S. Afr. J. Chem., 2010, **63**, 114–122,
<http://journals.sabinet.co.za/sajchem/>.

Synthesis and Biological Evaluation of some Novel 2-Mercaptobenzothiazoles Carrying 1,3,4-Oxadiazole, 1,3,4-Thiadiazole and 1,2,4-Triazole Moieties

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Received 3 May 2010, revised 1 June 2010, accepted 16 June 2010

ABSTRACT

Several 2-mercaptobenzothiazole derivatives containing 1,3,4-oxadiazoles, 1,2,4-triazoles and 1,3,4-thiadiazoles at the second position were synthesized. Some of these synthesized compounds were evaluated for their *in vivo* analgesic, anti-inflammatory, acute toxicity and ulcerogenic actions. Some of the tested compounds showed significant analgesic and anti-inflammatory activities. Two of the compounds showed significant gastrointestinal protection compared to the standard drug diclofenac sodium. The compounds were also tested for their *in vitro* antimicrobial activity with most displaying selective activity against the Gram-negative bacteria *Pseudomonas aeruginosa*. In the present investigation the tested compounds did not possess antifungal activity.

KEYWORDS

2-Mercaptobenzothiazoles, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, antimicrobial activity, anti-inflammatory activity.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) represent a heterogeneous family of pharmacologically active compounds used to alleviate acute and chronic inflammation, pain and fever. A major mechanism of action of NSAIDs is lowering prostaglandin (PG) production through the inhibition of cyclo-oxygenase (COX) enzyme that catalyses the conversion of arachidonic acid into PG.[1] Because PG has dual function; mediation of inflammation[2,3] and cytoprotection[4] in the stomach and intestine, long-term usage of NSAIDs to relieve the symptoms of inflammation and pain always results in gastrointestinal (GI) disorders and renal toxicity.[5,6] It is known that bacterial infections often produce pain and inflammation. In normal practice, chemotherapeutic, analgesic, and anti-inflammatory drugs are prescribed simultaneously which increases the risk for developing NSAIDs-related complications especially in the elderly, patients with a prior history of peptic ulcer disease and patients with impaired kidney functions. Hence, there is a pressing need for drugs having both antimicrobial and analgesic, anti-inflammatory activities with minimum adverse effects.

A literature survey revealed that substances containing 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole moieties have occupied a unique position in the design and synthesis of biologically active agents with remarkable analgesic, anti-inflammatory[7–9] and antimicrobial activities.[10–12] In addition, 2-mercaptobenzothiazoles are known to possess antiallergic[13], antimicrobial[14–17] and anti-inflammatory[18,19] properties. Based on the above observations it appeared of interest to link the 2-mercaptobenzothiazole nucleus at the second position to some 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole ring systems in an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules. In the present investigation 2-mercaptobenzothiazole derivatives (**6a–m**), (**7a–g**) and (**9a–m**) were synthesized and evaluated for their analgesic, anti-inflammatory, antimicrobial and ulcerogenic effects.

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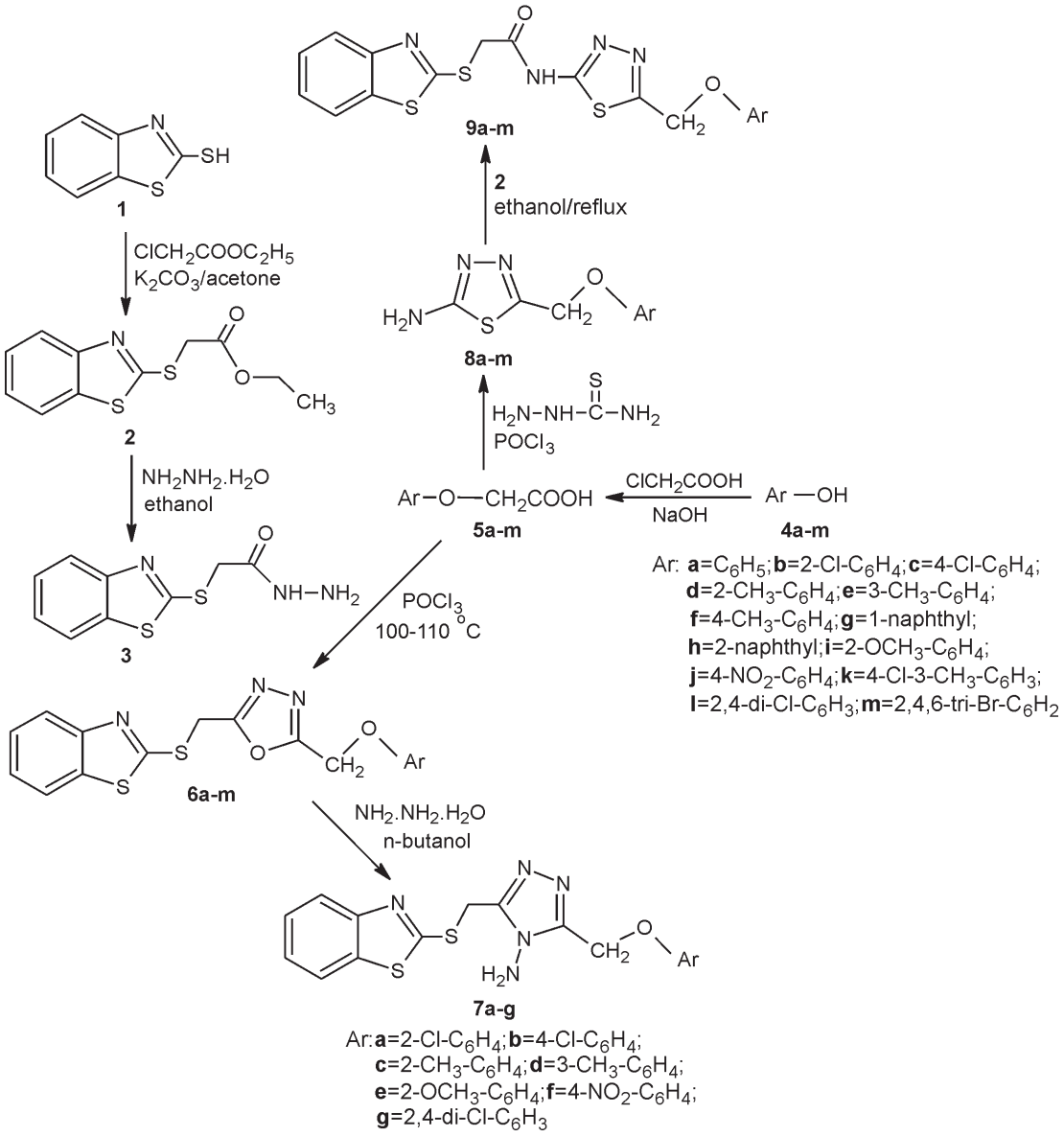
2. Results and Discussion

The synthesis of the title compounds (**6a–m**), (**7a–g**) and (**9a–m**) is described in Scheme 1. Ethyl (benzothiazol-2-ylthio)acetate (**2**) was prepared according to the reported method[16], and condensed with hydrazine hydrate in absolute ethanol to obtain benzothiazol-2-ylthio acetic acid hydrazide[20] (**3**). The reactions of phenols (**4a–m**) with chloroacetic acid in basic medium[21] resulted in the formation of corresponding aryloxyacetic acid derivatives (**5a–m**). Cyclo-condensation of these acids with hydrazide (**3**) in the presence of phosphorous oxychloride gave the desired 1,3,4-oxadiazol derivatives (**6a–m**). Further condensation of the 1,3,4-oxadiazol derivatives **6b–e**, **6i**, **6j** and **6l** with hydrazine hydrate (99 %) in n-butanol furnished the corresponding 1,2,4-triazol-4-amine derivatives (**7a–g**). On the other hand, cyclization of **5a–m** with thiosemicarbazide in the presence of phosphorous oxychloride gave the corresponding 1,3,4-thiadiazol-2-amine derivatives (**8a–m**). The reactions of compounds **8a–m** with ethyl (benzothiazol-2-ylthio)acetate (**2**) in refluxing absolute ethanol afforded the corresponding 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl] acetamides (**9a–m**). The structures of the newly synthesized compounds were confirmed by the analytical and spectral data.

The infrared (IR) spectrum of compounds **6a–m** showed C-O-C vibrations of the oxadiazole ring[12,22] in the region 1311–1249 cm^{-1}. The C=N stretching observed at 1660–1599 cm^{-1} is due to the ring closure. The proton magnetic resonance (^{1}H NMR) spectrum of **6a** showed multiplet signals at δ 7.99–7.68 and 7.54–6.98 ppm for four and five aromatic protons and singlet signals at δ 5.62 and 3.50 ppm due to the OCH_2 and SCH_2 fragments. In the ^{13}C NMR spectrum, the C=N carbon of the benzothiazole ring resonated at δ 166.33 ppm. The C-2 and C-5 carbons of the oxadiazole moiety gave singlets at δ 164.84 and



Scheme 1

157.75 ppm, respectively. The chemical shift values of the other aromatic carbons were observed in the expected region. The mass spectrum of **6a** showed $[M^+ - 1]$ peak at *m/z* 354 which is in agreement with its molecular formula $C_{17}H_{13}N_3O_2S_2$.

