European Journal of Medicinal Chemistry 51 (2012) 124–136

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Facile synthesis, characterization and pharmacological activities of 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles and 5,6-dihydro-3,6-disubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles

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ARTICLE INFO

Article history:
Received 26 October 2011
Received in revised form
15 February 2012
Accepted 16 February 2012
Available online 28 February 2012

Keywords:
Triazoles
Triazolo[3,4-*b*][1,3,4]thiadiazoles
Anti-inflammatory activity
Analgesic activity
Antioxidant activity
Anti-microbial activities

ABSTRACT

Two new series of compounds namely, 3,6-disubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadizoles (**5a**–**j**) and 5,6-dihydro-3,6-disubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadizoles (**7a**–**j**) were prepared. In continuation of a previously reported study, the first series (**5a**–**j**) were synthesized by the cyclo-condensation of 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**) with various substituted aromatic carboxylic acids in phosphorus oxychloride and the second series (**7a**–**j**) by the reaction of (**4**) with various substituted aromatic aldehydes in the presence of *p*-Toluene sulfonic acid. Reaction of (**4**) with the aldehyde (**9**) afforded the Schiff's base (**10**) and not the cyclised product (**11**) on treatment with *p*-Toluene sulfonic acid. Synthesized compounds were structurally confirmed by spectral analysis and studied for their anti-inflammatory, analgesic, anti-oxidant and antimicrobial activities. Some of the tested compounds showed significant pharmacological activities.

1. Introduction

The recent literature is enriched with progressive findings about the synthesis and pharmacological action of fused heterocycles. Various 1,2,4-triazole and their fused heterocyclic derivatives have received considerable attention due to their synthetic and biological importance. For example, various 1,2,4-triazole derivatives have been reported to possess versatile biological properties such as antibacterial [1,2], anti-fungal [3], antiviral [4], analgesic [5] and antimigraine [6] activities. The available therapeutically important medicines Terconazole, Itraconazole, Fluconazole, Cefazoline, Ribavarin, Triazolam, Alprazolam, Etizolam and Furacylin [7] are some of the examples which contain anyone of these heterocyclic nucleuses. The fused 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole and 5,6-dihydro-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives shows various biological properties such as anti-fungal [8,9], antibacterial [10,11], antiviral [12], anthelmentic [13,14], antitumour [15], analgesic [16] and anti-inflammatory activities [17,18].

Several methods were reported for the synthesis of 1,2,4–triazolo-[3,4-*b*][1,3,4]thiadiazole and 5,6-dihydro-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives and evaluated for their *in vitro* antimicrobial and anti-inflammatory activities [19–21].

In terms of compounds of interest in medicinal chemistry the most frequent cause of chirality results from the presence of a tetracoordinate carbon centre in a molecule to which four different groups are attached. The presence of one such centre in a molecule gives rise to a pair of enantiomers, the presence of n such centres gives rise to 2^n stereo isomers and half that number of pairs of enantiomers [22].

Prompted by these observations and continuation of previously reported [23,24] studies on heterocyclic derivatives of methyl 2-bromo-5-methoxybenzoate (**1**), it was planned to investigate a compositae system of bio labile components in a ring to give a compact and planar structure like fused 1,2,4–triazolo-[3,4-*b*][1,3,4]thiadiazole and optically active 5,6-dihydro-1,2,4-triazolo

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doi:10.1016/j.ejmech.2012.02.030

[3,4-*b*][1,3,4]thiadiazole derivatives and were screened for their biological activities.

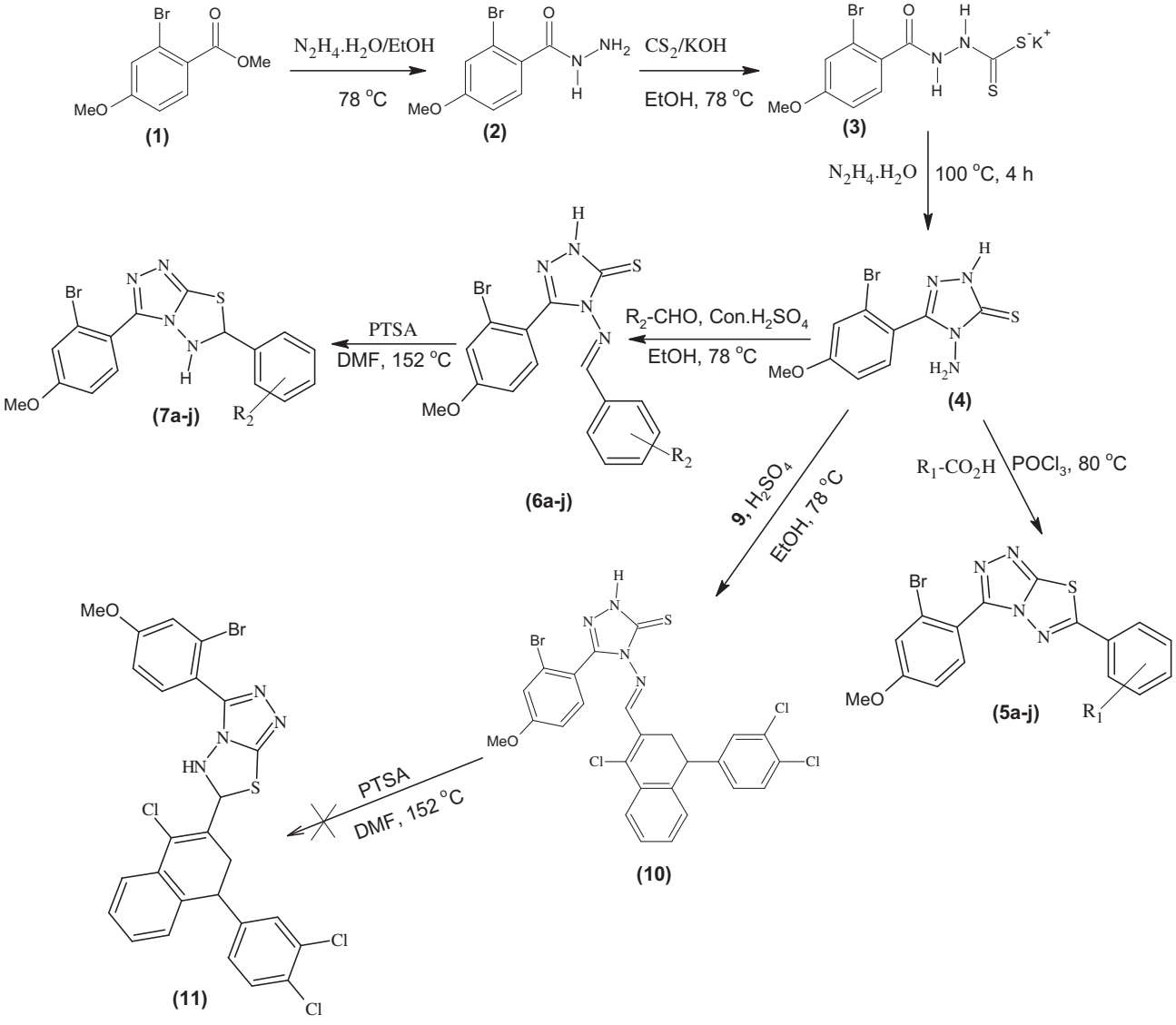
2. Results and discussion

2.1. Chemistry

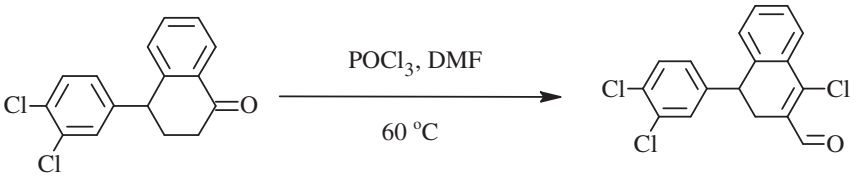
The acid hydrazide (**2**) was prepared by esterification of 2-bromo-5-methoxy benzoic acid followed by treatment with hydrazine hydrate in absolute ethanol. 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**) was prepared by following the procedure of Reid and Heindel [25]. The acid hydrazide (**2**) was allowed to react with carbon disulphide in the presence of potassium hydroxide in ethanol to afford the corresponding intermediate potassium dithiocarbazinate (**3**). This salt underwent ring closure with an excess of 99% hydrazine hydrate to give 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**). The resultant triazole (**4**) was further converted to 3,6-disubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles (**5a**–**j**) through one–pot reaction by condensation followed by cyclization with mono or disubstituted aromatic carboxylic acids in the presence of phosphorus oxychloride at reflux temperature. Phosphorus oxychloride was necessary for this condensation, which activate the carbonyl group of aromatic acids and increases its electrophilicity to enhance the addition of triazole to it.

5,6-dihydro-3,6-disubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles were also prepared by treating 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**) with different mono or disubstituted aromatic aldehydes in ethanol in the presence of few drops of Conc. H_2SO_4. The Schiff's base formed was further cyclised by refluxing in DMF in the presence catalytic amount of *p*-Toluene sulfonic acid. The reaction pathway is summarized in Schemes 1 and 2.

The structure of 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**) was confirmed by IR, NMR, MASS spectral data and microanalyses. Compound (**4**) can exist in two tautomeric forms, 4-amino-5-(2-bromo-5-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol and 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione. The spectral analysis shows that it exists in the latter form, *e.g.*, in the ^{1}H-NMR spectra, δ 13.96 can be only attributed to NH–C = S rather than to S–H and in the IR



Scheme 1. Synthetic route for the compounds **5a**–**j**, **7a**–**j** and **11**.



(8) (9)

Scheme 2. Synthetic route for the compound **9**.

spectrum, the S–H vibration band (2500 cm^{-1}) was absent. The ^{1}H-NMR spectrum showed singlet for NH_2 group at δ 5.47 ppm.

The absence of signals due to NH_2 and –N = C–SH (–NH–C = S of the tautomer) protons in the compounds (**5a**–**j**) confirmed the cyclization of triazole (**4**) by reacting with COOH group of substituted aromatic acids to afford triazolo-thiadiazoles (**5a**–**j**). An absorption band was observed for all the synthesized compounds in the range of 3046.45–3096.83 cm^{-1} may be attributed for aromatic stretching vibration, while that seen at 1575.12–1615.02 cm^{-1} corresponds to C=N linkage. Thus, the formation of iminomethine functional group in the compound was indicated.

On treatment of triazole (**4**) with the appropriate aromatic aldehydes in refluxing ethanol afforded only open chain hydrazones (**6a**–**j**). The structures of compounds (**6a**–**j**) were confirmed on the basis of IR, ^{1}H-NMR, ^{13}C NMR and mass spectral data. The IR spectra of these compounds showed common characteristic absorption peaks at 3306.92–3317.24 cm^{-1} (NH) and 3059.92–3090.46 cm^{-1} (Ar C–H). The ^{1}H-NMR spectra are characterized by the presence of the CH=N proton appeared as a singlet at δ 9.43–10.59 ppm, while NH/tautomeric with SH proton appeared around δ 13.38–14.43 ppm. The ^{13}C NMR spectra are characterized by the presence of the CH=N carbon at δ 158.85–158.96 ppm.

