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Synthesis of Some New *S*-Alkylated 1,2,4-Triazoles, Their Mannich Bases and Their Biological Activities

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A series of 1-(4-methoxyphenyl)-2-[5-{(biphenyl-4-yloxy)methyl}4-(substituted phenyl)-3-mercapto-(4*H*)-1,2,4-triazol-3-ylthio)] ethanones **(6a–6s)** and 4-(substituted phenyl)-3-(morpholin/pyrrolidin-4-ylmethylthio)-5-(4-phenylphenoxymethyl)-4*H*-1,2,4-triazoles **(7a–7e)** were synthesized in order to obtain new compounds with potent anti-inflammatory and analgesic activity with insignificant ulceration. Among the synthesized compounds, **(6c), (6e), (6g)** and **(6l)** from triazole series and **(7b)** and **(7e)** from Mannich base series were found to exhibit significant anti-inflammatory activity with 59.69, 59.69, 64.69, 79.84, 54.54, 79.69% and 52.55, 57.50, 72.52, 83.03, 60.06, 84.08% inhibition of paw edema at 3 h and 5 h respectively, in comparison to the standard drug ibuprofen (78.93 and 82.58% at 3 h and 5 h). The active compounds were further tested for their analgesic activity and gastric ulceration study. Compounds **6g, 7b** and **7e** exhibited significant analgesic activity with reaction time (3.60, 3.22, 3.88 s) respectively at 60 min. without causing any gastric irritation. These compounds were also screened for their *in vitro* antimicrobial activity, Compounds **6f, 6g, 6h, 6l, 6o, 6p, 7a, 7b** and **7c** showed significant zone of inhibition against various antimicrobial stains. It is concluded that the compounds **6g, 7b** and **7e** possess a good spectrum of activities. Compound **7e** may be considered potent for development of better anti-inflammatory agent. The antimicrobial activity revealed that most of the compounds showed moderate to significant activity. Compounds containing nitro, chloro, bromo and fluoro group showing better activity. All the compounds from **7a, 7b and 7e** were active against gram positive bacteria (*S. aureus*).

Keywords: Analgesic / Antimicrobial activity / Anti-inflammatory / Mannich base / Triazole / Ulcerogenic

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Introduction

Heterocycles play an important role in all spheres of life including pharmaceuticals, natural resources, veterinary, analytical reagents, agriculture products, and dyes [1]. The development of new approaches for the synthesis of novel heterocycles substituted with unique functional groups forms the basis of an extensive research activity in synthetic organic chemistry. The 1,2,4-triazole derivatives and their Mannich bases represent an important class of heterocyclic compounds which are known for their broad spectrum of biological activities including antimicrobial, anti-inflammatory, analgesic and many other uses [2–8]. As resistance to anti-inflammatory and antimicrobial drugs is widespread, there is an increasing demand for the identification of novel structure leads that may be of use in designing new, potent and less toxic anti-inflammatory and antimicrobial agents.

Several 1,2,4-triazole and oxadiazole based antimicrobial agents have been synthesized to develop new molecular

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Abbreviations: NSAIDs: non-steroidal anti-inflammatory drugs; **HP-PLA2:** human platelet phospholipase A2; **$CDCl_3$:** deuterated chloroform; **DMSO-d_6:** deuterated dimethylsulfoxide; **ATCC:** American Type Culture Collection; **MTCC:** Microbial Type Culture Collection; **AIIMS:** All India Institute of Medical Science; **CDRI:** Central Drug Research Institute; **SDA:** Sabouraud dextrose agar; **MH:** Muller Hinton; **CMC:** carboxymethyl cellulose.

entities with high potential against various microbial strains. Further several non-steroidal anti-inflammatory drugs (NSAIDs) have been developed so far to heal the inflammation. There are a number of NSAIDs present in the market to heal the inflammation but majority of them causes adverse side effects like gastric ulcer [9], kidney damage [10] and some of the NSAIDs also cause hepatotoxicity [11]. During the last few decades, a considerable attention has been devoted to the synthesis of various 1,2,4-triazole derivatives [12–16]. Several examples of NSAIDs having triazole moieties have been cited in the literature. Among them, 1,2,4-triazole-3-thiol derivatives are of particular interest and have been studied in recent years [17–20]. Most of the NSAIDs having biphenyl derivatives substituted with an aromatic or heteroaromatic aryl nucleus show better anti-inflammatory activity. Some of the biaryldiacid derivatives have been evaluated as potential anti-inflammatory agents through the inhibition of 14 kDa human platelet phospholipase A2 (HP-PLA2) [21]. Some of the biphenylic compounds that contain free carboxyl groups cause ulceration [22–23].

In view of the importance of biaryl and 1,2,4-triazole moieties as anti-inflammatory and antimicrobial agents, we aim to develop some novel biologically interesting moieties encompassing biaryl and 1,2,4-triazoles moieties conjugated through an ether linkage. A series of novel *S*-substituted 1,2,4-triazoles and Mannich bases of 1,2,4-triazoles were synthesized starting from *p*-hydroxy biphenyl. The structures of new compounds were confirmed by chemical and spectroscopic methods like FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and mass spectrometry. All the synthesized compounds were screened for their biological activities *viz.* anti-inflammatory, analgesic, ulcerogenic and antimicrobial activity. The synthetic path of the target compounds is shown in Scheme 1.