In the IR spectra of compounds **7a–g** absorption bands in the region 3200–3149 cm^{-1}, characteristic of a NH_2 group, were observed. The formation of triazole ring in **7a** was supported by its ^1H NMR spectrum, which showed a singlet signal at δ 5.53 ppm due to the NH_2 fragment of 1,2,4-triazol-4-amine. The protons belonging to the aromatic ring and substitutent groups were observed within the expected chemical shift values.

In the IR spectra of compounds **8a–m**, the disappearance of C=O stretching bands of the aryloxyacetic acids and detection of strong C=N stretching bands in the region 1653–1620 cm^{-1} are evidence for the ring closure of thiadiazol ring. The ^1H NMR spectrum of **8a** showed a multiplet signal for five aromatic protons at δ 7.37–6.98 ppm and singlet signals at δ 7.24 and 5.23 ppm due to the NH_2 and OCH_2 fragments, respectively.

In the IR spectrum of compound **9a** characteristic absorption bands at 3261, 3100 (NH), 1654 (C=O), and 1605 (C=N) cm^{-1} were observed. Its ^1H NMR spectrum showed multiplet signals at δ 7.33–7.27 and 7.04–6.94 ppm for four and five aromatic protons and singlet signals at δ 7.25, 5.26 and 4.12 ppm due to the CONH, OCH_2 and SCH_2 fragments, respectively. In the ^{13}C NMR spectrum, the carbonyl carbon exhibited a singlet at δ 169.07 ppm and the C=N carbon of the benzothiazole ring resonated at δ 166.14 ppm. The C-2 and C-5 carbons of the thiadiazole moiety gave singlets at δ 166.82 and 162.61 ppm, respectively. The mass spectrum of compound **9a** displayed the molecular ion peak at *m/z* 414.

The analgesic activity of the synthesized compunds was evaluated by the tail flick method[23] using mice. The analgesic activity results are summarized in Table 1. The tested compounds **6h**, **6k**, **9a**, **9d**, **9h** and **9i** exhibited fast analgesic activity (40.0–69.2 %) as evident from observation at 30 min following oral administration. At 1 and 2 h compounds **6d**, **6h, 6k, 9b, 9h** and **9i** exhibited potent analgesic activity (40.2–76.8 %) compared to the standard drug paracetamol (43.8 and 36.4 %, respectively, at a dose of 50 mg kg^{-1}). In general, a sharp decline in the activity was observed at 3 h following oral administration. It was observed that substitution with naphthalen-2-yloxymethyl (**6h**) group at fifth position of the oxadiazole nucleus or phenyloxymethyl, naphthalen-2-yloxymethyl or ortho-methoxyphenyloxymethyl (**9a, 9h** and **9i**) groups at fifth position of the thiadiazole nucleus

Table 1 Analgesic activity of some selected compounds in mice by the tail flick method.

Compound	Percentage analgesic activity			
	30 min	1 h	2 h	3 h
	% Analgesia /mean ± SEM	% Analgesia /mean ± SEM	% Analgesia /mean ± SEM	% Analgesia /mean ± SEM
6b	12.5 ± 0.8 [b]	34.9 ± 1.5 [b]	15.8 ± 0.9 [b]	12.5 ± 1.0 [b]
6d	27.2 ± 0.9 [c]	54.3 ± 1.4 [b]	44.7 ± 1.4 [b]	24.8 ± 1.1 [b]
6h	61.3 ± 1.4 [b]	76.8 ± 0.7 [b]	60.3 ± 1.2 [c]	20.7 ± 1.3 [c]
6i	32.7 ± 1.4 [b]	51.1 ± 1.1 [b]	31.8 ± 1.1 [b]	16.2 ± 1.2 [b]
6j	17.3 ± 1.4 [b]	24.5 ± 1.1 [b]	32.2 ± 1.5 [c]	15.4 ± 1.4 [b]
6k	55.9 ± 1.4 [c]	55.8 ± 1.2 [c]	40.5 ± 1.1 [c]	19.8 ± 0.7 [d]
7a	19.5 ± 1.2 [b]	23.8 ± 1.5 [b]	29.7 ± 0.9 [a]	11.4 ± 0.8 [b]
7c	37.5 ± 1.6 [b]	55.0 ± 0.7 [c]	33.5 ± 1.1 [b]	16.9 ± 1.5 [c]
9a	59.3 ± 1.4 [b]	66.9 ± 1.2 [b]	35.6 ± 1.3 [b]	23.8 ± 1.3 [b]
9b	37.5 ± 1.4 [b]	45.2 ± 1.3 [b]	58.7 ± 1.4 [b]	22.9 ± 1.3 [b]
9d	40.0 ± 1.3 [b]	53.7 ± 1.0 [c]	27.4 ± 1.2 [c]	18.1 ± 0.7 [a]
9h	40.3 ± 1.2 [b]	54.3 ± 1.0 [c]	40.9 ± 1.4 [b]	24.8 ± 1.4 [b]
9i	69.2 ± 0.9 [b]	66.8 ± 0.7 [a]	46.0 ± 1.1 [b]	19.0 ± 1.9 [b]
9j	32.1 ± 1.3 [d]	40.4 ± 1.6 [b]	29.7 ± 0.8 [b]	11.5 ± 1.5 [b]
Paracetamol	28.0 ± 0.8 [a]	43.8 ± 0.9 [c]	36.4 ± 1.0 [b]	24.1 ± 1.2 [b]

Test compounds and paracetamol were tested at 100 mg $kg^{-1}$and 50 mg kg^{-1} body weight, respectively.
Results are expressed in mean ± SEM (n = 6).
Significance levels [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ compared with the respective control.

enhances the activity. Moreover, it was also observed that substitution of ortho-Cl (**6b**, **7a** and **9b**) or para-NO_2 (**6j** and **9j**) in the phenyl ring of the phenyloxymethyl group at fifth position of the oxadiazole, triazole or thiadiazole nucleus resulted in a marked decrease in analgesic activity.

The anti-inflammatory activity results determined using the carrageenan-induced paw oedema method[24] in rats are summarized in Table 2. The tested compounds **6b**, **6j**, **6k**, **9d**, **9i** and **9j** showed rapid onset of action (50.6–67.9 %) as evident from observation at 30 min after carrageenan injection. At 1 h compounds **6b**, **6d**, **6i**, **7c**, **9b**, **9d**, **9h**, **9i** and **9j** were nearly effective in inhibiting the paw oedema (72.8–81.6 %), when compared with the reference drug (72.1 % at a dose of 20 mg kg^{-1}). The highest activity was found in derivative **9d** having 2-methylphenyloxymethyl group at fifth position of the thiadiazole ring. At 2 h compounds **9h**, **9i** and **9j** showed significant anti-inflammatory activity ranging from 76.0 % to 78.7 % inhibition. In general, a marked decrease in activity was observed at 3 h following carrageenan injection. It was observed that the anti-inflammatory activity is dependent on both the nature of substituents and the basic skeleton of the molecules.

Compounds **6d**, **6i, 9d** and **9i** were tested for their ulcerogenic potential according to the method reported by Cioli *et al.*[13] The tested compounds showed low severity index (2.2 ± 0.3 to 3.2 ± 0.5) compared to the standard drug diclofenac sodium (4.4 ± 0.6). The maximum reduction in the ulcerogenic activity was found in the thiadiazole derivatives **9d** and **9i** (2.2 ± 0.3 and 2.3 ± 0.3, respectively). The other tested compounds also exhib-

Table 2 Anti-inflammatory activity of some selected compounds by the carrageenan-induced rat paw oedema method.

Compound	Percentage protection			
	30 min	1 h	2 h	3 h
	% Protection /mean ± SEM	% Protection /mean ± SEM	% Protection /mean ± SEM	% Protection /mean ± SEM
6b	67.6 ± 1.1 [a]	80.0 ± 0.8 [a]	44.3 ± 0.9 [b]	15.3 ± 1.3 [b]
6d	3.4 ± 1.4 [b]	80.0 ± 1.4 [c]	49.7 ± 1.1 [c]	38.5 ± 1.5 [a]
6h	37.8 ± 1.1 [c]	66.0 ± 1.1 [a]	19.6 ± 1.3 [b]	12.0 ± 0.8 [b]
6i	39.4 ± 1.3 [b]	78.1 ± 0.9 [c]	61.8 ± 1.2 [b]	54.2 ± 1.1 [b]
6j	67.9 ± 1.3 [b]	52.6 ± 1.2 [c]	50.5 ± 1.2 [b]	32.1 ± 1.3 [b]
6k	66.3 ± 1.3 [b]	65.8 ± 1.4 [b]	71.8 ± 1.0 [b]	62.1 ± 1.1 [b]
7a	27.8 ± 1.1 [b]	45.0 ± 0.8 [a]	23.5 ± 1.3 [a]	15.7 ± 1.2 [c]
7c	46.8 ± 1.0 [b]	72.8 ± 1.2 [c]	29.3 ± 1.0 [b]	15.8 ± 1.0 [c]
9a	48.1 ± 1.0 [a]	69.7 ± 1.0 [b]	42.3 ± 1.2 [b]	17.9 ± 1.1 [c]
9b	30.1 ± 1.4 [b]	75.7 ± 1.3 [b]	35.2 ± 1.1 [b]	12.8 ± 1.0 [b]
9d	54.5 ± 1.3 [a]	81.6 ± 1.3 [b]	54.9 ± 0.8 [b]	23.6 ± 0.9 [b]
9h	4.9 ± 1.1 [c]	75.7 ± 1.1 [c]	78.7 ± 0.9 [c]	35.2 ± 0.7 [c]
9i	50.6 ± 1.3 [b]	73.0 ± 1.3 [b]	75.2 ± 0.9 [c]	43.0 ± 1.1 [b]
9j	51.6 ± 1.2 [b]	73.7 ± 1.0 [b]	76.0 ± 1.1 [b]	43.1 ± 1.4 [b]
Diclofenac sodium	47.1 ± 1.2 [b]	72.1 ± 0.8 [b]	72.3 ± 1.1 [b]	68.1 ± 0.8 [b]

Test compounds and diclofenac sodium were tested at 100 mg kg^{-1} and 20 mg kg^{-1} body weight, respectively.
Result are expressed in mean ± SEM. (n = 6).
Significance levels [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ compared with the respective control.