These hydrazones undergoes intramolecular Mannich reaction in the presence of catalytic amount of *p*-Toluene sulfonic acid to give corresponding 5,6-dihydro-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives (**7a**–**j**). The compounds (**7a**–**j**) contain one stereocenter at C_6, to which four different groups are attached. Due to this chirality, the molecule can exist in two enantiomeric forms. Hence the compounds (**7a**–**j**) are optically active. The IR spectra of the cyclized products (**7a**–**j**) showed absorption peaks at 3208.42–3220.04 cm^{-1} (thiadiazole NH) and 3186.73–3194.89 cm^{-1} (Ar–C–H). The absorption bands were observed for all the synthesized compounds in the range of 1581.46–1591.61 cm^{-1} attributed for C=N linkage. Thus, the formation of iminomethine functional group in the compound was indicated. In the ^{1}H-NMR spectra, the absence of CH=N proton in the region δ 9.43–10.59 ppm confirms the intramolecular cyclization. The ^{1}H-NMR spectra of compounds (**7a**–**j**) also showed singlet in the region δ 5.46–5.52 ppm due to the presence C_6 proton of thiadiazole ring. The NH proton of thiadiazole ring resonated as broad singlet at 13.42–13.98 ppm. The IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses supported the structure of various synthesized dihydro analogues of triazolo thiadiazoles.

We also prepared a new compound 1-Chloro-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalene-2-carbaldehyde (**9**) in good yield by Vilsmeier–Haack reaction of 4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-1(2*H*)-one (**8**). The structure of the compound (**9**) was confirmed by spectral and X-ray crystallographic data [26,27]. The intermediate compound 4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-1(2*H*)-one (**8**) was prepared by the previously reported procedure [28]. The above carbaldehyde further reacted with 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**) to give triazolo Schiff's base (**10**). Further we attempted intramolecular cyclization of (**10**) with *p*-Toluene sulfonic acid in refluxing DMF to the respective 5,6-dihydro-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole (**11**) was unsuccessful under conventional conditions.

The spectral values for all the compounds and C, H, N analyses are given in the experimental part. Both analytical and spectral data (IR, NMR, MS and elemental analysis) of all the synthesized compounds were in full agreement with the proposed structure. The physicochemical properties of synthesized compounds were presented in Tables 1and 2.

3. Pharmacological screening

3.1. Pharmacology

All newly synthesized compounds were evaluated for anti-inflammatory and analgesic activities. The tested compounds were administered in the form of a suspension (1% carboxy methyl cellulose as vehicle). Anti-inflammatory and analgesic activities of the tested compounds were measured with respect to the control and compared with the standard drugs Diclo and pentazocine respectively. All the pharmacological data are expressed as

Table 1
Characterization data of the compounds **2**, **4** and **5a**–**j**.

Comp. no.	R_1	Recryst. solvent	Mol. Formula (mol. wt.)	M.p. (°C)	Yield (%)
2	–	Ethanol	$C_8H_9BrN_2O_2$ (245.07)	190–192	98
4	–	Ethanol	$C_9H_9BrN_4OS$ (301.16)	184–186	74
5a	4-methoxy phenyl	Ethanol	$C_{17}H_{13}BrN_4O_2S$ (417.28)	142–146	68
5b	4-methyl phenyl	Ethanol	$C_{17}H_{13}BrN_4OS$ (401.28)	148–150	72
5c	phenyl	Ethanol	$C_{16}H_{11}BrN_4OS$ (387.25)	110–112	65
5d	3,5-dichlorophenyl	Ethanol	$C_{16}H_9BrCl_2N_4OS$ (456.14)	188–190	70
5e	4-amino phenyl	Ethyl acetate	$C_{16}H_{12}BrN_5OS$ (402.27)	190–192	66
5f	3,5-dimethyl phenyl	Ethanol	$C_{18}H_{15}BrN_4OS$ (415.30)	166–168	64
5g	4-nitro phenyl	Ethyl acetate	$C_{16}H_{10}BrN_5O_3S$ (432.25)	204–206	72
5h	3,5-dinitro phenyl	Ethyl acetate	$C_{16}H_9BrN_6O_5S$ (477.25)	178–180	78
5i	2-hydroxy-4-methyl phenyl	Ethanol	$C_{17}H_{13}BrN_4O_2S$ (417.28)	180–182	62
5j	2,4-diiodo phenyl	Ethyl acetate	$C_{16}H_9BrI_2N_4OS$ (639.04)	196–198	69

Table 2
Characterization data of the compounds **6a**–**j**, **7a**–**j**, **9** and **10**.

Comp. no.	R_2	Recryst. solvent	Mol. Formula (mol. wt.)	M.p. (°C)	Yield (%)
6a	2-chloro phenyl	Ethanol	$C_{16}H_{12}BrClN_4OS$ (423.71)	164–166	70
6b	phenyl	Ethanol	$C_{16}H_{13}BrN_4OS$ (389.23)	142–144	68
6c	4-chloro phenyl	Ethanol	$C_{16}H_{12}BrClN_4OS$ (423.71)	178–180	72
6d	3-chloro phenyl	Ethanol	$C_{16}H_{12}BrClN_4OS$ (423.71)	180–182	69
6e	2,4-dimethoxy phenyl	Ethanol	$C_{18}H_{17}BrN_4O_3S$ (449.32)	222–224	75
6f	4-methoxy phenyl	Ethanol	$C_{17}H_{15}BrN_4O_2S$ (419.29)	192–194	78
6g	2-methoxy phenyl	Ethanol	$C_{17}H_{15}BrN_4O_2S$ (419.29)	194–196	76
6h	4-nitro phenyl	Ethyl acetate	$C_{16}H_{12}BrN_5O_3S$ (434.26)	172–174	69
6i	biphenyl	Ethanol	$C_{22}H_{17}BrN_4OS$ (465.36)	162–164	74
6j	4-methyl phenyl	Ethanol	$C_{17}H_{15}BrN_4OS$ (403.29)	128–130	67
7a	2-chloro phenyl	Ethanol	$C_{16}H_{12}BrClN_4OS$ (423.71)	190–192	72
7b	phenyl	Ethanol	$C_{16}H_{13}BrN_4OS$ (389.23)	260–264	66
7c	4-chloro phenyl	Ethanol	$C_{16}H_{12}BrClN_4OS$ (423.71)	146–148	78
7d	3-chloro phenyl	Ethanol	$C_{16}H_{12}BrClN_4OS$ (423.71)	176–178	75
7e	2,4-dimethoxy phenyl	Ethanol	$C_{18}H_{17}BrN_4O_3S$ (449.32)	180–186	73
7f	4-methoxy phenyl	Ethanol	$C_{17}H_{15}BrN_4O_2S$ (419.29)	172–174	76
7g	2-methoxy phenyl	Ethanol	$C_{17}H_{15}BrN_4O_2S$ (419.29)	170–172	72
7h	4-nitro phenyl	Ethyl acetate	$C_{16}H_{12}BrN_5O_3S$ (434.26)	210–212	70
7i	biphenyl	Ethanol	$C_{22}H_{17}BrN_4OS$ (465.36)	202–204	79
7j	4-methyl phenyl	Ethanol	$C_{17}H_{15}BrN_4OS$ (403.29)	186–188	68
9	–	Ethyl acetate	$C_{17}H_{11}Cl_3O$ (337)	108–110	78
10	–	Ethanol	$C_{26}H_{18}BrCl_3N_4OS$ (620.77)	152–154	75

mean ± SEM; statistical analysis was applied to determine the significance of the difference between the control group and group of animals tested with the tested compounds. The compounds which showed potent anti-inflammatory and analgesic activities also screened for their *in vitro* anti-oxidant activity by DPPH scavenging method. Ascorbic acid was used as a positive control and measurements was run in triplicate. The percentage of scavenging activity was calculated.

We investigated all the newly synthesized compounds for their antibacterial activity against four bacterial strains, *viz*., *Staphylococcus aureus* (ATTC-25923), *Escherichia coli* (ATTC-25922), *Pseudomonas aeruginosa* (ATTC-27853) and *Klebsiella pneumoniae* (recultured). Newly synthesized compounds were also tested for anti-fungal activity against *Penicillium marneffei* (recultred), *Trichophyton mentagrophytes* (recultured), Aspergillus flavus (NICM No.524) and *Aspergillus fumigatus* (NCIM No.902) fungal strains.

3.2. Anti-inflammatory activity

The 1,2,4-triazolo[3,4-*b*][1,3,4]thiadizoles **5d**, **5e**, **5g** and **5h** containing 3,5-dichlorophenyl, 4-amino phenyl, 4-nitro phenyl, 3,5-dinitro phenyl groups and 5,6-dihydro-1,2,4-triazolo[3,4-*b*][1,3,4] thiadizoles **7a**, **7c**, **7d** and **7h** containing 2-chloro phenyl, 4-chloro phenyl, 3-chloro phenyl and4-nitro phenyl groups respectively are the most potent agents of this series against rat-foot inflammation. According to the study, 1,2,4-triazole (**4**) itself showed good activity, but it is observed that activity is further increased by the formation of thiadiazole ring. Therefore according to structure activity relationship, the functional groups like chloro, nitro and amino are responsible for the excellent activity against rat-foot inflammation. The compounds **5a**, **5b**, **5i**, **7e**, **7f**, **7g** and **7j** containing 4-methoxy phenyl, 4-methyl phenyl, 2-hydroxy-4-methyl phenyl, 2,4-dimethoxy phenyl, 4-methoxy phenyl, 2-methoxy and 4-methyl phenyl substituent's respectively showed good anti-inflammatory activity. The results were presented in Table 3.

3.3. Analgesic activity

The 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**) itself showed potent analgesic activity compared to a standard drug pentazocine. From the analgesic study it was observed that the formation of thiadiazole ring will not further enhanced the activity of synthesized compounds. Like 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**), the compounds **5d**, **5e**, **5g** and **5h** showed potent activity compared to a standard drug pentazocine. The good activity of these compounds are attributed in the presence of 3,5-dichlorophenyl, 4-amino phenyl, 4-nitro phenyl, 3,5-dinitro phenyl groups. Similarly 5,6-dihydro-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles **7a**, **7c**, **7d**, **7f** and **7h** containing 2-chloro phenyl, 4-chloro phenyl, 3-chloro phenyl, 4-methoxy phenyl and 4-nitro phenyl respectively showed potent activity. The compounds **5a**, **5b**, **5i**, **7b**, **7e**, **7f**, **7g** and **7j** containing 4-methoxy phenyl, 4-methyl phenyl, 2-hydroxy-4-methyl phenyl, phenyl, 2,4-dimethoxy phenyl, 4-methoxy phenyl, 2-methoxy and 4-methyl phenyl substituent's respectively showed good analgesic activity. The results were illustrated in Table 4.

3.4. Anti-oxidant activity

3.4.1. DPPH radical scavenging activity

The compounds having potent anti-inflammatory and analgesic activity are also tested for their anti-oxidant activity. The compounds **5e**, **5g**, **5h**, **7h** and **7f** showed comparatively significant anti-oxidant activities. The good activity of these compounds may be due to the presence of strong electron withdrawing group (NO_2) or para substituted phenyl groups. The remaining compounds **4, 5d**, **7a**, **7c** and **7d** showed moderate activity compared to the positive control ascorbic acid. These compounds contain chloro/methoxy functional groups in their structure. The results were tabulated in Table 5. Fig. 1 depicts the percentage of free radical scavenging activity of tested compounds using DPPH method.

3.5. Antibacterial activity

The investigation of antibacterial screening data revealed that all the tested compounds **4**, **5a**–**j** and **7a**–**j** showed only moderate activities. The compound 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **4** alone showed moderate activity against *E. coli* bacterial strain. The

Table 3
Anti-inflammatory activity of the compounds **4**, **5a–j** and **7a–j**.

