of CH_3), 3042 (aromatic C–H), 1623 (C=N), 887 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 7.34 (d, 2H, $J = 8.1$ Hz, 4-methylphenyl), 7.65 (d, 2H, $J = 8.1$ Hz, 4-methylphenyl), 7.75 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 7.85 (d, 1H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl); FAB MS (*m/z*, %): 394 (M^+, 10), 395 (M + 1, 10), 396 (M + 2, 28), 398 (M + 4, 28), 400 (M + 6, 6).

6.5.2. 6-(4-Methoxyphenyl)-3-(2,3,5-trichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (**7b**)

IR (KBr, cm^{-1}): 3055 (aromatic C–H), 1616 (C=N), 890 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 7.20 (d, 2H, $J = 8.7$ Hz, 4-methoxyphenyl), 7.82 (d, 2H, $J = 8.7$ Hz, 4-methoxyphenyl), 7.81 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 8.10 (d, 2H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl); FAB MS (*m/z*, %): 394 (M^+, 10), 395 (M + 1, 10), 396 (M + 2, 28), 398 (M + 4, 28), 400 (M + 6, 6).

6.5.3. 6-(3,5-Dichlorophenyl)-3-(2,3,5-trichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7c**)

IR (KBr, cm^{-1}): 3075 (aromatic C–H), 1616 (C=N), 894 (C–Cl); FAB MS (*m/z*, %): 450 (M^+, 5), 451 (M + 1, 60), 452 (M + 2, 10), 454 (M + 4, 7), 456 (M + 6, 10), 417 (10), 289 (15), 165 (5), 155 (20), 120 (5), 107 (20).

6.5.4. 6-(3,5-Methylphenyl)-3-(2,3,5-trichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (**7d**)

IR (KBr, cm^{-1}): 3019 (aromatic C–H), 1640 (C=N), 893 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 6H, CH_3), 7.26 (s, 1H, 2,6-dimethylphenyl), 7.48 (s, 1H, 2,6-dimethylphenyl), 7.49 (d, 1H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl), 7.7 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl).

6.5.5. 6-(Phenoxymethyl)-3-(2,3,5-trichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (**7e**)

IR (KBr, cm^{-1}): 3066 (aromatic C–H), 1632 (C=N), 872 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 5.21 (s, 2H, OCH_2), 7.64 (d, 1H, 2,3,5-trichlorophenyl), 7.26–7.38 (m, 5H, 2,6-phenoxy), 7.86 (d, 1H, 2,3,5-trichlorophenyl).

6.5.6. 6-(5-Quinolyl)-3-(2,3,5-trichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (**7f**)

IR (KBr, cm^{-1}): 3044 (aromatic C–H), 1648 (C=N), 868 (C–Cl); FAB MS (*m/z*, %): 432 ($M^+ + 1$, 90), 431 (M^+, 50), 289 (10), 154 (30), 136 (40), 120 (10).

6.5.7. 6-(Phenyl)-3-(2,3,5-trichlorophenyl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole (**7g**)

IR (KBr, cm^{-1}): 3053 (aromatic C–H), 1655 (C=N), 842 (C–Cl); FAB MS (*m/z*, %): 381 ($M^+ + 1$, 100), 8031 (M^+, 60), 165 (10), 136 (40), 107 (30).

*6.5.8. 6-Pyridin-4-yl-3-(2,3,5-trichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (**7h**)*

IR (KBr, cm^{-1}): 3056 (aromatic C–H), 864 (C–Cl), 1648 (C=N); ^{1}H NMR (DMSO-d_6): δ 7.59 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 7.62 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 7.72 (d, 1H, $J = 1.7$ Hz, pyridyl), 8.29 (d, 1H, $J = 1.7$ Hz, pyridyl), 8.85 (d, 1H, $J = 1.7$ Hz, pyridyl), 9.91 (d, 1H, $J = 1.7$ Hz, pyridyl); FAB MS (*m/z*, %): 382 (M^+, 96), 383 (M + 1, 20), 384 (M + 2, 100), 386 (M + 4, 30), 388 (M + 6, 5), 367 (20), 341 (15), 305 (10), 279 (50), 240 (70), 197 (60), 179 (60), 149 (50), 123 (20), 105 (40), 91 (70), 80 (20).

*6.5.9. 6-(5-Isoquinolyl)-3-(2,3,5-trichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (**7i**)*

IR (KBr, cm^{-1}): 3076 (aromatic C–H), 1624 (C=N), 884 (C–Cl); FAB MS (*m/z*, %): 431 (M^+, 100), 289 (25), 155 (56), 120 (10), 107 (20).

*6.5.10. 6-(3-Bromopyridin-4-yl)-3-(2,3,5-trichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7j**)*

IR (KBr, cm^{-1}): 3072 (aromatic C–H), 1593 (C=N), 892 (C–Cl), 753 (C–Br); ^{1}H NMR (DMSO-d_6): δ 7.71 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 7.73 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 8.36 (d, 1H, $J = 2.4$ Hz, 3-bromonicotyl), 8.89 (d, 1H, $J = 2.0$ Hz, 3-bromopyridyl), 9.02 (d, 1H, $J = 1.7$ Hz, 3-bromopyridyl); FAB MS (*m/z*, %): 460 (M^+, 50), 461 (M + 1, 5), 462 (M + 2, 75), 464 (M + 4, 55), 466 (M + 6, 15), 429 (50), 411 (100), 391 (40), 223 (25), 229 (25), 217 (60), 181 (25), 123 (5), 109 (20), 77 (5).