Table 3 Ulcerogenic effects of some selected compounds by the Cioli *et al.*'s method.

Compound	Control 1 % CMC	**6d**	**6i**	**9d**	**9i**	Diclofenac sodium
Severity Index	0.25 ± 0.2 [c]	3.2 ± 0.5 [b]	2.7 ± 0.2 [b]	2.2 ± 0.3 [b]	2.3 ± 0.3 [b]	4.4 ± 0.6 [b]

Test compounds and diclofenac sodium were tested at 200 and 20 mg kg^{-1} body weight, respectively.
Results are expressed in mean ± SEM ($n = 6$)
Significance levels [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.001$ compared with the respective control.

ited better GI safety profile as compared to the standard drug diclofenac sodium (Table 3).

The antimicrobial screening by the cup plate method[26] indicated significant inhibitory activity (inhibition zone >19 mm) of the tested compounds **6h**, **7a**, **8a**, **9d, 9h, 9i** and **9j** against the Gram-negative bacteria *Pseudomonas aeruginosa* whereas compounds **6b**, **6d**, **6e**, **6i**, **6j**, **6r**, **7c**, **9a** and **9b** were found to be moderately active (inhibition zone 16–19 mm) against the same microorganism (Table 4). Furthermore, the maximum inhibitory activity (inhibition zone 26 mm) was observed in derivative **9i** having 2-methoxyphenyloxymethyl group at fifth position of the thiadiazole ring. The tested compounds exhibited no activity against Gram-positive microorganism except compounds **7c** and **9j** which displayed weak inhibitory activity against *Staphylococcus epidermidis* (inhibition zones 17 and 16 mm, respectively). In the present investigation the tested compounds did not posses antifungal activity. It was observed that compound bearing ortho-OCH_3 or para-NO_2 in the phenyl ring of the phenyloxymethyl group at fifth position of the thiadiazole nucleus exhibited significant inhibitory activity against *Pseudomonas aeruginosa.* While a notable decrease or loss in activity was seen when these substituents were replaced with 2-Cl, 4-Cl, 3-CH_3 or 4-CH_3.

In summary, various 2-mercaptobenzothiazole derivatives were prepared with the objective of developing dual anti-inflammatory-antimicrobial agents with minimum ulcerogenic activity. Among these, a thiadiazole derivative 2-(1,3-benzothiazol-2-ylsulfanyl)-N-{5-[(2-methoxyphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9i**) showed the most prominent and consistent activity with a significant reduction of gastrointestinal toxicity. Therefore compound **9i** would represent a fruitful matrix for the development of a new class of dual anti-inflammatory-antimicrobial agents.

Table 4 Antimicrobial screening of some selected compounds by the cup plate method.

Compound	Zone of inhibition (mm) [a,b]					
	P. a.	*S. d.*	*S. e.*	*E. f.*	*C. a.*	*A. n.*
6a	12	–	–	14	–	–
6b	17	–	–	–	–	–
6c	13	–	–	–	–	–
6d	18	–	–	–	–	–
6e	16	–	–	–	–	–
6f	–	–	–	–	–	–
6g	12	–	–	–	–	–
6h	20	–	–	–	–	–
6i	18	–	–	–	–	–
6j	19	–	–	–	–	–
6k	18	–	–	–	–	–
6l	–	–	–	–	–	–
6m	–	–	–	–	–	–
7a	23	–	–	–	–	–
7b	12	–	–	–	–	–
7c	18	17	19	–	–	–
8a	23	–	–	–	–	–
8b	14	–	–	–	–	–
9a	18	–	–	–	–	–
9b	19	–	–	–	–	–
9c	–	16	–	–	–	–
9d	20	–	–	–	–	–
9e	12	–	–	–	–	–
9f	13	–	–	12	–	–
9g	12	–	–	–	–	–
9h	21	–	–	–	–	–
9i	26	–	–	–	–	–
9j	24	–	16	–	–	–
DMSO	–	–	–	–	–	–
Ciprofloxacin	34	53	55	52	–	–
Ketoconazole	–	–	–	–	32	28

Test compounds, ciprofloxacin and ketoconazole were tested at 100, 10 and 20 μg mL^{-1}concentrations, respectively.
[a] Average of three readings.
[b] Indicates no activity.
P.a., Pseudomonas aeruginosa; S. d., Shigella dysenteriae; S. e., Staphylococcus epidermidis; E. f., Enterococcus faecalis; C. a., Candida albicans; A. n., Aspergillus niger.

3. Experimental

3.1. Synthesis

Melting points were determined in open capillaries and were uncorrected. The reaction progress was routinely monitored by thin layer chromatography (TLC) on silica gel plates. The IR spectra were recorded on a Shimadzu 8400S FT-IR spectrometer (Tokyo, Japan) in KBr pellets. The ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker AV-III 400 (Rheinstetten/Karlsruhe, Germany) spectrometer in $CDCl_3$/DMSO-d_6 and referenced to TMS. The mass spectra were measured with a Shimadzu 2010A LC-MS spectrometer (Tokyo, Japan). Elemental analyses of compounds were carried out on a Flash EA 1112 series instrument (Thermo Finnigan, Italy).

3.1.1. *Synthesis of* 2-*{(benzo[d]thiazol-2-ylthio)methyl}-5-(aryloxymethyl)-1,3,4-oxadiazoles* (**6a–m**)

General Procedure. To a mixture of appropriate aryloxyacetic acids (**5a–m**) (0.01 mol) and benzothiazol-2-ylthio acetic acid hydrazide (**3**) (0.01 mol), phosphorous oxychloride (5 mL) was added and the reaction mixture was heated under reflux for 5 to 6 h. After completion of the reaction the content of the flask was cooled and poured onto crushed ice. It was then neutralized by 5 % sodium bicarbonate solution and the separated solid was filtered, washed several times with water, dried and recrystallized from appropriate solvent to give the title compounds **6a–m**.

2-*{(Benzo[d]thiazol-2-ylthio)methyl}-5-(phenyloxymethyl)-1,3,4-oxadiazole* (**6a**): Solv. crystallization: ethanol. M.p.: 160 °C. % Yield 44. IR (KBr, cm^{-1}): 3061 (CH aromatic), 2848 (CH_2), 1599 (C=N), 1583 (C=C aromatic), 1276 (C-O-C oxadiazole), 1219, 1080 (C-O-C), 752 (C-S-C), 727, 690 (monosubstituted benzene). ^{1}H NMR ($CDCl_3$): δ 7.99–7.68 (m , 4H, ArH), 7.54–6.98 (m, 5H, ArH), 5.62 (s, 2H, OCH_2), 3.50 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6):