Compound	Dose (mg/kg)	60 min	120 min	180 min
Control	100	2.175 ± 0.1797	2.375 ± 0.1436	2.275 ± 0.1181
DICLO	100	0.6 ± 0.04082***	0.625 ± 0.04787***	0.625 ± 0.04787***
4	100	1.491 ± 0.1913*	1.64 ± 0.1423**	1.72 ± 0.1358*
5a	100	1.605 ± 0.1402*	1.70 ± 0.0921**	1.73 ± 0.1552*
5b	100	1.576 ± 0.1701*	1.66 ± 0.1324**	1.77 ± 0.1260*
5c	100	1.88 ± 0.1688	1.985 ± 0.1802	2.035 ± 0.0915
5d	100	1.575 ± 0.0677**	1.65 ± 0.06455**	1.6 ± 0.07071**
5e	100	1.6 ± 0.0778**	1.75 ± 0.1936**	1.875 ± 0.1315
5f	100	1.845 ± 0.08739	1.85 ± 0.2041	2.055 ± 0.1011
5g	100	1.45 ± 0.06455**	1.8 ± 0.108*	1.975 ± 0.1109
5h	100	1.51 ± 0.06564**	1.78 ± 0.106*	1.899 ± 0.1010
5i	100	1.577 ± 0.1722*	1.68 ± 0.1383**	1.77 ± 0.1263*
5j	100	2.15 ± 0.09574	2.25 ± 0.0866	2.25 ± 0.06455
7a	100	1.45 ± 0.06455**	1.75 ± 0.1555**	1.9 ± 0.1581
7b	100	2 ± 0.09129	2.05 ± 0.0866	2.2 ± 0.1213
7c	100	1.515 ± 0.1552**	1.89 ± 0.098	1.98 ± 0.1743
7d	100	1.525 ± 0.1652**	1.9 ± 0.108	1.95 ± 0.1443
7e	100	1.545 ± 0.2012*	1.68 ± 0.1601**	1.73 ± 0.1451*
7f	100	1.581 ± 0.1801*	1.59 ± 0.0843**	1.69 ± 0.1336*
7g	100	1.585 ± 0.1803*	1.59 ± 0.1323**	1.69 ± 0.1828*
7h	100	1.45 ± 0.1658**	1.875 ± 0.1548	2.075 ± 0.1548
7i	100	2.125 ± 0.06292	2.15 ± 0.06455	1.9 ± 0.178
7j	100	1.498 ± 0.1692*	1.60 ± 0.1463**	1.80 ± 0.0154*

Results were expressed in mean ± SE M. ($n = 6$) significance levels $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the respective control.

compound **5a** showed moderate activity towards all the four bacterial strains. This may be attributed because of the presence of methoxy group in para position of the phenyl ring. The compound **5b** is moderately active towards *Staphyllococcus aureus*, *E. coli* and *K. pneumoniae* bacterial strains. This compound contains para-methyl phenyl substituent in their structure. The compound **5h** showed moderate activity against *Staphyllococcus aureus*, *E. coli* and *K. pneumoniae*. The compounds **5d**, **5f**, **5i**, **7c**, **7e** and **7j** are moderately active towards three bacterial strains namely, *E. coli*, *Psedomonus Aeruginosa P. aeruginosa* and *K. pneumoniae*. All these compounds contain electron releasing or electron withdrawing groups in their structure either in 3,5 or para positions. The compound **5e** is active against *E. coli* and *P. aeruginosa* bacterial strains. The compound **7g** showed moderate activity towards *E. coli* bacterial strain compared to the standard drug ampicillin. The compounds **5e** and **7g** contain electron emancipating groups like $-NH_2$ or $-OCH_3$ respectively. The compound **7h** showed moderate activity towards *P. aeruginosa* and *K. pneumoniae* bacterial strains. The compounds **5h** and **7h** contain electron diminishing $-NO_2$ group in their structure. The compound **7i** showed moderate activity against *K. pneumoniae* bacterial strain. On after going through structure activity relationship, it is observed that the construction of the thiadiazole ring fused to the triazole moiety widen the activity of the compound compared to 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **4** alone. The results were summarized in Table 6.

Table 4
Analgesic activity of the newly synthesized compounds **4**, **5a–j** and **7a–j**.

Compound	Dose (mg/kg)	60 min	120 min	180 min
Control	100	1.363 ± 0.1297	1.363 ± 0.1297	1.363 ± 0.1297
Pentazocine	100	7.075 ± 0.1164***	6.863 ± 0.1919***	7.025 ± 0.2504***
4	100	3.463 ± 0.3085**	3.388 ± 0.2968**	2.338 ± 0.1329**
5a	100	2.379 ± 0.09035*	2.674 ± 0.2902**	2.440 ± 0.0834
5b	100	2.384 ± 0.08962*	2.681 ± 0.2808**	2.470 ± 0.161
5c	100	1.963 ± 0.2202	2.125 ± 0.09242	2.5 ± 0.1399
5d	100	3.421 ± 0.2653**	3.075 ± 0.5622*	2.388 ± 0.08985*
5e	100	3.675 ± 0.8125**	3.563 ± 0.7031**	2.425 ± 0.2385**
5f	100	2.175 ± 0.08539	2.075 ± 0.101	2.238 ± 0.0875
5g	100	3.375 ± 0.4789**	3.263 ± 0.3243**	2.663 ± 0.2831**
5h	100	3.675 ± 0.8125**	3.563 ± 0.7031**	2.425 ± 0.2385**
5i	100	2.388 ± 0.08985*	3.321 ± 0.1603**	3.121 ± 0.1501
5j	100	2.038 ± 0.07739	2.088 ± 0.07739	2.225 ± 0.07773
7a	100	3.6 ± 0.1242**	3.25 ± 0.1581*	2.938 ± 0.1784*
7b	100	3.181 ± 0.6140*	3.460 ± 0.2710**	3.391 ± 0.2401
7c	100	3.675 ± 0.8125**	3.563 ± 0.7031**	2.425 ± 0.2385**
7d	100	3.546 ± 0.6835**	3.623 ± 0.7631**	2.235 ± 0.0485*
7e	100	2.390 ± 0.09014*	2.642 ± 0.2730**	2.492 ± 0.2342
7f	100	3.468 ± 0.5719**	3.334 ± 0.3953**	2.841 ± 0.4611*
7g	100	2.387 ± 0.0765*	2.652 ± 0.3436**	2.501 ± 0.1922
7h	100	3.675 ± 0.8125**	3.563 ± 0.7031**	2.425 ± 0.2385**
7i	100	2.102 ± 0.0673	2.067 ± 0.0803	2.455 ± 0.2616
7j	100	2.403 ± 0.0902*	2.643 ± 0.2713**	2.438 ± 0.1924

Results were expressed in mean ± SE M. ($n = 6$) significance levels $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the respective control.

Table 5
DPPH radical scavenging assay for some selective derivatives.

Compounds	Concentration (ug/mL)		
	200	400	800
	% inhibition	% inhibition	% inhibition
4	52.67	56.84	67.45
5d	49.75	54.29	66.82
5e	60.32	67.98	75.92
5g	62.91	68.14	71.86
5h	58.55	68.40	78.01
7a	47.96	51.81	62.73
7c	42.30	48.06	63.39
7d	35.85	44.72	62.55
7h	53.24	72.42	82.65
7f	40.56	65.87	77.92
Ascorbic acid	87.57	94.97	96

3.6. Antifungal activity

The anti-fungal screening data also revealed that all the newly synthesized compounds were moderate anti-fungal agents. The compounds **4**, **5g** and **7c** showed moderate activity against all the four fungal strains. The compound **5d** showed moderate activity against three fungal strains namely, *P. marneffei*, *T. mentagrophytes* and *A. fumigatus*. The compounds **7b** and **7f** are active against *P. marneffei* and *A. fumigatus* fungal strains. The compounds **5f**, **5h**, **5i** and **7g** showed moderate activity towards *T. mentagrophytes*, Aspergillus flavus and *A. fumigatus* fungal strains. The compound **7h** moderately active against three fungal strains, *P. marneffei*, Aspergillus flavus and *A. fumigatus*. The compounds **7a** and **7j** showed moderate activity against Aspergillus flavus and *A. fumigatus* fungal strains compared to a standard drug itraconozole. The compound **5a** showed moderate anti-fungal activity against *T. mentagrophytes* and *A. fumigatus*. Similarly the compounds **5c**, **5b** and **7i** showed moderate activity against *P. marneffei*, *T. mentagrophytes* and Aspergillus flavus respectively. The anti-fungal study revealed that, the activities of triaolothiadiazoles are not enhanced by the formation of thiadiazole ring fused to triazole moiety. Results of anti-fungal studies are presented in Table 7.

4. Conclusion

Several 3-(2-bromo-5-methoxyphenyl)-6-(substituted)[1,2,4]-triazolo [3,4-*b*][1,3,4]thiadiazole (**5a**–**j**) and 3-(2-bromo-5-methoxyphenyl)-6-(sustituted phenyl)-5,6-dihydro[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazole (**7a**–**j**) derivatives were successfully synthesized in 62–79% yields. The newly synthesized compounds are characterized by ^{1}H NMR, ^{13}C NMR, Mass spectrometry and IR studies. The compounds **5d**, **5e**, **5g**, **5h**, **7a**, **7c**, **7d** and **7h** showed potent anti-inflammatory activity. Whereas **4**, **5d**, **5e**, **5g**, **5h**, **7a**, **7c**, **7d**, **7f** and **7h** are potent analgesic compounds. Thus the anti-inflammatory and analgesic study revealed that, the compounds having strong electron withdrawing groups (NO_2, Cl) in their structures are potent anti-inflammatory and analgesic agents.

The anti-oxidant study results revealed that the compounds **5e**, **5g**, **5h**, **7h** and **7f** are very good antioxidants. The significant activity of these compounds may be attributed to the presence of strong electron withdrawing group (NO_2) or para substituted phenyl groups. The remaining compounds **4**, **5d**, **7a**, **7c** and **7d** showed moderate activity when compared to the positive control ascorbic acid. These compounds contain chloro/methoxy functional groups in their structure.

The majority of synthesized compounds showed moderate antibacterial activity compared to a standard drug ampicillin. On the view of structure activity relationship, it is observed that the construction of thiadiazole ring fused to triazole moiety widen the activity of the compound compared to 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **4** alone. There is no considerable increase in the anti-fungal activity is observed by the formation of thiadiazole ring fused to triazole moiety compared to 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **4** alone.

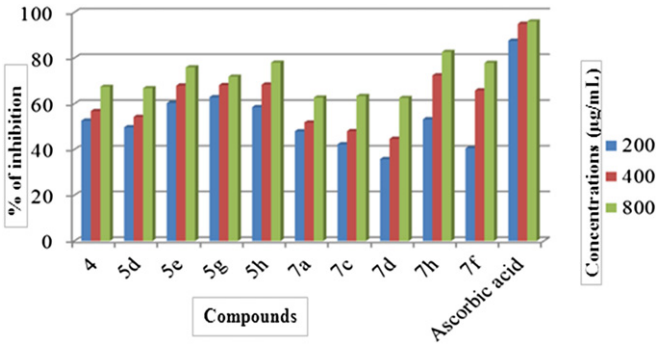


Fig. 1. DPPH radical scavenging assay for some selective compounds. a) Concentrations are given in µg/mL Blue-200 µg/mL Red-400 µg/mL Green-800 µg/mL b) Compound numbers are shown in *X*-axis c) Radical scavenging activity was expressed as the percentage of inhibition on *Y*-axis d) Ascorbic acid was used as a positive control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

5. Experimental section

5.1. Materials and methods

All solvents used were of analytical grade and the reagents were used was purchased. DPPH was procured from Sigma–Aldrich. All melting points were determined by open capillary method and are uncorrected. IR spectra were obtained in KBr disc on a Shimadzu FT-IR 157 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded either on a Perkin–Elmer EM-390 or on a Bruker WH-200 (400 MHz or 300 MHz) in $CDCl_3$ or DMSO-d_6 as a solvent, using TMS as an internal standard and chemical shifts are expressed as ppm. Mass spectra were determined on a Jeol SX 102/Da-600 mass spectrometer/Data System using Argon/Xenon (6kv, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. The LCMS were recorded in MDSSCIEX, API 4000 spectrometer. The elemental analyses (CHN) were performed on CHNS-O-analyser Flash EA 1112 series. The progress of the reaction was monitored by TLC on pre-coated silica gel G plates.