*6.6. General procedure for the preparation of 6-aryl-3-(2,3,5-trichlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazines (**8a–g**)*

A mixture 4-amino-5-(2,3,5-trichlorophenyl)-4*H*-[1,2,4] triazole-3-thiol **6** (1 mmol) and substituted phenacyl bromides (1.2 mmol) in 10 mL of absolute ethanol was refluxed for 6–7 h. The reaction mass was poured into crushed ice and neutralized with sodium bicarbonate. Solid product obtained was filtered, washed with water, dried and recrystallized from absolute ethanol.

*6.6.1. 6-(Biphenyl)-3-(2,3,5-trichlorophenyl)-7H-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazine (**8a**)*

IR (KBr, cm^{-1}): 3033 (aromatic C–H), 866 (C–Cl), 1636 (C=N); ^{1}H NMR (DMSO-d_6): 4.06 (s, 2H, $-S-CH_2$), 7.60 and 7.66 (2d, 2H, 2,3,5-trichlorophenyl), 7.42–7.86 (m, 9H, biphenyl); FAB MS (*m/z*, %): 470 (M^+), 472 (M + 2, 45), 474 (M + 4, 25), 476 (M + 6, 10).

*6.6.2. 6-(3-Amido-4-hydroxyphenyl)-3-(2,3,5-trichlorophenyl)-7H-[1,2,4]triazolo-[3,4-b][1,3,4]thiadiazine (**8b**)*

IR (KBr, cm^{-1}): 3356 ($CONH_2$), 3041 (aromatic C–H), 866 (C–Cl), 1636 (C=N); FAB MS (*m/z*, %): 454 ($M^+ + 1$, 100), 453 (M^+, 40).

*6.6.3. 6-(4-Chlorophenyl)-3-(2,3,5-trichlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**8c**)*

^{1}H NMR (DMSO-d_6): δ 4.35 (s, 2H, CH_2), 7.46 (d, 2H, $J = 8.6$ Hz, *p*-chlorophenyl), 7.6 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 7.73 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 7.83 (d, 2H, $J = 8.6$ Hz, *p*-chlorophenyl); FAB MS (*m/z*, %): 430 (M^+, 120), 431 (M + 1, 100), 432 (M + 2, 25), 434 (M + 4, 10), 436 (M + 6, 5), 395 (15), 325 (3), 272 (10), 180 (5), 165 (10), 120 (10), 107 (20), 89 (20).

*6.6.4. 6-(4-Methylthiophenyl)-3-(2,3,5-trichlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**8d**)*

IR (KBr, cm^{-1}): 3039 (aromatic C–H), 861 (C–Cl), 1653 (C=N); ^{1}H NMR (DMSO-d_6): δ 2.52 (s, 3H, SCH_3), 7.22 (d, 2H, 4-methylthiophenyl), 7.36 (d, 2H, 4-methylthiophenyl), 7.54 (d, 1H, 2,3,5-trichlorophenyl), 7.74 (d, 1H, 2,3,5-trichlorophenyl); FAB MS (*m/z*, %): 440 (M^+, 100), 431 (M + 1, 100), 432 (M + 2, 25), 434 (M + 4, 10), 395 (10), 165 (20), 155 (10), 120 (10).

*6.6.5. 6-(Phenyl)-3-(2,3,5-trichlorophenyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazine (**8e**)*

IR (KBr, cm^{-1}): 3062 (aromatic C–H), 1649 (C=N), 863 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 4.48 (s, 2H, SCH_2), 7.10–7.28 (m, 5H, phenyl), 7.60 (d, 1H, 2,3,5-trichlorophenyl), 7.84 (d, 1H, 2,3,5-trichlorophenyl).

*6.6.6. 6-(4-Methoxyphenyl)-3-(2,3,5-trichlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**8f**)*

IR (KBr, cm^{-1}): 1689 (C=O), 1592 (C=N), 1230 (N–N=C), 688 (C–S–C); ^{1}H NMR (DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 4.43 (s, 2H, CH_2), 7.08 (d, 2H, $J = 8.4$ Hz, 4-methoxyphenyl), 7.82 (d, 1H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl), 7.83 (d, 2H, $J = 8.4$ Hz, 4-methoxyphenyl), 8.14 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl); FAB MS (*m/z*, %): 424 (M^+, 10), 425 (M + 1, 80), 426 (M + 2, 25), 428 (M + 4, 20), 430 (M + 6, 5), 391 (70), 373 (15), 220 (5), 178 (5), 167 (15), 120 (30), 107 (50), 89 (40).

*6.6.7. 6-(4-Methylphenyl)-3-(2,3,5-trichlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**8g**)*

IR (KBr, cm^{-1}): 2944 (C–H of CH_3), 3014 (aromatic C–H), 1620 (C=N), 872 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 4.06 (s, 2H, CH_2), 7.28 (d, 2H, $J = 8.0$ Hz, 4-methylphenyl), 7.61 (d, 1H, $J = 2.4$ Hz, 2,3,5-trichlorophenyl), 7.67 (d, 1H, $J = 2.7$ Hz, 2,3,5-trichlorophenyl), 7.69 (d, 2H, $J = 8.2$ Hz, 4-methylphenyl).

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