δ 166.33, 164.84, 159.92, 157.75, 153.80, 136.12, 131.66, 129.63, 125.64, 121.73, 121.12, 120.92, 114.32, 60.98, 55.73. MS: *m/z* 354 (M^+-1), 262, 221, 208, 180, 166, 135, 125, 110, 93, 76, 58. Calcd. for $C_{17}H_{13}N_3O_2S_2$: C, 57.46; H, 3.66; N, 11.83. Found: C, 57.42; H, 3.69; N, 11.86 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2-chlorophenyloxymethyl)-1,3,4-oxadiazole (**6b**): Solv. crystallization: ethanol. M.p.: 184 °C. % Yield 56. IR (KBr, cm^{-1}): 3063 (CH aromatic), 2926, 2852 (CH_2), 1599 (C=N), 1587 (C=C aromatic), 1276 (C-O-C oxadiazole), 1228, 1058 (C-O-C), 750 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.43–7.99 (m, 4H, ArH), 7.82–6.88 (m, 4H, ArH), 5.13 (s, 2H, OCH_2), 3.46 (s, 2H, SCH_2). MS: *m/z* 388 (M^+-2). Calcd. for $C_{17}H_{12}ClN_3O_2S_2$: C, 52.30; H, 3.33; N, 10.76. Found: C, 52.27; H, 3.35; N, 10.72 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(4-chlorophenyloxymethyl)-1,3,4-oxadiazole (**6c**): Solv. crystallization: ethanol. M.p.: 155 °C. % Yield 67. IR (KBr, cm^{-1}): 3063 (CH aromatic), 2928 (CH_2), 1605 (C=N), 1600 (C=C aromatic), 1301 (C-O-C oxadiazole), 1240, 1092 (C-O-C), 825 (para-substituted benzene), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.38–7.92 (m, 4H, ArH), 7.79–6.82 (m, 4H, ArH), 5.21 (s, 2H, OCH_2), 3.32 (s, 2H, SCH_2). MS: *m/z* 388 (M^+-2). Calcd. for $C_{17}H_{12}ClN_3O_2S_2$: C, 52.30; H, 3.33; N, 10.76. Found: C, 52.36; H, 3.29; N, 10.75 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2-methylphenyloxymethyl)-1,3,4-oxadiazole (**6d**): Solv. crystallization: ethanol. M.p.: 175 °C. % Yield 54. IR (KBr, cm^{-1}): 3061 (CH aromatic), 2978 (CH_3), 2924 (CH_2), 1595 (C=N), 1587 (C=C aromatic), 1307 (C-O-C oxadiazole), 1288, 1053 (C-O-C), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.53–7.90 (m, 4H, ArH), 7.86–6.78 (m, 4H, ArH), 4.68 (s, 2H, OCH_2), 3.17 (s, 2H, SCH_2), 2.18 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 166.23, 164.54, 160.27, 157.41, 153.25, 136.16, 131.25, 129.54, 129.2, 125.44, 124.24, 121.80, 121.55, 121.26, 114.35, 60.64, 55.57, 21.48. MS: *m/z* 369 (M^+). Calcd. for $C_{18}H_{15}N_3O_2S_2$: C, 58.53; H, 4.06; N, 11.38. Found: C, 58.57; H, 4.02; N, 11.34 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(3-methylphenyloxymethyl)-1,3,4-oxadiazole (**6e**): Solv. crystallization: DMF and ethanol (1:1). M.p.: 170 °C. % Yield 54. IR (KBr, cm^{-1}): 3053 (CH aromatic), 2912 (CH_2), 2864 (CH_3), 1602 (C=N), 1585 (C=C aromatic), 1249 (C-O-C oxadiazole), 1157, 1082 (C-O-C), 756 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.23–7.89 (m, 4H, ArH), 7.79–6.98 (m, 4H, ArH), 4.73 (s, 2H, OCH_2), 3.31 (s, 2H, SCH_2), 1.98 (s, 3H, CH_3). MS: *m/z* 368 (M^+-1). Calcd. for $C_{18}H_{15}N_3O_2S_2$: C, 58.53; H, 4.06; N, 11.38. Found: C, 58.56; H, 4.02; N, 11.36 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(4-methylphenyloxymethyl)-1,3,4-oxadiazole (**6f**): Solv. crystallization: DMF and water (1:1). M.p.: 167 °C. % Yield 43. IR (KBr, cm^{-1}): 3063 (CH aromatic), 2918 (CH_2), 2868 (CH_3), 1660 (C=N), 1598 (C=C aromatic), 1311 (C-O-C oxadiazole), 1238, 1078 (C-O-C), 815 (para-substituted benzene), 756 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.17–7.83 (m, 4H, ArH), 7.74–6.97 (m, 4H, ArH), 4.82 (s, 2H, OCH_2), 3.22 (s, 2H, SCH_2), 1.85 (s, 3H, CH_3). MS: *m/z* 370 (M^++1). Calcd. for $C_{18}H_{15}N_3O_2S_2$: C, 58.53; H, 4.06; N, 11.38. Found: C, 58.57; H, 4.12; N, 11.35 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(naphthalen-1-yloxymethyl)-1,3,4-oxadiazole (**6g**): Solv. crystallization: ethanol. M.p.: 135 °C. % Yield 63. IR (KBr, cm^{-1}): 3024 (CH aromatic), 2978 (CH_3), 2931(CH_2), 1627 (C=N), 1595 (C=C aromatic), 1263 (C-O-C oxadiazole), 1234, 1087 (C-O-C), 756 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.18–7.83 (m, 4H, ArH), 7.73–6.92 (m, 7H, ArH), 5.12 (s, 2H, OCH_2), 3.35 (s, 2H, SCH_2). MS: *m/z* 406 (M^++1). Calcd. for $C_{21}H_{15}N_3O_2S_2$: C, 62.22; H, 3.70; N, 10.37. Found: C, 62.27; H, 3.67; N, 10.33 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(naphthalen-2-yloxymethyl)-1,3,4-oxadiazole (**6h**): Solv. crystallization: DMF and ethanol (1:1). M.p.: 160 °C. % Yield 55. IR (KBr, cm^{-1}): 3059 (CH aromatic), 2926 (CH_2), 1627 (C=N), 1599 (C=C aromatic), 1254 (C-O-C oxadiazole), 1215, 1087 (C-O-C), 752 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.23–7.85 (m, 4H, ArH), 7.73–6.82 (m, 7H, ArH), 4.53 (s, 2H, OCH_2), 3.35 (s, 2H, SCH_2). MS: *m/z* 405 (M^+). Calcd. for $C_{21}H_{15}N_3O_2S_2$: C, 62.22; H, 3.70; N, 10.37. Found: C, 62.26; H, 3.74; N, 10.34 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2-methoxyphenyloxymethyl)-1,3,4-oxadiazole (**6i**): Solv. crystallization: DMF and ethanol (1:1). M.p.: 160 °C. % Yield 72. IR (KBr, cm^{-1}): 3057 (CH aromatic), 2970 (CH_3), 2833 (CH_2), 1660 (C=N), 1599 (C=C aromatic), 1250 (C-O-C oxadiazole), 1219, 1109 (C-O-C), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.30–7.94 (m, 4H, ArH), 7.81–7.23 (m, 4H, ArH), 4.62 (s, 2H, OCH_2), 3.68 (s, 3H, OCH_3), 3.26 (s, 2H, SCH_2). MS: *m/z* 385 (M^+). Calcd. for $C_{18}H_{15}N_3O_3S_2$: C, 56.10; H, 3.89; N, 10.90. Found: C, 56.15; H, 3.92; N, 10.95 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(4-nitrophenyloxymethyl)-1,3,4-oxadiazole (**6j**): Solv. crystallization: DMF and acetone (1:1). M.p.: 182 °C. % Yield 66. IR (KBr, cm^{-1}): 3074 (CH aromatic), 2927 (CH_2), 1615 (C=N), 1591 (C=C aromatic), 1514, 1342 (NO_2), 1300 (C-O-C oxadiazole), 1253, 1111 (C-O-C), 846 (para-substituted benzene), 750 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.15-7.98 (m, 4H, ArH), 7.78–7.33 (m, 4H, ArH), 4.63 (s, 2H, OCH_2), 3.29 (s, 2H, SCH_2). MS: *m/z* 400 (M^+). Calcd. for $C_{17}H_{12}N_4O_4S_2$: C, 51.00; H, 3.00; N, 14.00. Found: C, 50.88; H, 3.04; N, 13.87 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(4-chloro-3-methylphenyloxy methyl)-1,3,4-oxadiazole (**6k**): Solv. crystallization: methanol. M.p.: 119 °C. % Yield 61. IR (KBr, cm^{-1}): 3053 (CH aromatic), 2949 (CH_3), 2924 (CH_2), 1656 (C=N), 1597 (C=C aromatic), 1305 (C-O-C oxadiazole), 1238, 1166 (C-O-C), 1039 (C-Cl), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.98–7.86 (m, 4H, ArH), 7.76–6.98 (m, 3H, ArH), 4.52 (s, 2H, OCH_2), 3.33 (s, 2H, SCH_2), 1.87 (s, 3H, CH_3). MS: *m/z* 404 (M^+). Calcd. for $C_{18}H_{14}ClN_3O_2S_2$: C, 53.46; H, 3.46; N, 10.39. Found: C, 53.43; H, 3.44; N, 10.42 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2,4-dichlorophenyloxymethyl)-1,3,4-oxadiazole (**6l**): Solv. crystallization: methanol. M.p.: 137 °C. % Yield 65. IR (KBr, cm^{-1}): 3058 (CH aromatic), 2925 (CH_2), 1660 (C=N), 1597 (C=C aromatic), 1288 (C-O-C oxadiazole), 1246, 1101 (C-O-C), 1060 (C-Cl), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.32–7.85 (m, 4H, ArH), 7.79–7.23 (m, 3H, ArH), 5.12 (s, 2H, OCH_2), 3.34 (s, 2H, SCH_2). MS: *m/z* 424 (M^+). Calcd. for $C_{17}H_{11}Cl_2N_3O_2S_2$: C, 48.11; H, 2.59; N, 9.90. Found: C, 48.13; H, 2.55; N, 9.84 %.

2-{(Benzo[d]thiazol-2-ylthio)methyl}-5-(2,4,6-tribromophenyloxymethyl)-1,3,4-oxadiazole (**6m**): Solv. crystallization: methanol. M.p.: 181 °C. % Yield 60. IR (KBr, cm^{-1}): 3066 (CH aromatic), 2924 (CH_2), 1654 (C=N), 1597 (C=C aromatic), 1309 (C-O-C oxadiazole), 1240, 1099 (C-O-C), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.17–7.89 (m, 4H, ArH), 7.83–7.65 (m, 2H, ArH), 4.78 (s, 2H, OCH_2), 3.42 (s, 2H, SCH_2). MS: *m/z* 593 (M^++1). Calcd. for $C_{17}H_{10}Br_3N_3O_2S_2$: C, 34.45; H, 1.68; N, 7.09. Found: C, 34.47; H, 1.64; N, 7.07 %.