All the spectral data's of newly synthesized compounds are consistent with proposed structure and microanalysis within ±0.3 of the calculated values.

5.2. Conventional synthesis of 2-bromo-5-methoxy-benzoic acid hydrazide (*2*)

A mixture of 2-bromo-5-methoxy-benzoic acid methyl ester (**1**) (0.01 mol) in ethanol (10 mL) and hydrazine hydrate (0.01 mol) was refluxed for 4 h. The completion of reaction was monitored by TLC. After reaction completion the excess solvent was removed by distillation. The reaction mass was then cooled to 5 °C, the solid separated was filtered, dried and recrystallized from ethanol.

IR (KBr) γ/cm^{-1}: 3352.4 (-NH_2 Stretch), 3230.3 (>NH Stretch), 2845 (-OCH_3), 1640.65 (>C=O stretch), 764 (>C–Br). ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.77 (ss, 3H, –OCH_3), 4.45 (ss, 2H, –NH_2),

Table 6
Antibacterial activity of the newly synthesized compounds **4**, **5a**–**j** and **7a**–**j**.

Compounds	MIC (in μM) and zone of inhibition (mm) in parentheses			
	Staphylococcus aureus (ATTC-25923)	*Escherichia coli* (ATTC-25922)	*Pseudomonas aeruginosa* (ATTC-27853)	*Klebsiella pneumoniae* (recultured)
4	41.50 (11–15)	20.75 (16–20)	41.50 (11–15)	20.75 (16–20)
5a	14.97 (16–20)	14.97 (16–20)	14.97 (16–20)	14.97 (16–20)
5b	15.75 (16–20)	15.75 (16–20)	31.15 (11–15)	15.75 (16–20)
5c	32.27 (11–15)	32.27 (11–15)	32.27 (11–15)	32.27 (11–15)
5d	27.40 (11–15)	13.70 (16–20)	13.70 (16–20)	13.70 (16–20)
5e	31.07 (11–15)	31.07 (11–15)	15.53 (16–20)	31.07 (11–15)
5f	30.09 (11–15)	15.05 (16–20)	15.05 (16–20)	15.05 (16–20)
5g	28.92 (11–15)	28.92 (11–15)	28.92 (11–15)	28.92 (11–15)
5h	13.09 (16–20)	13.09 (16–20)	26.19 (11–15)	13.09 (16–20)
5i	29.95 (11–15)	14.97 (16–20)	14.97 (16–20)	14.97 (16–20)
5j	19.56 (11–15)	19.56 (11–15)	19.56 (11–15)	19.56 (11–15)
7a	14.75 (16–20)	29.50 (11–15)	29.50 (11–15)	29.50 (11–15)
7b	32.11 (11–15)	16.05 (16–20)	32.11 (11–15)	32.11 (11–15)
7c	29.50 (11–15)	14.75 (16–20)	14.75(16–20)	14.75 (16–20)
7d	29.50 (11–15)	29.50 (11–15)	29.50 (11–15)	29.50 (11–15)
7e	27.81 (11–15)	13.90 (16–20)	13.90 (16–20)	13.90 (16–20)
7f	29.81 (11–15)	29.81 (11–15)	29.81 (11–15)	29.81 (11–15)
7g	29.81 (11–15)	14.90 (16–20)	29.81 (11–15)	29.81 (11–15)
7h	28.78 (11–15)	28.78 (11–15)	14.39 (16–20)	14.39 (16–20)
7i	26.86 (11–15)	26.86 (11–15)	26.86 (11–15)	13.43 (16–20)
7j	30.99 (11–15)	15.49 (16–20)	15.49 (16–20)	15.49 (16–20)
Standard (Ampicillin)	4.46 (22–30)	17.88 (30–40)	17.88 (25–33)	17.88 (23–27)

6.89–6.92 (m, 1H, ArH), 6.94–6.95 (d, 1H, J = 3.09 Hz, ArH), 7.49–7.52 (d, 1H, J = 8.67 Hz, ArH), 9.52 (ss, 1H, NH); ^{13}C NMR: (100 MHz, DMSO-d6): δ 55.04 (OCH_3 carbon), 113.73, 119.42, 121.91, 129.13, 131.39, 159.45, 166.32, ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 55.01(OCH_3 carbon), 113.69, 119.39, 121.87; MS: m/z = 246 (M+1); Anal. calcd. for $C_8H_9BrN_2O_2$: C, 39.28; H, 3.77; N, 11.48; Found: C, 39.20; H, 3.70; N, 11.43%.

5.3. Conventional synthesis of 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (*4*)

The acid hydrazide (**2**) (0.01 mol) was added to absolute alcohol (15 mL) containing KOH (1.6 g) at ambient temperature. Carbon disulphide (0.013 mol) was added and the mixture was stirred at ambient temperature for 10 h. The mixture was diluted with ether (10 mL) and stirred for further 1 h. The potassium salt (**3**) separated was filtered and washed with ether (5 mL). The potassium salt (**3**) was used for the next stage without further purification. Hydrazine hydrate (99%) (0.02 mol) was gradually added to the above potassium salt (0.01 mol) dissolved in water (12 mL) with stirring and the mixture was refluxed gently for 3 h during which hydrogen sulphide evolved and the colour of the reaction mixture changed to dark green colour. It was then cooled to 5 °C and acidified with Conc. HCl to pH 1.00. A yellow solid separated out was filtered, washed with water and crystallized from ethanol to obtain pure triazole (**4**).

Table 7
Antifungal activity of the newly synthesized compounds **4**, **5a**–**j** and **7a**–**j**.

Compounds	MIC (in μM) and zone of inhibition (mm) in parentheses			
	Penicillium marneffei (recultured)	*Trichophyton mentagrophytes* (recultured)	*Aspergillus flavus* (NCIM No.524)	*Aspergillus fumigatus* (NCIM No.902)
4	20.75 (16–20)	20.75 (16–20)	20.75 (16–20)	20.75 (16–20)
5a	29.95 (11–15)	14.97 (16–20)	29.95 (11–15)	14.97 (16–20)
5b	31.15 (11–15)	15.75 (16–20)	31.15 (11–15)	31.15 (11–15)
5c	16.13 (16–20)	32.27 (11–15)	32.27 (11–15)	32.27 (11–15)
5d	13.70 (16–20)	13.70 (16–20)	27.40 (11–15)	13.70 (16–20)
5e	31.07 (11–15)	15.53 (16–20)	31.07 (11–15)	31.07 (11–15)
5f	30.09 (11–15)	15.05 (16–20)	15.05 (16–20)	15.05 (16–20)
5g	14.46 (16–20)	14.46 (16–20)	14.46 (16–20)	14.46 (16–20)
5h	26.19 (11–15)	13.09 (16–20)	13.09 (16–20)	13.09 (16–20)
5i	29.95 (11–15)	14.97 (16–20)	14.97 (16–20)	14.97 (16–20)
5j	19.56 (11–15)	19.56 (11–15)	19.56 (11–15)	19.56 (11–15)
7a	29.50 (11–15)	29.50 (11–15)	14.75 (16–20)	14.75 (16–20)
7b	6.25 (16–20)	12.5 (11–15)	12.5 (11–15)	6.25 (16–20)
7c	16.05 (11–15)	16.05 (16–20)	16.05 (16–20)	16.05 (16–20)
7d	29.50 (11–15)	29.50 (11–15)	29.50 (11–15)	29.50 (11–15)
7e	13.90 (16–20)	27.81 (11–15)	27.81 (11–15)	27.81 (11–15)
7f	14.90 (16–20)	29.81 (11–15)	29.81(11–15)	14.90 (16–20)
7g	29.87 (11–15)	14.90 (16–20)	14.90 (16–20)	14.90 (16–20)
7h	14.39 (16–20)	28.78 (11–15)	14.39 (16–20)	14.39 (16–20)
7i	26.86 (11–15)	26.86 (11–15)	13.43 (16–20)	26.86 (11–15)
7j	30.99 (11–15)	30.99 (11–15)	15.49 (16–20)	15.49 (16–20)
Standard (Itraconozole)	2.21 (22–30)	8.86 (30–40)	8.86 (25–33)	8.86 (23–27)

IR (KBr) γ/cm^{-1}: 3171.36–3300.57 (NH_2 stretch), 3071.36 (aromatic CH stretching), 2939.95 (methyl CH stretch), 1611.23 (C=N stretching), 1567.84, 1549.52 and 1428.03 (C=C ring stretching), 1472.38 (tautomeric C=S), 1337.39 (C–N stretching), 1223.61 (N–N = C), 722.21(>C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.76 (ss, 3H, $-OCH_3$), 5.46 (ss, 2H, $-NH_2$), 7.0580–7.1788 (m, 1H, ArH), 7.34–7.35 (d, 1H, J = 2.96 Hz, ArH), 7.62–7.72 (dd, 1H, J = 8.88 and 8.84 Hz, ArH), 13.95 (ss, 1H, SH); ^{13}C NMR: (100 MHz, DMSO-d6): δ 56.31 (OCH_3 carbon), 116.79, 118.74, 120.20, 128.43, 133.98, 150.23, 159.03, 166.96; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 56.32 (OCH_3 carbon), 116.81, 118.74, 120.20; MS: m/z = 303.1 (M+2); Anal. calcd. for $C_8H_9BrN_2O_2$: C, 35.89; H, 3.01; N, 18.60; Found: C, 35.94; H, 3.06; N, 18.54%.

5.4. General procedure for the conventional synthesis of 3-(2-bromo-5-methoxyphenyl)-6-(substituted)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**5a–j**)

An equimolar mixture of compound (**4**) (0.0035 mol) and appropriate mono or disubstituted aromatic carboxylic acids (0.0035 mol) in phosphorus oxychloride (15 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature and poured onto crushed ice (50 g) with stirring. Finally, to remove the excess of phosphorus oxychloride powdered potassium carbonate and the required amount of potassium hydroxide were added till the pH of the mixture was raised to 8. The solid was collected by vacuum filtration, dried and recrystallized from appropriate solvent.

5.4.1. 3-(2-Bromo-5-methoxyphenyl)-6-(4-methoxyphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**5a**)

IR (KBr) γ/cm^{-1}: 3090.68 (Ar C–H str), 2943.83 and 2840.63 (methyl C–H str), 1605.45 (C=N str), 1475.28 (C=C str), 851.41(Ar–H bend) and 769.4 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.84 (ss, 3H, $-OCH_3$ of 2-Br-5-OCH_3 phenyl), 3.88 (ss, 3H, $-OCH_3$ of p-OCH_3 phenyl), 7.26–7.28 (d, 1H, J = 10.42 Hz, 2-Br-5-OCH_3 phenyl), 6.97–7.00 (m, 3H, ArH), 7.62–7.64 (d, 1H, J = 8.78 Hz, 2-Br-5-OCH_3 phenyl), 7.80–7.83 (d, 2H, J = 8.26, p-OCH_3 phenyl); ^{13}C NMR: (100 MHz, DMSO-d6): δ 55.63 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 55.75 (OCH_3 carbon of p-OCH_3 phenyl), 113.48, 114.83, 117.27, 118.57, 121.81, 126.53, 127.83, 129.01, 134.37, 158.79, 163.22, 166.36, 167.01; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 55.63 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 55.75 (OCH_3 carbon of p-OCH_3 phenyl), 114.83, 117.27, 118.57, 129.01, 134.37; MS: m/z = 418 (M+1); Anal. calcd. for $C_{17}H_{13}BrN_4O_2S$: C, 48.93; H, 3.14; N, 13.43; Found: C, 48.96; H, 3.13; N, 13.46%.