3.1.2. *Synthesis of 3-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-5-(aryloxymethyl)-4H- 1,2,4-triazol-4-amines* (**7a–g**)

General Procedure. To a solution of approriate 2-{(benzo-[d]thiazol-2-ylthio)methyl}-5-(aryloxymethyl)-1,3,4-oxadiazoles (**6a–m**) (0.05 mol) in n-butanol (25 mL), hydrazine hydrate (99 %, 0.15 mol) was added and the reaction mixture was heated under reflux for 5–6 h. Then, potassium hydroxide (0.10 mol) was

added to the reaction mixture and the precipitate formed was filtered. The solid obtained was acidified with Conc. HCl to pH 3, washed several times with water and dried. The resultant solid was recrystallized from appropriate solvent to give the title compounds **7a–g**.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(2-chlorophenoxy)methyl]-4H-1,2,4-triazol-4-amine (**7a**): Solv. crystallization: ethanol. M.p.: 205 °C. % Yield 69. IR (KBr, cm^{-1}): 3167, 3149 (NH_2), 3061 (CH aromatic), 2922 (CH_2), 1665 (C=N), 1599 (C=C aromatic), 1236, 1016 (C-O-C), 1080 (C-Cl), 752 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.23–7.98 (m, 4H, ArH), 7.35–6.88 (m, 4H, ArH), 5.53 (s, 2H, NH_2, D_2O exchangeable), 5.12 (s, 2H, OCH_2), 3.48 (s, 2H, SCH_2). MS: *m/z* 403 (M^+–1), 276, 262, 222, 219, 206, 180, 166, 134, 128, 112, 97, 82, 78, 58. Calcd. for $C_{17}H_{14}ClN_5OS_2$: C, 50.49; H, 3.46; N, 17.32. Found: C, 50.52; H, 3.42; N, 17.36 %.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(4-chlorophenoxy)methyl]-4H-1,2,4-triazol-4-amine (**7b**): Solv. crystallization: ethanol. M.p.: 219 °C. % Yield 65. IR (KBr, cm^{-1}): 3184, 3167 (NH_2), 3063 (CH aromatic), 2924 (CH_2), 1645 (C=N), 1600 (C=C aromatic), 1238, 1022 (C-O-C), 1091 (C-Cl), 752 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.15–7.93 (m, 4H, ArH), 7.85–6.77 (m, 4H, ArH), 5.48 (s, 2H, NH_2, D_2O exchangeable), 5.14 (s, 2H, OCH_2), 3.46 (s, 2H, SCH_2). MS: *m/z* 422 ($M^+ + H_2O$). Calcd. for $C_{17}H_{14}ClN_5OS_2$: C, 50.49; H, 3.46; N, 17.32. Found: C, 50.51; H, 3.43; N, 17.35 %.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(2-methylphenoxy)methyl]-4H-1,2,4-triazol-4-amine (**7c**): Solv. crystallization: chloroform. M.p.: >300 °C. % Yield 46. IR (KBr, cm^{-1}): 3186, 3165 (NH_2), 3061 (CH aromatic), 2933 (CH_2), 1645 (C=N), 1596 (C=C aromatic), 1251, 1020 (C-O-C), 1080 (C-Cl), 752 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.08-7.97 (m, 4H, ArH), 7.83–6.68 (m, 4H, ArH), 5.49 (s, 2H, NH_2, D_2O exchangeable), 4.98 (s, 2H, OCH_2), 3.51 (s, 2H, SCH_2), 2.38 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 166.32, 160.51, 155.84, 154.23, 153.54, 136.12, 131.52, 129.61, 125.60, 122.12, 121.55, 121.11, 114.23, 60.82, 55.58. MS: *m/z* 383 (M^+). Calcd. for $C_{18}H_{17}N_5OS_2$: C, 56.39; H, 4.43; N, 18.27. Found: C, 56.36; H, 4.47; N, 18.31 %.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(3-methylphenoxy)methyl]-4H-1,2,4-triazol-4-amine (**7d**): Solv. crystallization: chloroform. M.p.: 218 °C. % Yield 57. IR (KBr, cm^{-1}): 3200, 3173 (NH_2), 3061 (CH aromatic), 2956 (CH_3), 2926 (CH_2), 1652 (C=N), 1602 (C=C aromatic), 1249, 1018 (C-O-C), 815, 852 (meta-substituted benzene), 756 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.98–7.88 (m, 4H, ArH), 7.78–6.86 (m, 4H, ArH), 5.64 (s, 2H, NH_2, D_2O exchangeable), 4.86 (s, 2H, OCH_2), 3.47 (s, 2H, SCH_2), 2.33 (s, 3H, CH_3). MS: *m/z* 383 (M^+). Calcd. for $C_{18}H_{17}N_5OS_2$: C, 56.39; H, 4.43; N, 18.27. Found: C, 56.34; H, 4.39; N, 18.22 %.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(2-methoxyphenoxy)methyl]-4H-1,2,4-triazol-4-amine (**7e**): Solv. crystallization: ethanol. M.p.: 185 °C. % Yield 88. IR (KBr, cm^{-1}): 3186, 3165 (NH_2), 3061 (CH aromatic), 2958 (CH_3), 2933 (CH_2), 1645 (C=N), 1596 (C=C aromatic), 1251, 1215, 1020 (C-O-C), 752 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.12–7.92 (m, 4H, ArH), 7.82–7.23 (m, 4H, ArH), 5.71 (s, 2H, NH_2, D_2O exchangeable), 4.92 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.52 (s, 2H, SCH_2). MS: *m/z* 440 ($M^+ + CH_3CN$). Calcd. for $C_{18}H_{17}N_5O_2S_2$: C, 54.13; H, 4.26; N, 17.54. Found: C, 54.17; H, 4.21; N, 17.59 %.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(4-nitrophenoxy)methyl]-4H-1,2,4-triazol-4-amine (**7f**): Solv. crystallization: ethanol. M.p.: >300 °C. % Yield 62. IR (KBr, cm^{-1}): 3186, 3167 (NH_2), 3064 (CH aromatic), 2920 (CH_2), 1665 (C=N), 1598 (C=C aromatic), 1494, 1315 (NO_2), 1236, 1020 (C-O-C), 831 (para-substituted benzene), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.98-7.85 (m, 4H, ArH), 7.78–7.65 (m, 4H, ArH), 6.13 (s, 2H, NH_2, D_2O exchangeable), 5.22 (s, 2H, OCH_2), 3.61 (s, 2H, SCH_2). MS: *m/z* 368 (M^+-NO_2). Calcd. for $C_{17}H_{14}N_6O_3S_2$: C, 49.27; H, 3.38; N 20.28. Found: C, 49.31; H, 3.36; N, 20.25 %.

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-5-[(2,4-dichlorophenoxy)methyl]-4H-1,2,4-triazol-4-amine (**7g**): Solv. crystallization: ethanol. M.p.: 226 °C. % Yield 72. IR (KBr, cm^{-1}): 3196, 3167 (NH_2), 3063 (CH aromatic), 2924 (CH_2), 1652 (C=N), 1605 (C=C aromatic), 1247, 1016 (C-O-C), 1058 (C-Cl), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.05–7.79 (m, 4H, ArH), 7.86–7.62 (m, 3H, ArH), 5.82 (s, 2H, NH_2, D_2O exchangeable), 5.17 (s, 2H, OCH_2), 3.72 (s, 2H, SCH_2). MS: *m/z* 438 (M^+-1). Calcd. for $C_{17}H_{13}Cl_2N_5OS_2$: C, 46.46; H, 2.96; N, 15.94. Found: C, 46.43; H, 2.94; N, 15.90 %.

3.1.3. *Synthesis of 5-(aryloxymethyl)-1,3,4-thiadiazol-2-amines* (**8a–m**)

General Procedure. A mixture of appropriate aryloxyacetic acids (**5a–m**) (0.01 mol), thiosemicarbazide (0.01 mol) and phosphorous oxychloride (5 mL) was gently refluxed for 30 min. After cooling water (10 mL) was added and the reaction mixture was further refluxed for 4 h and filtered. The filtrate was made alkaline with 10 % potassium hydroxide solution; the formed precipitate was filtered, washed several times with water, dried and recrystallized from appropriate solvent to give compounds **8a–m**.

5-(Phenoxymethyl)-1,3,4-thiadiazol-2-amine (**8a**): Solv. crystallization: ethanol. M.p.: 185 °C. % Yield 66. IR (KBr, cm^{-1}): 3275, 3269 (NH_2), 3099 (CH aromatic), 2916 (CH_2), 1635 (C=N), 1599 (C=C aromatic), 1247, 1043 (C-O-C), 840 (C-S-C), 750, 688 (mono-substituted benzene). ^{1}H NMR (DMSO-d_6): δ 7.37–6.98 (m, 5H, ArH), 7.24 (s, 2H, NH_2, D_2O exchangeable), 5.23 (s, 2H, OCH_2). MS: *m/z* 207 (M^+). Calcd. for $C_9H_9N_3OS$: C, 52.17; H, 4.34; N, 20.28. Found: C, 52.14; H, 4.38; N, 20.28 %.