5.4.2. 3-(2-Bromo-5-methoxyphenyl)-6-(4-methylphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**5b**)

IR (KBr) γ/cm^{-1}: 3081.65 (Ar C–H str), 2936.09 and 2840.63 (methyl C–H str), 1595.81 (C=N str), 1474.31 (C=C str), 862.98 (Ar–H bend) and 775.24 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 2.43 (ss, 3H, $-CH_3$ of p-CH_3 phenyl), 3.84 (ss, 3H, $-OCH_3$ of 2-Br-5-OCH_3 phenyl), 6.97–7.00 (dd, 1H, J = 8.8 Hz and 2.9 Hz, 2-Br-5-OCH_3 phenyl), 7.26–7.32 (m, 3H, ArH), 7.62–7.64 (d, 1H, J = 8.87 Hz, 2-Br-5-OCH_3 phenyl), 7.76–7.78 (d, 2H, J = 8.01, p-CH_3 phenyl); ^{13}C NMR: (100 MHz, DMSO-d6): δ 21.72 (CH_3 carbon of p-CH_3 phenyl), 55.78 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 113.47, 117.28, 118.71, 126.53, 127.23, 127.57, 130.16, 134.41, 143.82, 146.46, 158.79, 167.01; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 21.72 (-CH_3 carbon of p-CH_3 phenyl), 55.78 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 117.28, 118.71, 127.24, 130.16, 134.41; MS: m/z = 402 (M+1); Anal. calcd. for $C_{17}H_{13}BrN_4OS$: C, 50.88; H, 3.27; N, 13.96; Found: C, 50.84; H, 3.30; N, 13.99%.

5.4.3. 3-(2-Bromo-5-methoxyphenyl)-6-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5c**)

IR (KBr) γ/cm^{-1}: 3096.83 (Ar C–H str), 2931.27 and 290.05 (methyl C–H str), 1592.91 (C=N str), 1471.42 (C=C str), 862.98 (Ar–H bend) and 760.78 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, $-OCH_3$ of 2-Br-5-OCH_3 phenyl), 7.48–7.60 and 7.02–7.423 (m, 5H, ArH), 7.423–7.429 (d, 1H, J = 2.4 Hz, 2-Br-5-OCH_3 phenyl), 7.43–7.45 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.63–7.65 (d, 1H, J = 7.6 Hz, 2-Br-5-OCH_3 phenyl); MS: m/z = 388 (M+1); Anal. calcd. for $C_{16}H_{11}BrN_4OS$: C, 49.62; H, 2.86; N, 14.47; Found: C, 49.59; H, 2.88; N, 14.50%.

5.4.4. 3-(2-Bromo-5-methoxyphenyl)-6-(3,5-dichlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**5d**)

IR (KBr) γ/cm^{-1}: 3086.42 (Ar C–H str), 2838.24 (methyl C–H str), 1597.32 (C=N str), 1472.79 (C=C str), 860.21 (Ar–H bend) and 774.49 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.86 (ss, 3H, $-OCH_3$ of 2-Br-5-OCH_3 phenyl), 6.95–6.98 (dd, 1H, J = 8.8 Hz and 2.9 Hz, 2-Br-5-OCH_3 phenyl), 6.99–7.02 (m, 1H, 2-Br-5-OCH_3 phenyl), 7.25 (s, 1H, 3,5-dichlorophenyl), 7.30 (s, 1H, 3,5-dichlorophenyl), 7.60–7.62 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.74 (s, 1H, 3,5-dichlorophenyl); ^{13}C NMR: (100 MHz, DMSO-d6): δ 55.64 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 112.98, 117.18, 117.94, 121.54, 122.53, 128.70, 130.42, 136.15, 141.57, 147.64, 159.92, 167.12; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 55.64 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 117.18, 117.94, 122.53, 128.70, 130.42, 136.15; MS: m/z = 457.1 (M+1), 458.1 (M+2); Anal. calcd. for $C_{16}H_9BrCl_2N_4OS$: C, 42.17; H, 2.03; N, 12.30; Found: C, 42.13; H, 1.99; N, 12.28%.

5.4.5. 4-[3-(2-Bromo-5-methoxyphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6-yl]aniline (**5e**)

IR (KBr) γ/cm^{-1}:3286.00 (NH stretching), 3090.21 (Ar C–H str), 2942.90 (methyl C–H str), 1575.12 (C=N str), 1456.58 (C=C str), 859.41 (Ar C–H bend) and 781.78 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.76 (ss, 3H, $-OCH_3$ of 2-Br-5-OCH_3 phenyl), 6.61 (bs, 2H, NH_2 of p-amino phenyl), 6.92–6.94 (dd, 1H, J = 8.8 Hz and 2.9 Hz, 2-Br-5-OCH_3 phenyl), 7.13–7.25 (m, 4H, p-amino phenyl), 7.26 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.55–7.58 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl); MS: m/z = 403.27 (M+1), 404.29 (M+2); Anal. calcd. for $C_{16}H_{12}BrN_5OS$: C, 47.75; H, 3.05; N, 17.44; Found: C, 47.77; H, 3.01; N, 17.41%.

5.4.6. 3-(2-Bromo-5-methoxyphenyl)-6-(3,5-dimethylphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**5f**)

IR (KBr) γ/cm^{-1}: 3090.01 (Ar C–H str), 2940.89 and 2845.23 (methyl C–H str), 1594.03 (C=N str), 1482.08 (C=C str), 863.81 (Ar–H bend) and 780.35 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 2.45 (ss, 3H, $-CH_3$ of 3,5-$(CH_3)_2$ phenyl), 2.50 (ss, 3H, $-CH_3$ of 3,5-$(CH_3)_2$ phenyl), 3.78 (ss, 3H, $-OCH_3$ of 2-Br-5-OCH_3 phenyl), 6.93–6.96 (dd, 1H, J = 8.8 Hz and 2.9 Hz, 2-Br-5-OCH_3 phenyl), 7.14 (s, 1H, 3,5-$(CH_3)_2$ phenyl), 7.24 (s, 1H, 3,5-$(CH_3)_2$ phenyl), 7.56–7.58 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.27 (d, 1H, J = 2.5 Hz, 2-Br-5–OCH_3–phenyl), 7.70 (s, 1H, 3,5-$(CH_3)_2$ phenyl); ^{13}C NMR: (100 MHz, DMSO-d6): δ 21.78 (CH_3 carbon of3,5-$(CH_3)_2$ phenyl), 21.81 (CH_3 carbon of3,5-$(CH_3)_2$ phenyl), 55.66 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 112.98, 117.18, 117.94, 121.54, 122.53, 128.70, 130.42, 136.15, 141.57, 147.64, 159.92, 167.12; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 21.78 (CH_3 carbon of 3,5-$(CH_3)_2$ phenyl), 21.81 (CH_3 carbon of3,5-$(CH_3)_2$ phenyl), 55.66 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 117.34, 118.54, 127.98, 130.36, 134.25; MS: m/z = 416.31 (M+1), 417.30 (M+2); Anal. calcd. for $C_{18}H_{15}BrN_4OS$: C, 52.09; H, 3.68; N, 13.52; Found: C, 52.06; H, 3.64; N, 13.49%.

*5.4.7. 3-(2-Bromo-5-methoxyphenyl)-6-(4-nitrophenyl)[1,2,4] triazolo[3,4-b][1,3,4] thiadiazole (**5g**)*

IR (KBr) γ/cm^{-1}: 3092.01 (Ar C–H str), 2938.98 (methyl C–H str), 1576.42 (C=N str), 1455.80 (C=C str), 860.65 (Ar C–H bend) and 778.48 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 6.91–6.93 (dd, 1H, *J* = 8.79 Hz and 2.88 Hz, 2-Br-5-OCH_3 phenyl), 7.12–7.23 (m, 4H, *p*-nitro phenyl), 7.24 (d, 1H, *J* = 2.5 Hz, 2-Br-5–OCH_3–phenyl), 7.53–7.56 (d, 1H, *J* = 8.8 Hz, 2-Br-5-OCH_3 phenyl); MS: *m*/*z* = 433.25 (M +1); Anal. calcd. for $C_{16}H_{10}BrN_5O_3S$: C, 44.48; H, 2.36; N, 16.22; Found: C, 44.46; H, 2.33; N, 16.20%.

*5.4.8. 3-(2-Bromo-5-methoxyphenyl)-6-(3,5-dinitrophenyl)[1,2,4] triazolo[3,4-b][1,3,4] thiadiazole (**5h**)*

IR (KBr) γ/cm^{-1}: 3088.91 (Ar C–H str), 2945.42 (methyl C–H str), 1580.23 (C=N str), 1455.78 (C=C str), 861.58 (Ar C–H bend) and 779.78 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 6.93 (dd, 1H, *J* = 8.8 Hz and 2.9 Hz, 2-Br-5-OCH_3 phenyl), 7.11–7.24 (m, 4H, 3,5-$(NO_2)_2$ phenyl), 7.25 (d, 1H, *J* = 2.48 Hz, 2-Br-5–OCH_3–phenyl), 7.54–7.56 (d, 1H, *J* = 8.8 Hz, 2-Br-5-OCH_3 phenyl); MS: *m*/*z* = 478.3 (M+1); Anal. calcd. for $C_{16}H_9BrN_6O_5S$: C, 40.30; H, 2.01; N, 17.63; Found: C, 40.27; H, 1.90; N, 17.61%.

*5.4.9. 2-[3-(2-Bromo-5-methoxyphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6-yl]-5-methylphenol (**5i**)*

IR (KBr) γ/cm^{-1}: 3454.23 (OH stretching), 3046.45 (aromatic CH stretching), 1615.02 (C=N stretching), 1573.12, 1485.36, 1457.75 (C=C ring stretch), 2972.54, 2921.75 (methyl CH stretch), 1240.34 (C–O stretching), 1278.47 (N–N = C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 2.64 (s, 3H, CH_3 of 2–OH–4–CH_3–phenyl), 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 6.3 (s, 1H, OH of 2–OH–4–CH_3–phenyl), 6.88–6.90 (dd, 1H, *J* = 8.8 Hz and 2.9 Hz, 2-Br-5-OCH_3 phenyl), 7.01–7.20 (m, 4H, 2–OH–4–CH_3–phenyl), 7.30 (d, 1H, *J* = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.53–7.55 (d, 1H, *J* = 8.8 Hz2-Br-5-OCH_3 phenyl); MS: *m*/*z* = 418.3 (M+1), 419.29 (M+2); Anal. calcd. for $C_{17}H_{13}BrN_4O_2S$: C, 48.96; H, 3.17; N, 13.47; Found: C, 48.93; H, 3.14; N, 13.43%.

*5.4.10. 3-(2-Bromo-5-methoxyphenyl)-6-(2,4-diiodophenyl)[1,2,4] triazolo[3,4-b][1,3,4] thiadiazole (**5j**)*

IR (KBr) γ/cm^{-1}: 3050.89 (aromatic CH stretching), 1620.78 (C=N stretching), 1574.56, 1482.56, 1453.43 (C=C ring stretch), 2980.43, 2925.76 (methyl CH stretch), 1269.21 (N–N = C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 6.90–6.92 (dd, 1H, *J* = 8.8 Hz and 2.9 Hz, 2-Br-5-OCH_3 phenyl), 7.08–7.14 (m, 3H, 2,4-$(I)_2$ phenyl), 7.24 (d, 1H, *J* = 2.5 Hz, 2-Br-5–OCH_3–phenyl), 7.53–7.55 (d, 1H, *J* = 8.78 Hz, 2-Br-5-OCH_3 phenyl); MS: *m*/*z* = 640.04 (M+1); Anal. calcd. for $C_{16}H_9BrI_2N_4OS$: C, 30.10; H, 1.44; N, 8.80; Found: C, 30.07; H, 1.42; N, 8.77%.

*5.5. General procedure for the conventional synthesis of 5-(2-bromo-5-methoxyphenyl)-4-{[(substituted)methylene]amino}-4H-1,2,4-triazole-3-thiol (**6a**–**h**) and (**10**)*

Equimolar mixture of compound (**4**) (0.003 mol) and substituted aromatic aldehydes/(**9**) (0.003 mol) in ethanol (15 mL) along with few drops Conc. H_2SO_4 (0.1 mL) was refluxed for 4 h. The completion of reaction was monitored by TLC. After reaction completion, the reaction mass was cooled to 30 °C. The solid separated was filtered off, washed with ethanol (8 mL) and dried. Recrystallization was done using appropriate solvent to obtain (**6a**–**h**) and (**10**).

*5.5.1. 5-(2-Bromo-5-methoxyphenyl)-4-{[(2-chlorophenyl) methylene]amino}-4H-1,2,4-triazole-3-thiol (**6a**)*

IR (KBr) γ/cm^{-1}: 3307.32 (NH tautomeric with SH), 3065.3 (Ar C–H str), 2960.2 and 2830.03 (methyl C–H str), 1591.95 (C=N str), 1475.28 (C=C str), 860.09 (Ar–H bend) and 752.10 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.74 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.07–7.10 (dd, 1H, *J* = 8.8 Hz and 2.6 Hz, 2-Br-5-OCH_3 phenyl), 7.271–7.277 (d, 1H, *J* = 2.49 Hz, 2-Br-5-OCH_3 phenyl), 7.37–7.40 (t, 1H, *J* = 6.9, 2-Cl-phenyl), 7.63–7.66 (d, 1H, *J* = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.52–7.58 (m, 2H, 2-Cl-phenyl), 7.71–7.73 (d, 1H, *J* = 7.59, 2-Cl-phenyl), 10.59 (ss, 1H, CH=N), 14.43 (bs, NH/tautomeric with SH); ^{13}C NMR: (100 MHz, DMSO-d6): δ 56.20 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 113.43, 118.83, 119.18, 126.49, 127.28, 127.75, 128.43, 130.71, 134.06, 134.56, 143.92, 146.76, 158.71(CH=N), 158.76, 167.08; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 56.3 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 118.83, 119.18, 127.75, 128.43, 130.77, 134.06, 134.56, 158.71 (CH=N); MS: *m*/*z* = 424 (M+1); Anal. calcd. for $C_{16}H_{12}BrClN_4OS$: C, 45.35; H, 2.85; N, 13.22; Found: C, 45.38; H, 2.83; N, 13.25%.

*5.5.2. 5-(2-Bromo-5-methoxyphenyl)-4-{[(phenyl)methylene] amino}-4H-1,2,4-triazole-3-thiol (**6b**)*

IR (KBr) γ/cm^{-1}: 3310.42 (NH tautomeric with SH), 3090.46(Ar C–H str), 2958.4 and 2835.63 (methyl C–H str), 1586.45 (C=N str), 1465.99 (C=C str), 859.93 (Ar–H bend) and 765.03 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.03–7.06 (dd, 1H, *J* = 8.8 Hz and 2.44 Hz, 2-Br-5-OCH_3 phenyl), 7.19–7.20 (d, 1H, *J* = 2.46 Hz, 2-Br-5–OCH_3–phenyl), 7.63–7.65 (d, 1H, *J* = 8.79 Hz, 2-Br-5–OCH_3–phenyl), 7.66–7.68 and 7.5–7.6 (m, 5H, phenyl ring), 9.43 (ss, 1H, CH=N), 13.73 (bs, NH/tautomeric with SH); ^{13}C NMR: (100 MHz, DMSO-d6): δ 56.22 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 112.10, 117.32, 118.91, 126.54, 127.42, 128.25, 130.96, 134.86, 143.86, 146.72, 149.74, 158.96 (CH=N), 166.99; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 56.22 (-OCH_3 of 2-Br-5–OCH_3–phenyl), 117.32, 118.91, 127.42, 128.25, 130.96, 134.86, 158.96 (CH=N); MS: *m*/*z* = 390 (M+1); Anal. calcd. for $C_{16}H_{13}BrN_4OS$: C, 49.37; H, 3.37; N, 14.39; Found: C, 49.40; H, 3.35; N, 14.42%.

*5.5.3. 5-(2-Bromo-5-methoxyphenyl)-4-{[(4-chlorophenyl) methylene]amino}-4H-1,2,4-triazole-3-thiol (**6c**)*

IR (KBr) γ/cm^{-1}: 3306.92 (NH tautomeric with SH), 3092.64 (Ar C–H str), 2958.42 and 2848.85 (methyl C–H str), 1586.55 (C=N str), 1480.73 (C=C str), 875.65 (Ar–H bend) and 757.98 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.75 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.08–7.10 (d, 1H, *J* = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.24–7.25 (d, 1H,*J* = 2.8 Hz, 2-Br-5–OCH_3–phenyl), 7.54–7.56 (m, 2H, 4-Cl-phenyl), 7.62–7.64 (d, 1H,*J* = 8.8 Hz, 2-Br-5–OCH_3–phenyl), 7.71–7.73 (d, 2H, *J* = 8.0, 4-Cl-phenyl), 9.91 (ss, 1H, CH=N), 13.90 (bs, NH/tautomeric with SH); LC MS: *m*/*z* = 424 (M+1); Anal. calcd. for $C_{16}H_{12}BrClN_4OS$: C, 45.35; H, 2.85; N, 13.22; Found: C, 45.32; H, 2.84; N, 13.24%.

*5.5.4. 5-(2-Bromo-5-methoxyphenyl)-4-{[(3-chlorophenyl) methylene] amino}-4H-1,2,4-triazole-3-thiol (**6d**)*

IR (KBr) γ/cm^{-1}: 3310.28 (NH tautomeric with SH), 3064.25 (Ar C–H str), 2956.22 and 2828.32 (methyl C–H str), 1588.89 (C=N str), 1480.82 (C=C str), 861.29 (Ar–H bend) and 751.20 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.73 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.04–7.08 (dd, 1H, *J* = 8.8 Hz and 2.6 Hz, 2-Br-5-OCH_3 phenyl), 7.27–7.28 (d, 1H, *J* = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.32–7.37 (t, 1H, *J* = 6.9, 3-Cl-phenyl), 7.59–7.61 (d, 1H, *J* = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.50–7.54 (m, 2H, 3-Cl-phenyl), 7.68–7.72 (d, 1H, *J* = 7.59, 3-Cl-phenyl), 10.55 (ss, 1H, CH=N), 14.42 (bs, NH/

tautomeric with SH); MS: m/z = 424 (M+1); Anal. calcd. for $C_{16}H_{12}BrClN_4OS$: C, 45.35; H, 2.85; N, 13.22; Found: C, 45.37; H, 2.85; N, 13.21%.

*5.5.5. 5-(2-Bromo-5-methoxyphenyl)-4-{[(2,4-dimethoxyphenyl) methylene]amino}-4H-1,2,4-triazole-3-thiol (**6e**)*

IR (KBr) γ/cm^{-1}: 3314.58 (NH tautomeric with SH), 3068.82 (Ar C–H str), 2960.4 and 2830.20 (methyl C–H str), 1584.35 (C=N str), 1478.79 (C=C str), 868.42 (Ar–H bend) and 754.94 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.82 (s, 3H, –OCH_3 of 2,4-$(OCH_3)_2$ phenyl), 3.84 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 3.85 (s, 3H, –OCH_3 of 2,4-$(OCH_3)_2$ phenyl), 7.10–7.12 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.25–7.27 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.31 (s, 1H, 2,4-$(OCH_3)_2$ phenyl), 7.60–7.61 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.62–7.69 (m, 2H, 2,4-$(OCH_3)_2$ phenyl), 10.48 (ss, 1H, CH=N), 13.38 (bs, NH/tautomeric with SH); MS: m/z = 450 (M+1); Anal. calcd. for $C_{18}H_{17}BrN_4O_3S$: C, 48.12; H, 3.81; N, 12.47; Found: C, 48.14; H, 3.84; N, 12.50%.

*5.5.6. 5-(2-Bromo-5-methoxyphenyl)-4-{[(4-methoxyphenyl) methylene]amino}-4H-1,2,4-triazole-3-thiol (**6f**)*

IR (KBr) γ/cm^{-1}: 3309.94 (NH tautomeric with SH), 3062.92 (Ar C–H str), 2964.8 and 2827.43 (methyl C–H str), 1590.43 (C=N str), 1474.42 (C=C str), 860.49 (Ar–H bend) and 758.46 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.65 (s, 3H, –OCH_3 of 4-OCH_3 phenyl), 3.77 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.09–7.12 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.22–7.24 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.61–7.62 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.63–7.70 (m, 3H, 4-OCH_3 phenyl), 10.41 (ss, 1H, CH=N), 14.04 (bs, NH/tautomeric with SH); MS: m/z = 420 (M+1), 421 (M+2); Anal. calcd. for $C_{17}H_{15}BrN_4O_2S$: C, 48.70; H, 3.61; N, 13.36; Found: C, 48.72; H, 3.64; N, 13.38%.

*5.5.7. 5-(2-Bromo-5-methoxyphenyl)-4-{[(2-methoxyphenyl) methylene]amino}-4H-1,2,4-triazole-3-thiol (**6g**)*

IR (KBr) γ/cm^{-1}: 3312.86 (NH tautomeric with SH), 3059.47 (Ar C–H str), 2958.84 and 2834.03 (methyl C–H str), 1592.94 (C=N str), 1472.52 (C=C str), 861.50 (Ar–H bend) and 760.06 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.62 (s, 3H, –OCH_3 of 2-OCH_3 phenyl), 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.10–7.12 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.21–7.25 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.60–7.61 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.62–7.69 (m, 3H, 2-OCH_3 phenyl), 10.42 (ss, 1H, CH=N), 13.99 (bs, NH/tautomeric with SH); MS: m/z = 420 (M+1); Anal. calcd. for $C_{17}H_{15}BrN_4O_2S$: C, 48.70; H, 3.61; N, 13.36; Found: C, 48.73; H, 3.59; N, 13.34%.

*5.5.8. 5-(2-Bromo-5-methoxyphenyl)-4-{[(4-nitrophenyl) methylene]amino}-4H-1,2,4-triazole-3-thiol (**6h**)*

IR (KBr) γ/cm^{-1}: 3310.76 (NH tautomeric with SH), 3065.24 (Ar C–H str), 2960.56 and 2835.13 (methyl C–H str), 1590.04 (C=N str), 1475.92 (C=C str), 859.98 (Ar-H bend) and 761.56 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.84 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 6.92–6.94 (dd, 1H, J = 8.79 Hz and 2.88 Hz, 2-Br-5-OCH_3 phenyl), 7.13–7.22 (m, 4H, *p*-nitro phenyl), 7.23 (d, 1H, J = 2.5 Hz, 2-Br-5–OCH_3–phenyl), 7.54–7.58 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 10.44 (ss, 1H, CH=N), 14.05 (bs, NH/tautomeric with SH); MS: m/z = 435 (M+1), 436 (M+2); Anal. calcd. for $C_{16}H_{12}BrN_5O_3S$: C, 44.25; H, 2.79; N, 16.13; Found: C, 44.23; H, 2.76; N, 16.11%.