5-[(2-Chlorophenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8b**): Solv. crystallization: THF. M.p.: 219 °C. % Yield 51. IR (KBr, cm^{-1}): 3258, 3250 (NH_2), 3097 (CH aromatic), 2929 (CH_2), 1641 (C=N), 1593 (C=C aromatic), 1247, 1062 (C-O-C), 842 (C-S-C), 744 (ortho-substituted benzene), 690 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 7.42–7.12 (m, 4H, ArH), 7.22 (s, 2H, NH_2, D_2O exchangeable), 5.17 (s, 2H, OCH_2). MS: *m/z* 242 (M^+). Calcd. for $C_9H_8ClN_3OS$: C, 44.62; H, 3.30; N, 17.35. Found: C, 44.59; H, 3.34; N, 17.39 %.

5-[(4-Chlorophenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8c**): Solv. crystallization: THF. M.p.: 204-205 °C. % Yield 58. IR (KBr, cm^{-1}): 3313, 3290 (NH_2), 3099 (CH aromatic), 2926 (CH_2), 1620 (C=N), 1597 (C=C aromatic), 1244, 1039 (C-O-C), 821 (para-substituted benzene), 804 (C-S-C), 660 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 7.35–7.00 (m, 4H, ArH), 7.28 (s, 2H, NH_2, D_2O exchangeable), 5.28 (s, 2H, OCH_2). MS: *m/z* 243 (M^++1). Calcd. for $C_9H_8ClN_3OS$: C, 44.62; H, 3.30; N, 17.35. Found: C, 44.65; H, 3.34; N, 17.32 %.

5-[(2-Methylphenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8d**): Solv. crystallization: THF. M.p.: 208 °C. % Yield 65. IR (KBr, cm^{-1}): 3252, 3246 (NH_2), 3099 (CH aromatic), 2974 (CH_3), 2918 (CH_2), 1641 (C=N), 1593 (C=C aromatic), 1247, 1037 (C-O-C), 846 (C-S-C), 746 (otho-substituted benzene). ^{1}H NMR (DMSO-d_6): δ 7.52–7.14 (m, 4H, ArH), 7.26 (s, 2H, NH_2, D_2O exchangeable), 5.12 (s, 2H, OCH_2), 2.22 (s, 3H, CH_3). MS: *m/z* 223 (M^++2). Calcd. for $C_{10}H_{11}N_3OS$: C, 54.29; H, 4.97; N, 19.00. Found: C, 54.33; H, 4.95; N, 18.97 %.

5-[(3-Methylphenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8e**): Solv. crystallization: THF. M.p.: 181 °C. % Yield 59. IR (KBr, cm^{-1}): 3287, 3248 (NH_2), 3093 (CH aromatic), 2969 (CH_3), 2932 (CH_2), 1647

(C=N), 1599 (C=C aromatic), 1259, 1019 (C-O-C), 849 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.48–6.98 (m, 4H, ArH), 7.21 (s, 2H, NH_2, D_2O exchangeable), 4.98 (s, 2H, OCH_2), 2.31 (s, 3H, CH_3). MS: *m/z* 221(M^+). Calcd. for $C_{10}H_{11}N_3OS$: C, 54.29; H, 4.97; N, 19.00. Found: C, 54.33; H, 4.95; N, 19.06 %.

5-[(4-Methylphenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8f**): Solv. crystallization: THF. M.p.: 208 °C. % Yield 53. IR (KBr, cm^{-1}): 3272, 3256 (NH_2), 3085 (CH aromatic), 2958 (CH_3), 2926 (CH_2), 1653 (C=N), 1596 (C=C aromatic), 1267, 1034 (C-O-C), 846 (C-S-C), 815 (para-substituted benzene). ^{1}H NMR (DMSO-d_6): δ 7.56–6.92 (m, 4H, ArH), 7.31 (s, 2H, NH_2, D_2O exchangeable), 5.08 (s, 2H, OCH_2), 2.37 (s, 3H, CH_3). MS: *m/z* 221(M^+). Calcd. for $C_{10}H_{11}N_3OS$: C, 54.29; H, 4.97; N, 19.00. Found: C, 54.33; H, 4.95; N, 18.96 %.

5-[(Naphthalen-1-yloxy)methyl]-1,3,4-thiadiazol-2-amine (**8g**): Solv. crystallization: THF. M.p.: 236–238 °C. % Yield 72. IR (KBr, cm^{-1}): 3265, 3240 (NH_2), 3088 (CH aromatic), 2925 (CH_2), 1635 (C=N), 1599 (C=C aromatic), 1252, 1025 (C-O-C), 849 (C-S-C). MS: *m/z* 257 (M^+). Calcd. for $C_{13}H_{11}N_3OS$: C, 60.70; H, 4.28; N, 16.34. Found: C, 60.75; H, 4.18; N, 16.31 %.

5-[(Naphthalen-2-yloxy)methyl]-1,3,4-thiadiazol-2-amine (**8h**): Solv. crystallization: THF. M.p.: 188 °C. % Yield 73. IR (KBr, cm^{-1}): 3276, 3257 (NH_2), 3093 (CH aromatic), 2927 (CH_2), 1646 (C=N), 1597 (C=C aromatic), 1258, 1032 (C-O-C), 849 (C-S-C). MS: *m/z* 257 (M^+). Calcd. for $C_{13}H_{11}N_3OS$: C, 60.70; H, 4.28; N, 16.34. Found: C, 60.75; H, 4.25; N, 16.37 %.

5-[(2-Methoxyphenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8i**): Solv. crystallization: THF. M.p.: 183 °C. % Yield 61. IR (KBr, cm^{-1}): 3277, 3268 (NH_2), 3090 (CH aromatic), 2968 (CH_3), 2928 (CH_2), 1632 (C=N), 1598 (C=C aromatic), 1258, 1026 (C-O-C), 844 (C-S-C). MS: *m/z* 237 (M^+). Calcd. for $C_{10}H_{11}N_3O_2S$: C, 50.63; H, 4.64; N, 17.72. Found: C, 50.68; H, 4.61; N, 17.75 %.

5-[(4-Nitrophenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8j**): Solv. crystallization: THF. M.p.: 198 °C. % Yield 56. IR (KBr, cm^{-1}): 3298, 3266 (NH_2), 3087 (CH aromatic), 2928 (CH_2), 1637 (C=N), 1595 (C=C aromatic), 1525, 1310 (NO_2), 1254, 1018 (C-O-C), 850 (C-S-C), 830 (para-substituted benzene). MS: *m/z* 252 (M^+). Calcd. for $C_9H_8N_4O_3S$: C, 42.85; H, 3.17; N, 22.22. Found: C, 42.81; H, 3.20; N, 22.26 %.

5-[(4-Chloro-3-methylphenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8k**): Solv. crystallization: THF. M.p.: 206 °C. % Yield 54. IR (KBr, cm^{-1}): 3274, 3268 (NH_2), 3095 (CH aromatic), 2969 (CH_3), 2958 (CH_2), 1646 (C=N), 1598 (C=C aromatic), 1249, 1029 (C-O-C), 849 (C-S-C), 665 (C-Cl). MS: *m/z* 256 (M^+). Calcd. for $C_{10}H_{10}ClN_3OS$: C, 46.87; H, 3.90; N, 16.40. Found: C, 46.85; H, 4.02; N, 16.37 %.

5-[(2,4-Dichlorophenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8l**): Solv. crystallization: THF. M.p.: 219–220 °C. % Yield 47. IR (KBr, cm^{-1}): 3287, 3259 (NH_2), 3078 (CH aromatic), 2929 (CH_2), 1649 (C=N), 1598 (C=C aromatic), 1253, 1028 (C-O-C), 849 (C-S-C), 755, 665 (C-Cl). MS: *m/z* 277 (M^+). Calcd. for $C_9H_7Cl_2N_3OS$: C, 38.98; H, 2.52; N, 15.16. Found: C, 39.02; H, 2.56; N, 15.13 %.

5-[(2,4,6-Tribromophenoxy)methyl]-1,3,4-thiadiazol-2-amine (**8m**): Solv. crystallization: THF. M.p.: 162 °C. % Yield 62. IR (KBr, cm^{-1}): 3272, 3266 (NH_2), 3095 (CH aromatic), 2929 (CH_2), 1649 (C=N), 1602 (C=C aromatic), 1247, 1037 (C-O-C), 847 (C-S-C). MS: *m/z* 444 (M^+). Calcd. for $C_9H_6Br_3N_3OS$: C, 24.32; H, 1.34; N, 9.45. Found: C, 24.36; H, 1.31; N, 9.48 %.