*5.5.9. 4-{[Biphenyl-4-ylmethylene]amino}-5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazole-3-thiol (**6i**)*

IR (KBr) γ/cm^{-1}: 3316.09 (NH tautomeric with SH), 3070.14 (Ar C–H str), 2964.63 and 2840.28 (methyl C–H str), 1593.14 (C=N str), 1480.34 (C=C str), 858.38 (Ar–H bend) and 762.49 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.04–7.08 (dd, 1H, J = 8.8 Hz and 2.44 Hz, 2-Br-5-OCH_3 phenyl), 7.12–7.21 (d, 1H, J = 2.46 Hz, 2-Br-5–OCH_3–phenyl), 7.60–7.63 (d, 1H, J = 8.79 Hz, 2-Br-5–OCH_3–phenyl), 7.64–7.69 and 7.70–7.74 (m, 9H, biphenyl ring), 9.82 (ss, 1H, CH=N), 13.96 (bs, NH/tautomeric with SH); MS: m/z = 466 (M+1); Anal. calcd. for $C_{22}H_{17}BrN_4OS$: C, 56.78; H, 3.68; N, 12.04; Found: C, 56.75; H, 3.70; N, 12.01%.

*5.5.10. 5-(2-Bromo-5-methoxyphenyl)-4-{[(4-methylphenyl) methylene]amino}-4H-1,2,4-triazole-3-thiol (**6j**)*

IR (KBr) γ/cm^{-1}: 3317.24 (NH tautomeric with SH), 3070.31 (Ar C–H str), 2961.06 and 2834.83 (methyl C–H str), 1594.14 (C=N str), 1480.32 (C=C str), 857.38 (Ar–H bend) and 762.48 (C–Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 2.25 (ss, 3H, 4-methyl phenyl), 3.78 (ss, 3H, –OCH_3), 6.90–6.92 (d, 1H, J = 8.79 Hz, 2-Br-5-OCH_3 phenyl), 7.14–7.19 (m, 4H, 4-methyl phenyl), 7.24 (d, 1H, J = 2.5 Hz, 2-Br-5–OCH_3–phenyl), 7.52–7.56 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 10.42 (ss, 1H, CH=N), 14.01 (bs, NH/tautomeric with SH); MS: m/z = 404 (M+1); Anal. calcd. for $C_{17}H_{15}BrN_4OS$: C, 50.63; H, 3.75; N, 13.89; Found: C, 50.60; H, 3.77; N, 13.90%.

*5.5.11. 5-(2-Bromo-5-methoxyphenyl)-4-{[1-chloro-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-2-yl]amino}-4H-1,2,4-triazole-3-thiol (**10**)*

IR (KBr) γ/cm-1: 3322.28 (NH tautomeric with SH), 3078.25 (Ar C–H str), 2958.26 and 2842.53 (methyl C–H str), 1592.44 (C=N str), 1482.02 (C=C str), 856.28 (Ar–H bend), 838.883 (C–Cl)and 762.48 (C-Br); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.70–3.72(d, 2H, J = 6.84 Hz, –CH_2 proton of fused cyclohexene ring), 3.80 (ss, 3H, –OCH_3), 4.11 (m, 1H, –CH proton of fused cyclohexene ring), 6.84 (s, 1H, CH of dichlorophenyl ring), 7.34–7.45 (m, 3H, Benzene ring), 7.18–7.19 (d, J = 2.1 Hz, 1H, Dichlorophenyl ring), 6.93–7.78 (m, 1H, Dichloro phenyl ring), 7.84 (s, 1H, Benzene ring), 8.18 (s, 1H, Benzene ring), 8.48 (ss, 1H, =CH proton), 10.67 (NH/tautomeric with SH); ^{13}C NMR: (100 MHz, DMSO-d6): δ 29.70 (CH_2 carbon of fused cyclohexene ring), 41.68 (CH carbon of fused cyclohexene ring), 55.86 (-OCH3 of 2-Br-5-OCH3 phenyl), 113.35, 117.30, 118.97, 126.62, 126.74, 127.48, 127.82, 127.87, 128.00, 128.16, 128.24, 130.07, 130.13, 31.52, 132.76, 134.23, 142.02, 142.27, 149.52, 158.60, 158.85 (CH=N), 159.12, 159.29; MS: m/z = 621 (M+1); Anal. calcd. for $C_{26}H_{18}BrCl_3N_4OS$: C, 50.30; H, 2.92; N, 9.03; Found: C, 50.28; H, 2.94; N, 9.06%.

*5.6. General procedure for the conventional synthesis of 3-(2-bromo-5-methoxyphenyl)-6-sustituted phenyl-5,6-dihydro[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (**7a**–**j**)*

The compounds (**6a**–**j**) (0.002 mol), a catalytic amount of *p*-Toluene sulfonic acid (0.025 g) and DMF (12 mL) were taken in a round bottom flask. The reaction mixture was refluxed for about 10–12 h. The reaction mixture was concentrated to half of its volume and cooled to room temperature. Poured the cooled mixture gradually into crushed ice cubes (30 g) with stirring. Allowed the mixture to stand till solid separated. It was filtered, washed thoroughly with cold water, dried and recrystallized from hot ethanol.

*5.6.1. 3-(2-Bromo-5-methoxyphenyl)-6-(2-chlorophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7a**)*

IR (KBr) γ/cm^{-1}: 3307.32 (NH), 3065.30, 2987.20, 2960.20 (Ar C–H str), 2937.06 and 2830.03 (methyl C–H str), 1591.95 (C=N str), 1475.28 (C=C str), 860.09 (Ar–H bend), 752.10 (C–Br) and 616.14 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.74 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.08–7.11 (dd, 1H, J = 8.8 Hz and 2.6 Hz, 2-Br-

5-OCH_3 phenyl), 5.48 (s, 1H, –CH–NH), 7.27–7.28 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.37–7.41 (t, 1H, J = 6.9, 2-Cl-phenyl), 7.64–7.66 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.52–7.58 (m, 2H, 2-Cl-phenyl), 7.72–7.74 (d, 1H, J = 7.59, 2-Cl-phenyl), 13.73 (bs, NH–CH–); ^{13}C NMR: (100 MHz, DMSO-d6): δ 56.20 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 60.24 (CH–NH), 67.08, 113.43, 118.83, 119.18, 126.49, 127.28, 127.75, 128.43, 130.71, 134.06, 134.56, 143.92, 146.76, 158.76; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 56.3 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 60.24 (CH–NH), 118.83, 119.18, 127.75, 128.43, 130.77, 134.06, 134.56; MS: m/z = 424 (M+1); Anal. calcd. For $C_{16}H_{12}BrClN_4OS$: C, 45.35; H, 2.85; N, 13.22; Found: C, 45.35; H, 2.82; N, 13.19%.

5.6.2. 3-(2-Bromo-5-methoxyphenyl)-6-(phenyl)-5,6-dihydro [1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**7b**)

IR (KBr) γ/cm^{-1}:3094.23 (NH), 2921.28 (Ar C–H str), 1583.27 (C=N str), 1477.21 (C=C str), 868.77 (Ar–H bend), 728.96 (C–Br) and 627.71 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH3 phenyl), 5.52 (s, 1H, –CH–NH-), 7.04–7.06 (dd, 1H, J = 8.8 Hz and 2.44 Hz, 2-Br-5-OCH_3 phenyl), 7.24 (d, 1H, J = 2.46 Hz, 2-Br-5–OCH_3–phenyl), 7.64–7.66 (d, 1H, J = 8.79 Hz, 2-Br-5–OCH_3–phenyl), 7.62–7.68 and 7.52–7.68 (m, 5H, phenyl ring), 13.66 (bs, NH); ^{13}C NMR: (100 MHz, DMSO-d6): δ 56.22 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 59.90 (–CH–NH-), 112.10, 117.32, 118.91, 126.54, 127.42, 128.25, 130.96, 134.86, 143.86, 146.72, 149.74, 166.99; ^{13}C-NMR-DEPT-135: (100 MHz, DMSO-d6), 56.22 (-OCH_3 of 2-Br-5–OCH_3–phenyl), 59.90 (–CH–NH-), 117.32, 118.91, 127.42, 128.25, 130.96, 134.86; MS: m/z = 390 (M+1); Anal. calcd. for $C_{16}H_{13}BrN_4OS$: C, 49.37; H, 3.37; N, 14.39; Found: C, 49.38; H, 3.39; N, 14.40%.

5.6.3. 3-(2-Bromo-5-methoxyphenyl)-6-(4-chlorophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7c**)

IR (KBr) γ/cm^{-1}: 3208.42 (NH), 3191.32 (Ar C–H str), 2960.65 and 2840.67 (methyl C–H str), 1588.44 (C=N str), 1481.42 (C=C str), 863.04 (Ar–H bend), 752.81 (C–Br) and 613 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 7.10–7.12 (dd, 1H, J = 8.8 Hz and 2.6 Hz, 2-Br-5-OCH_3 phenyl), 5.46 (s, IH, –CH–NH), 7.24–7.27 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.34–7.38 (t, 1H, J = 6.9, 4-Cl-phenyl), 7.63–7.65 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.50–7.56 (m, 2H, 4-Cl-phenyl), 7.70–7.72 (d, 1H, J = 7.59, 4-Cl-phenyl), 13.92 (bs, NH–CH–); ^{13}C NMR: (100 MHz, DMSO-d6): δ 56.21 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 60.42 (CH–NH), 69.12, 113.13, 118.53, 119.72, 126.53, 127.30, 127.82, 128.81, 130.93, 134.28, 134.87, 143.87, 146.05, 159.06; ^{13}C NMR-DEPT: (100 MHz, DMSO-d6): δ 56.21 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 60.42 (CH–NH), 118.53, 119.72, 127.82, 128.81, 130.93, 134.28, 134.87; MS: m/z = 424 (M+1), 425 (M+2); Anal. calcd. for $C_{16}H_{12}BrClN_4OS$: C, 45.35; H, 2.85; N, 13.22; Found: C, 45.34; H, 2.88; N, 13.20%.

5.6.4. 3-(2-Bromo-5-methoxyphenyl)-6-(3-chlorophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7d**)

IR (KBr) γ/cm^{-1}: 3214.85 (NH), 3194.06 (Ar C–H str), 2962.64 and 2838.17 (methyl C–H str), 1582.53 (C=N str), 1478.82 (C=C str), 861.14 (Ar–H bend), 750.94 (C–Br) and 614.98 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.84 (ss, 3H, –OCH_3 of 2-Br-5-OCH3 phenyl), 7.08–7.10 (dd, 1H, J = 8.8 Hz and 2.6 Hz, 2-Br-5-OCH_3 phenyl), 5.51 (s, IH, –CH–NH), 7.21–7.23 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.32–7.35 (t, 1H, J = 6.9, 3-Cl-phenyl), 7.65–7.68 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.51–7.58 (m, 2H, 3-Cl-phenyl), 7.68–7.70 (d, 1H, J = 7.59, 3-Cl-phenyl), 13.80 (bs, NH–CH–); MS: m/z = 424 (M+1); Anal. calcd. for $C_{16}H_{12}BrClN_4OS$: C, 45.35; H, 2.85; N, 13.22; Found: C, 45.38; H, 2.83; N, 13.24%.

5.6.5. 3-(2-Bromo-5-methoxyphenyl)-6-(2,4-dimethoxyphenyl)-5,6-dihydro[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole (**7e**)

IR (KBr) γ/cm^{-1}: 3210.58 (NH), 3186.73 (Ar C–H str), 2961.59 and 2840.12 (methyl C–H str), 1581.46 (C=N str), 1480.92 (C=C str), 859.54 (Ar–H bend), 756.66 (C–Br) and 612.62 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.82 (s, 3H, –OCH_3 of 2,4-$(OCH_3)_2$ phenyl), 3.84 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 3.85 (s, 3H, –OCH_3 of 2,4-$(OCH_3)_2$ phenyl), 5.48 (s, 1H, –CH–NH), 7.11–7.12 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.25–7.26 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.30 (s, 1H, 2,4-$(OCH_3)_2$ phenyl), 7.61–7.62 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.62–7.70 (m, 2H, 2,4-$(OCH_3)_2$ phenyl), 13.42 (bs, NH–CH–); MS: m/z = 450 (M+1); Anal. calcd. for $C_{18}H_{17}BrN_4O_3S$: C, 48.12; H, 3.81; N, 12.47; Found: C, 48.12; H, 3.79; N, 12.45%.

5.6.6. 3-(2-Bromo-5-methoxyphenyl)-6-(4-methoxyphenyl)-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7f**)

IR (KBr) γ/cm^{-1}: 3212.38 (NH), 3190.24 (Ar C–H str), 2960.03 and 2841.88 (methyl C–H str), 1582.26 (C=N str), 1484.62 (C=C str), 861.95 (Ar–H bend), 752.36 (C–Br) and 616.11 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.64 (s, 3H, –OCH_3 of 4-OCH_3 phenyl), 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 5.51 (s, IH, –CH–NH), 7.11–7.12 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.21–7.23 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.60–7.62 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.63–7.69 (m, 3H, 4-OCH_3 phenyl), 13.86 (bs, NH–CH–); MS: m/z = 420 (M+1); Anal. calcd. for $C_{17}H_{15}BrN_4O_2S$: C, 48.70; H, 3.61; N, 13.36; Found: C, 48.68; H, 3.59; N, 13.37%.

5.6.7. 3-(2-Bromo-5-methoxyphenyl)-6-(2-methoxyphenyl)-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7g**)

IR (KBr) γ/cm^{-1}: 3210.97 (NH), 3191.48 (Ar C–H str), 2968.76 and 2846.28 (methyl C–H str), 1585.32 (C=N str), 1480.25 (C=C str), 858.69 (Ar–H bend), 756.93 (C–Br) and 610.24 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.63 (s, 3H, –OCH_3 of 2-OCH_3 phenyl), 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 5.46 (s, IH, –CH–NH), 7.11–7.12 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 7.22–7.23 (d, 1H, J = 2.49 Hz, 2-Br-5–OCH_3–phenyl), 7.59–7.60 (d, 1H, J = 8.84 Hz, 2-Br-5–OCH_3–phenyl), 7.61–7.68 (m, 3H, 2-OCH_3 phenyl), 13.78 (bs, NH–CH–); MS: m/z = 420 (M+1); Anal. calcd. for $C_{17}H_{15}BrN_4O_2S$: C, 48.70; H, 3.61; N, 13.36; Found: C, 48.71; H, 3.63; N, 13.35%.

5.6.8. 3-(2-Bromo-5-methoxyphenyl)-6-(4-nitrophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7h**)

IR (KBr) γ/cm^{-1}: 3220.04 (NH), 3194.89 (Ar C–H str), 2965.61 and 2848.14 (methyl C–H str), 1585.32 (C=N str), 1481.65 (C=C str), 856.87 (Ar–H bend), 751.86 (C–Br) and 612.84 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.84 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 5.52 (s, IH, –CH–NH), 6.91–6.93 (dd, 1H, J = 8.79 Hz and 2.88 Hz, 2-Br-5-OCH_3 phenyl), 7.14–7.21 (m, 4H, p-nitro phenyl), 7.22 (d, 1H, J = 2.5 Hz, 2-Br-5–OCH_3–phenyl), 7.53–7.56 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 13.95 (bs, NH–CH–); MS: m/z = 435 (M +1), 436 (M +2); Anal. calcd. for $C_{16}H_{12}BrN_5O_3S$: C, 44.25; H, 2.79; N, 16.13; Found: C, 44.22; H, 2.75; N, 16.15%.

5.6.9. 6-Biphenyl-4-yl-3-(2-bromo-5-methoxyphenyl)-5,6-dihydro [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7i**)

IR (KBr) γ/cm^{-1}: 3216.87 (NH), 3192.76 (Ar C–H str), 2963.74 and 2852.98 (methyl C–H str), 1582.78 (C=N str), 1486.24 (C=C str), 854.64 (Ar–H bend), 756.36 (C–Br) and 614.77 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 3.78 (ss, 3H, –OCH_3 of 2-Br-5-OCH_3 phenyl), 5.49 (s, IH, –CH–NH), 7.06–7.08 (dd, 1H, 2-Br-5-OCH_3 phenyl, J = 8.8 Hz and 2.44 Hz), 7.14–7.16 (d, 1H, J = 2.46 Hz, 2-Br-5–OCH_3–phenyl), 7.61–7.63 (d, 1H, J = 8.79 Hz, 2-Br-5–OCH_3–phenyl), 7.66–7.69 and 7.71–7.78 (m, 9H, biphenyl ring), 13.96 (bs, NH–CH–); MS: m/z = 466 (M+1), 467 (M+2); Anal.

calcd. for $C_{22}H_{17}BrN_4OS$: C, 56.78; H, 3.68; N, 12.04; Found: C, 56.80; H, 3.64; N, 12.07%.

5.6.10. 3-(2-Bromo-5-methoxyphenyl)-6-(4-methylphenyl)-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**7j**)

IR (KBr) γ/cm^{-1}: 3214.82 (NH), 3190.34 (Ar C–H str), 2958.15 and 2860.91 (methyl C–H str), 1584.88 (C=N str), 1482.24 (C=C str), 852.61 (Ar–H bend), 752.35 (C–Br) and 612.76 (C–S–C); ^{1}H NMR: (400 MHz, DMSO-d6): δ 2.26 (ss, 3H, 4-methyl phenyl), 3.78 (ss, 3H, $-OCH_3$), 5.48 (s, IH, –CH–NH), 6.90–6.92 (d, 1H, J = 8.79 Hz, 2-Br-5-OCH_3 phenyl), 7.14–7.17 (m, 4H, 4-methyl phenyl), 7.22 (d, 1H, J = 2.5 Hz, 2-Br-5–OCH_3–phenyl), 7.53–7.54 (d, 1H, J = 8.8 Hz, 2-Br-5-OCH_3 phenyl), 13.98 (bs, NH–CH–); MS: m/z = 404 (M+1), 405 (M+2); Anal. calcd. for $C_{17}H_{15}BrN_4OS$: C, 50.63; H, 3.75; N, 13.89; Found: C, 50.61; H, 3.76; N, 13.91%.

5.7. Synthesis of 1-chloro-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalene-2-carbaldehyde (**9**)

To the Vilsmeier–Haack complex prepared from DMF (0.12 mol) and $POCl_3$ (0.03 mol) at 0 °C, the compound (**8**) (0.01 mol) was added and the reaction mixture was stirred at 65 °C for 4 h. The reaction completion was monitored by TLC. The contents were cooled, poured in to ice-cold water and neutralized using Na_2CO_3 solution in water. The solid product that separated was filtered, washed with cold water and dried. The crude product was recrystallized from ethyl acetate.

IR (KBr) γ/cm^{-1}: 3443.28 (-CHO), 1662.34 (C=O of aldehyde), 1595.81 (C=C, aromatic),838.88 (C–Cl), 1255.43 (C–H stretch).^{1}H NMR (400 MHz, DMSO-d6): δ 2.86–3.01(m, 2H, $-CH_2$ proton of fused cyclohexene ring), 4.13 (t, 1H, J = 7.5 Hz, –CH proton of fused cyclohexene ring), 6.92–6.98 (m, 1H of dichloro phenyl ring and 1H of benzene ring), 7.34–7.45 (m, 3H, Benzene ring), 7.18–7.19 (d,1H, J = 2.1 Hz, Dichloro phenyl ring), 7.96–8.00 (m, 1H, Dichloro phenyl ring), 10.33 (s, 1H, –CHO); MS: m/z = 337 (M+); Anal. calcd. for $C_{17}H_{11}Cl_3O$: C, 60.48; H, 3.28; Found: C, 60.49; H, 3.28%.

5.8. Anti-inflammatory activity

The anti-inflammatory activity of newly synthesized 3,6-disubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles (**5a**–**j**) and 5,6-dihydro-3,6-disubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles (**7a**–**j**) were evaluated by applying carrageenan-induced paw oedema bioassay in rats of either sex (100-140 g) by following the method of Winter et al. [29] using Diclo as a reference standard. Rats were selected by random sampling technique. The test compounds were administrated at dose level of 100 mg/kg orally 30 min prior to the administration of carrageenan in the right hind paw of the rats. The paw thickness was measured using vernier callipers at regular intervals of 60, 120 and 180 min after carrageenan administration.

5.9. Analgesic activity

The analgesic activity of the above mentioned derivatives were also evaluated by applying tail flick method [30] using pentazocine as a standard reference. Wistar albino mice of either sex (20-30 g) in the groups of six animals each one was selected by random sampling technique. The test compounds at dose level of 100 mg/kg were administered orally by intragastric tube. The animals were held in position by a suitable restrained the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 50–55 °C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. The reading was recorded at regular intervals of 60, 80 and 120 min after administration of compounds. A cut off point of 10 s was observed to prevent the tail damage.

5.10. Anti-oxidant activity

5.10.1. DPPH radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical which has maximum optical absorbance at 517 nm. The reaction of DPPH with free radical scavenger causes decline in the absorbance value at 517 nm [31]. The newly synthesized compounds were dissolved in methylene dichloride at 200, 400 and 800 ug/mL concentrations and 4 mL of 0.1 mM methanolic solution of DPPH was added. The test tubes were kept at an ambient temperature for 20 min and the absorbances were measured at 517 nm against control. Ascorbic acid was used as a positive control. These measurements were run in triplicate. The percentage of scavenging activity was calculated as follows:

$$\text{Scavenging activity}(\%\text{ of inhibition})(\%) = [(A_{DPPH} - A_{TEST})/A_{DPPH}] \times 100$$

Where A_{DPPH} is the absorbance of DPPH without test sample (control) and A_{TEST} is the absorbance of DPPH in the presence of test sample.

5.11. Antibacterial activity

All the newly synthesized compounds were screened for their antibacterial activity against four bacterial strains, *viz*., *Staphyllococcus aureus* (ATTC-25923), *E. coli* (ATTC-25922), *P. aeruginosa* (ATTC-27853) and *K. pneumoniae* (recultured) by disc diffusion method [32,33]. Serial dilutions of the drug in Muller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using a 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 conspicuous growth.

A number of antibacterial discs were placed on the agar for the sole purpose of producing zone of inhibition in the bacterial lawn. Twenty millilitres of agar media was poured into each Petri dish. The excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for an h. Using a punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethyl sulfoxide (DMSO) was added into each labelled well. A control was also prepared for the plates in the same way using DMSO as a solvent. 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 taking ampicillin as standard.

5.12. Antifungal activity

All those compounds screened for antibacterial activity were also tested for their anti-fungal activity against *P. marneffei* (recultred), *T. mentagrophytes* (recultured), Aspergillus flavus (NICM No.524) and *A. fumigatus* (NCIM No.902) by serial plate dilution method [34]. Sabourands 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 sore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of the corresponding species. Twenty mL of agar media was poured in to each petri dish. The excess of suspension

was decanted and the plates were dried by placing in an incubator at 37 °C for 1hr. Using a punch, wells were made on these seeded agar plates. Minimum inhibitory concentrations of the test compounds in DMSO were added into each labelled well. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. Activity of each compound was compared with itraconozole as standard. Itraconozole has a MIC value of 0.04–22.67 μM. The minimum inhibitory concentrations of each compound were determined.

Acknowledgements

One of the authors, N. Chidananda, is grateful to the management of SeQuent Scientific Ltd., New Mangalore, India for encouraging research work. N. Chidananda is also thankful to Prof. A. Srikrishna, Department of Organic Chemistry, IISc, Bangalore for providing ^{1}H NMR and ^{13}C NMR spectral facilities. We are also thankful to Smt. Ashwini Srinivas, Department of Bioscience, Mangalore University for her sincere help during the anti-oxidant study. Thanks are also due to CDRI-Lucknow and Punjab University Chandigarh for providing ^{1}H NMR and MS spectral data.

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