3.1.4. *Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)-N-[5-(aryloxymethyl)-1,3,4-thiadiazol-2-yl]acetamides* (**9a–m**)

General Procedure. To a solution of appropriate 5-(aryloxymethyl)-1,3,4-thiadiazol-2-amines (**8a–m**) (0.05 mol) in absolute ethanol (50 mL), ethyl (benzothiazol-2-ylthio)acetate (**2**) (0.05 mol) was added and the reaction mixture was refluxed for 16 to18 h, distilled in vacuum and cooled. The separated solid was filtered, dried and recrystallized from appropriate solvent to give the titled compounds **9a–m**.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-[5-(phenoxymethyl)-1,3,4-thiadiazol-2-yl]acetamide (**9a**): Solv. crystallization: methanol. M.p.: 204 °C. % Yield 44. IR (KBr, cm^{-1}): 3261, 3100 (NH), 3064 (CH aromatic), 2916 (CH_2), 1654 (C=O), 1605 (C=N), 1589 (C=C aromatic), 1506 (amide II), 1247, 1043 (C-O-C), 742, 686 (monosubstituted benzene), 750 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.33–7.27 (m, 4H, ArH), 7.25 (s, 1H, CONH, D_2O exchangeable), 7.04–6.94 (m, 5H, ArH), 5.26 (s, 2H, OCH_2), 4.12 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ 169.07, 166.82, 166.14, 162.61, 159.62, 153.72, 136.12, 131.58, 129.86, 125.62, 124.32, 121.23, 121.05, 114.52, 60.68, 55.61. MS: *m/z* 414 (M^+), 321, 281, 267, 250, 208, 180, 165, 151, 133, 107, 93, 58. Calcd. for $C_{18}H_{14}N_4O_2S_3$: C, 52.17; H, 3.38; N, 13.52. Found: C, 52.14; H, 3.40; N, 13.56 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2-chlorophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9b**): Solv. crystallization: methanol. M.p.: 232 °C. % Yield 65. IR (KBr, cm^{-1}): 3252, 3097 (NH), 3062 (CH aromatic), 2926 (CH_2), 1641 (C=O), 1608 (C=N), 1593 (C=C aromatic), 1508 (amide II), 1247, 1062 (C-O-C), 744 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.52–7.43 (m, 4H, ArH), 7.24 (s, 1H, CONH, D_2O exchangeable), 7.03–6.95 (m, 4H, ArH), 5.33 (s, 4H, OCH_2), 4.20 (s, 2H, SCH_2). MS: *m/z* 449 (M^+). Calcd. for $C_{18}H_{13}ClN_4O_2S_3$: C, 48.10; H, 2.89; N, 12.47. Found: C, 48.15; H, 2.92; N, 12.43 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(4-chlorophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9c**): Solv. crystallization: methanol. M.p.: 213 °C. % Yield 71. IR (KBr, cm^{-1}): 3282, 3097 (NH), 3041 (CH aromatic), 2926 (CH_2), 1620 (C=O), 1602 (C=N), 1597 (C=C aromatic), 1525 (amide II), 1244, 1039 (C-O-C), 821 (para-substituted benzene), 680 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.58-7.47 (m, 4H, ArH), 7.26 (s, 1H, CONH, D_2O exchangeable), 7.12–6.93 (m, 4H, ArH), 5.22 (s, 2H, OCH_2), 4.32 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ 169.18, 166.78, 166.22, 162.46, 160.38, 153.62, 136.16, 133.21, 131.47, 129.27, 125.53, 121.33, 121.12, 114.33, 61.23, 55.78. MS: *m/z* 449 (M^+). Calcd. for $C_{18}H_{13}ClN_4O_2S_3$: C, 48.10; H, 2.89; N, 12.47. Found: C, 48.11; H, 2.85; N, 12.49 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2-methylphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9d**): Solv. crystallization: acetone and THF (1:1). M.p.: 209 °C. % Yield 56. IR (KBr, cm^{-1}): 3252, 3097 (NH), 3034 (CH aromatic), 2976 (CH_3), 2918 (CH_2), 1641 (C=O), 1604 (C=N), 1593 (C=C aromatic), 1508 (amide II), 1247, 1037 (C-O-C), 746 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.82-7.53 (m, 4H, ArH), 7.42 (s, 1H, CONH, D_2O exchangeable), 7.39–6.97 (m, 4H, ArH), 4.86 (s, 2H, OCH_2), 4.20 (s, 2H, SCH_2), 1.82 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 169.12, 167.24, 166.21, 162.65, 159.73, 153.69, 137.78, 135.92, 131.51, 130.24, 126.59, 125.54, 121.21, 121.08, 120.70, 114.15, 61.32, 55.69, 21.47. MS: *m/z* 428 (M^+). Calcd. for $C_{19}H_{16}N_4O_2S_3$: C, 53.27; H, 3.73; N, 13.08. Found: C, 53.25; H, 3.78; N, 13.12 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(3-methylphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9e**): Solv. crystallization: methanol. M.p.: 182 °C. % Yield 59. IR (KBr, cm^{-1}): 3267, 3100 (NH), 3062 (CH aromatic), 2972 (CH_3), 2924 (CH_2), 1637 (C=O), 1602 (C=N), 1593 (C=C aromatic), 1521 (amide II), 1261, 1045 (C-O-C), 860, 775 (meta-substituted benzene), 742 (C-S-C).

^{1}H NMR (DMSO-d_6): δ 7.86-7.72 (m, 4H, ArH), 7.51 (s, 1H, CONH, D_2O exchangeable), 7.33–6.86 (m, 4H, ArH), 4.98 (s, 2H, OCH_2), 4.15 (s, 2H, SCH_2), 1.76 (s, 3H, CH_3). MS: *m/z* 428 (M^+). Calcd. for $C_{19}H_{16}N_4O_2S_3$: C, 53.27; H, 3.73; N, 13.08. Found: C, 53.25; H, 3.76; N, 13.12 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(4-methylphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9f**): Solv. crystallization: methanol. M.p.: 225 °C. % Yield 47. IR (KBr, cm^{-1}): 3290, 3100 (NH), 3028 (CH aromatic), 2989 (CH_3), 2922, (CH_2), 1633 (C=O), 1612 (C=N), 1587 (C=C aromatic), 1514 (amide II), 1215, 1041 (C-O-C), 810 (para-substituted benzene), 754 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.92–7.86 (m, 4H, ArH), 7.48 (s, 1H, CONH, D_2O exchangeable), 7.42–6.88 (m, 4H, ArH), 5.03 (s, 2H, OCH_2), 4.26 (s, 2H, SCH_2), 1.80 (s, 3H, CH_3). MS: *m/z* 428 (M^+). Calcd. for $C_{19}H_{16}N_4O_2S_3$: C, 53.27; H, 3.73; N, 13.08. Found: C, 53.29; H, 3.72; N, 13.17 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(naphthalen-1-yloxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9g**): Solv. crystallization: methanol. M.p.: 245 °C. % Yield 62. IR (KBr, cm^{-1}): 3235, 3098 (NH), 3063 (CH aromatic), 2976 (CH_3), 2918 (CH_2), 1639 (C=O), 1606 (C=N), 1595 (C=C aromatic), 1508 (amide II), 1271, 1107 (C-O-C), 742 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.12–7.88 (m, 4H, ArH), 7.56–7.28 (m, 7H, ArH), 7.20 (s, 1H, NH, D_2O exchangeable), 5.48 (s, 2H, OCH_2), 4.35 (s, 2H, SCH_2). MS: *m/z* 465 (M^++1). Calcd. for $C_{22}H_{16}N_4O_2S_3$: C, 56.89; H, 3.44; N, 12.06. Found: C, 56.86; H, 3.41; N, 12.02 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(naphthalen-2-yloxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9h**): Solv. crystallization: THF. M.p.: 193 °C. % Yield 54. IR (KBr, cm^{-1}): 3244, 3100 (NH), 3057 (CH aromatic), 2920 (CH_2), 1629 (C=O), 1599 (C=N), 1597 (C=C aromatic), 1514 (amide II), 1217, 1045 (C-O-C), 744 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.96-7.65 (m, 4H, ArH), 7.58-7.45 (m, 7H, ArH), 7.39 (s, 1H, CONH, D_2O exchangeable), 5.11 (s, 2H, OCH_2), 4.23 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6): δ 169.08, 167.52, 166.32, 163.12, 157.63, 153.72, 136.26, 134.59, 131.48, 130.68, 129.42, 127.61, 126.69, 126.32, 125.63, 123.55, 121.23, 121.11, 118.71, 105.81, 62.22, 55.46. MS: *m/z* 464 (M^+). Calcd. for $C_{22}H_{16}N_4O_2S_3$: C, 56.89; H, 3.44; N, 12.06. Found: C, 56.86; H, 3.49; N, 12.03 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2-methoxyphenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9i**): Solv. crystallization: THF. M.p.: 196 °C. % Yield 68. IR (KBr, cm^{-1}): 3250, 3109 (NH), 3010 (CH aromatic), 2933 (CH_2), 1630 (C=O), 1602 (C=N aromatic), 1593 (C=C aromatic), 1506 (amide II), 1255, 1033 (C-O-C), 771 (ortho-substituted benzene), 745 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.67–7.55 (m, 4H, ArH), 7.42 (s, 1H, CONH, D_2O exchangeable), 7.24–6.98 (m, 4H, ArH), 5.16 (s, 2H, OCH_2), 4.24 (s, 2H, SCH_2), 3.76 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6): δ 169.70, 167.32, 166.27, 162.58, 154.10, 146.23, 145.62, 136.12, 131.48, 125.57, 121.86, 121.73, 121.21, 121.02, 106.42, 115.18, 61.74, 55.45, 54.16. MS: *m/z* 444 (M^+). Calcd. for $C_{19}H_{16}N_4O_3S_3$: C, 51.35; H, 3.60; N, 12.61. Found: C, 51.39; H, 3.57; N, 12.59 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(4-nitrophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9j**): Solv. crystallization: THF. M.p.: 214 °C. % Yield 51. IR (KBr, cm^{-1}): 3252, 3097 (NH), 3062 (CH aromatic), 2926 (CH_2), 1641 (C=O), 1610 (C=N), 1593 (C=C aromatic), 1506 (amide II), 1491, 1336 (NO_2), 1259, 1028 (C-O-C), 848 (para-substituted benzene), 744 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 7.94–7.75 (m, 4H, ArH), 7.52 (s, 1H, CONH, D_2O exchangeable), 7.42–6.96 (m, 4H, ArH), 4.97 (s, 2H, OCH_2), 4.32 (s, 2H, SCH_2). MS: *m/z* 459 (M^+). Calcd. for $C_{18}H_{13}N_5O_4S_3$: C, 47.05; H, 2.83; N, 15.25. Found: C, 47.02; H, 2.87; N, 15.22 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(4-chloro-3-methylphenoxy)methyl]-1,3,4-thiadiazol-2-yl}acetamide (**9k**): Solv. crystallization: THF. M.p.: 210 °C. % Yield 55. IR (KBr, cm^{-1}): 3284, 3100 (NH), 3010 (CH aromatic), 2956 (CH_3), 2928 (CH_2), 1625 (C=O), 1615 (C=N), 1573 (C=C aromatic), 1525 (amide II), 1244, 1041 (C-O-C), 796 (C-Cl), 746 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.13–7.82 (m, 4H, ArH), 7.74–7.57 (m, 3H, ArH), 7.26 (s, 1H, CONH, D_2O exchangeable), 5.20 (s, 2H, OCH_2), 3.89 (s, 2H, SCH_2), 2.22 (s, 3H, CH_3). MS: *m/z* 481(M^++H_2O). Calcd. for $C_{19}H_{15}ClN_4O_2S_3$: C, 49.24; H, 3.23; N, 12.09. Found: C, 49.27; H, 3.20; N, 12.04 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2,4-dichlorophenoxy)methyl]-1,3,4-thiadiazol-2-yl} acetamide (**9l**): Solv. crystallization: methanol. M.p.: 129 °C. % Yield 45. IR (KBr, cm^{-1}): 3252, 3105 (NH), 3054 (CH aromatic), 2928 (CH_2), 1637 (C=O), 1605 (C=N), 1595 (C=C aromatic), 1506 (amide II), 1246, 1033 (C-O-C), 799 (C-Cl), 750 (C-S-C). MS: *m/z* 484 (M^+). ^{1}H NMR (DMSO-d_6): δ 7.98–7.81 (m, 4H, ArH), 7.79–7.64 (m, 3H, ArH), 7.29 (s, 1H, CONH, D_2O exchangeable), 5.16 (s, 2H, OCH_2), 3.95 (s, 2H, SCH_2). Calcd. for $C_{18}H_{12}Cl_2N_4O_2S_3$: C, 44.62; H, 2.47; N, 11.57. Found: C, 44.59; H, 2.44; N, 11.53 %.

2-(1,3-Benzothiazol-2-ylsulfanyl)-N-{5-[(2,4,6-tribromophenoxy)methyl]-1,3,4-thiadiazol-2-yl}acetamide (**9m**): Solv. crystallization: THF. M.p.: 150 °C. % Yield 72. IR (KBr, cm^{-1}): 3290, 3111 (NH), 3063 (CH aromatic), 2940 (CH_2), 1635 (C=O), 1604 (C=N), 1597 (C=C aromatic), 1518 (amide II), 1244, 1062 (C-O-C), 740 (C-S-C). ^{1}H NMR (DMSO-d_6): δ 8.06–7.84 (m, 4H, ArH), 7.79–7.72 (m, 2H, ArH), 7.16 (s, 1H, CONH, D_2O exchangeable), 5.13 (s, 2H, OCH_2), 3.89 (s, 2H, SCH_2). MS: *m/z* 651 (M^+). Calcd. for $C_{18}H_{11}Br_3N_4O_2S_3$: C, 33.17; H, 1.68; N, 8.60. Found: C, 33.14; H, 1.62; N, 8.58 %.

4. Biological Activity

Animals were procured from M/S Venkatesh Enterprises, Bangalore, India and were maintained in colony cages at 23 ± 2 °C, relative humidity of 45–50 %, maintained under 12 h light and dark cycle and fed with the standard rat pellet diet (Hindustan Liver Ltd., Mumbai). Prior approval of the Local Animal Ethical Committee was obtained to carry out the experimental work on animals. The synthesized compounds **6b**, **6d**, **6h**, **6i**, **6j**, **6k**, **7a**, **7c**, **9a**, **9b**, **9d**, **9h**, **9i** and **9j** were evaluated for their analgesic and anti-inflammatory activities. Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Student's *t*-test to assess the statistical significance.

4. 1. Analgesic Activity

The analgesic activity of the synthesized compounds was evaluated by the tail flick method[23] using Wistar albino mice (n = 6) of either sex. The test compounds and paracetamol were administered orally suspended in carboxymethyl cellulose (CMC 1.0 % w/v solution in water). The animals were held in position by a suitable restrainer with the tail extending out. The lower 5 cm of tail was gently immersed into thermostatistically controlled water at 55 ± 0.5 °C. The time in seconds for the tail withdrawal was taken as the reaction time with a cut-off time of immersion, set at 10 second for both control as well as treated groups of animals. The reaction time was recorded at 0.5, 1, 2 and 3 h after treatment. The percentage analgesic activity was calculated by the following formula,

$$(T_2 - T_1/10 - T_1) \times 100 ,$$

where T_1 is the reaction time (second) before treatment, T_2 is the reaction time (second) after treatment. Results are presented in Table 1.

4.2. Anti-inflammatory Activity

The acute anti-inflammatory activity of the synthesized compounds was determined following the carrageenan-induced paw oedema method[24] in albino rats (n = 6) of either sex. The animals were fasted for 24 h before the experiment with free access to water. The test compounds and diclofenac sodium were administered orally suspended in 1 % CMC. The control rats received appropriate volumes of 1 % CMC orally. Thirty minutes after administration of the test compounds, 0.1 mL carrageenan solution (1.0 % w/v in sterile saline) was injected into the sub-planter tissue of right hind paw of each rat. The volume of paws was measured at different time intervals of 0.5, 1, 2 and 3 h after carrageenan injection by plethysmometer (UGO Basile 7140, India). The percentage protection against inflammation was calculated by the following formula,

$$(V_c - V_t)/V_c \times 100 ,$$

where V_c is the oedema volume in control group and V_t is the oedema volume in groups treated with the test compounds. Results are presented in Table 2.

4.3. Ulcerogenic Effects

The test compounds **6d, 6i, 9d** and **9i** were evaluated for their acute ulcerogenic effects according to the method of Cioli *et al.*[25] in albino rats (n = 6) of either sex. The test compounds and diclofenac sodium were administered orally suspended in 1 % CMC. Control group received appropriate volumes of 1 % CMC. Food but not water was removed 24 h before administration of the test compounds. After compound treatment, the rats were fed with normal diet for 17 h and then sacrificed. Their stomachs were removed, cut out along the greater curvature and washed with distilled water and then gently cleaned by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring systems: 0.5 redness; 1.0 spot ulcers; 1.5 haemorrhagic streaks; 2.0 ulcers >3 but ≤5; 3.0 ulcers >5. The mean score of each treated group minus the mean score of control group was regarded as the severity index of gastric mucosal damage. Results are presented in Table 3.

4.4. Acute Toxicity

Acute oral toxicity was performed for the synthesized compounds **6b**, **6d**, **6h**, **6i**, **6j**, **6k**, **7a**, **7c, 9a**, **9b**, **9d**, **9h, 9i** and **9j** following the Organization of Economic Cooperation and Development (OECD-423) guidelines (acute toxic class method). Swiss albino mice (n = 3) of either sex selected by random sampling were used for the study. The animals were fasted for 3–4 h with water *ad libitum*, after which the test compounds (suspension in 1 % CMC) were administered orally at the doses of 50, 100, 250, 500 and 1000 mg kg^{-1} and the mice were observed for three days. No behavioral changes in animals were observed during the experiment and at the end hematological parameters were estimated and there was no observable change. In the present study, mortality was not observed even at 1000 mg kg^{-1} indicating that the compounds are nontoxic to animals.

4.5. Antimicrobial

The synthesized compounds **6a–m**, **7a–c, 8a**, **8b** and **9a–j** were tested for their *in vitro* antimicrobial activity by the cup plate method[26]. The antibacterial activity was evaluated on Muller Hinton agar (Hi-media) plates (37 °C, 24 h) against *Staphylococcus epidermidis, Enterococcus faecalis, Shigella dysenteriae* and *Pseudomonas aeruginosa.* The test compounds were also evaluated for their antifungal potential on Sabouraud dextrose agar (Hi-media) plates (26 °C, 48–72 h) against *Candida albicans* and *Aspergillus niger.* Ciprofloxacin and ketoconazole were used as control drugs and the results (Table 4) were recorded as the average diameter of inhibition zones (three independent determinations) of bacterial or fungal growth around the disks in mm.

Acknowledgements

The authors are most grateful to His Holiness Jagadguru Sri Sri Sri Shivarathri Deshikendra Mahaswamigalavaru of Sri Suttur Mutt, Mysore, for providing facilities.

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