Results and discussion

Chemistry

Twenty-four new compounds **(6a–6s)** and **(7a–7e)** were synthesized starting from *p*-hydroxybiphenyl as outlined in Scheme 1. Reaction of *p*-hydroxybiphenyl **(1),** with ethylchloroacetate in presence of potassium carbonate in anhydrous acetone afforded ethyl 2-(biphenyl-4-yloxy) acetate **(2)** which readily yielded 2-(biphenyl-4-yloxy) acetohydrazide **(3)** by refluxing with hydrazine monohydrate in absolute alcohol. Compound **(3)** then was converted into corresponding thiosemicarbazide **(4a–4f)** by reacting with different substituted aryl isothiocyanate in absolute alcohol using the known protocol [24]. The thiosemicarbazides **(4a–4f)** were further converted into respective 5-[(biphenyl-4-yloxy)-methyl]4-(substituted phenyl)-3-mercapto-(*4H*)-1,2,4-triazoles **(5a–5f)** using triethyl amine in absolute alcohol. Finally *S*-alkylation of 3-mercapto-1,2,4-triazoles with substituted phenacyl bromides/benzyl bromide leads to the formation of target molecules **(6a–6s)**. The triazoles **(5c, 5d and 5f)** were also converted into corresponding Mannich bases **(7a–7e)** by reacting with different secondary amines and formaldehyde in presence of alcohol.

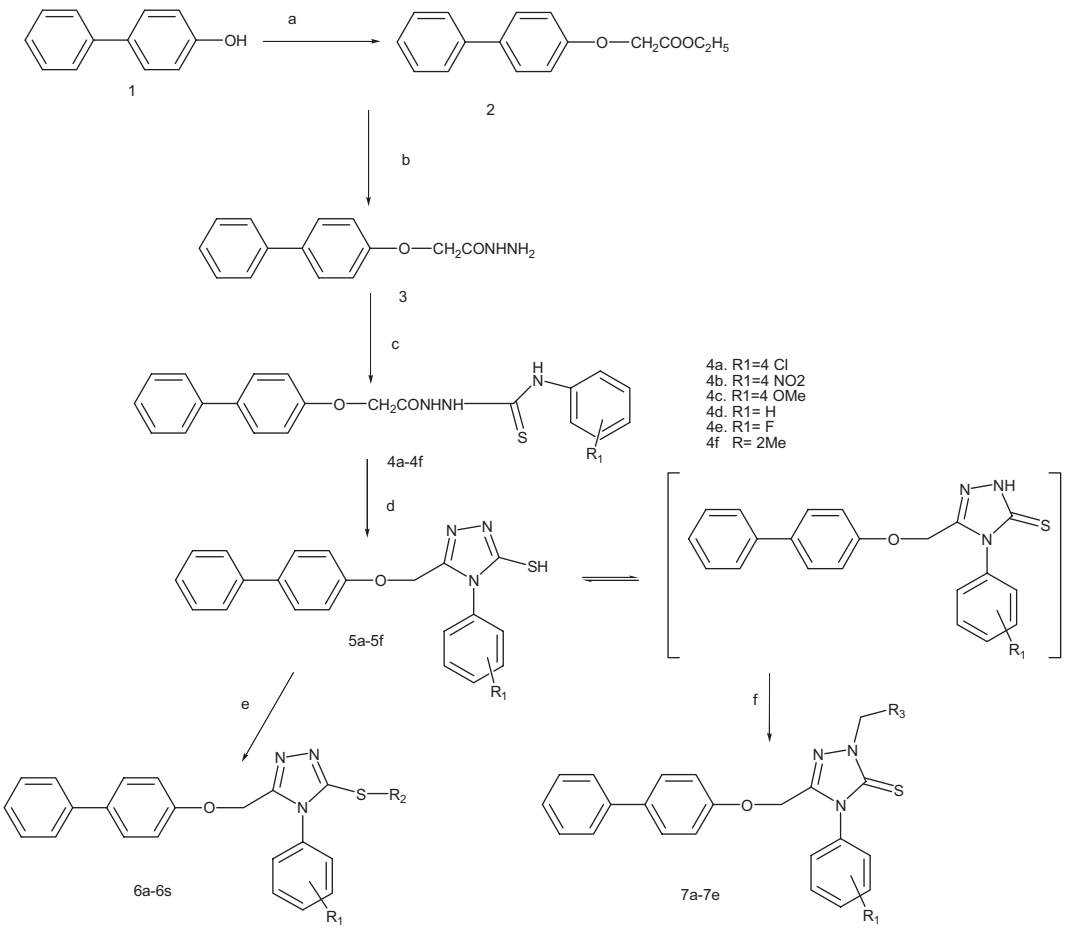
Cyclization of compounds **4(a–f)** to **5(a–f)** were confirmed from the IR (absorption bands for S–H, 2680–2690 and C=N, 1594–1607 cm^{-1} triazole ring stretching) and from the ^{1}H NMR spectra (singlet at δ 8.71–13.61 for S–H). *S*-Alkylation of compounds **5(a–e)** to **6(a–s)** was confirmed from IR (absorption bands for C=O, 1655–1687) and from ^{13}C NMR spectra (peaks at δ 190–192 for C=O).

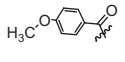
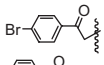
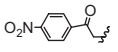
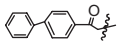
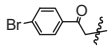
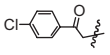
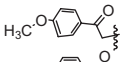
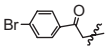
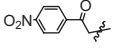
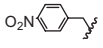
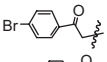
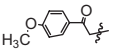
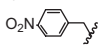
1,2,4-Triazole ring in compound **5(a–f)** will exist in two tautomeric forms. Under the Mannich reaction conditions the -NH proton of the triazole ring will participate in the reaction and will form the final Mannich base **7(a–e)**. Formation of Mannich base was confirmed from ^{1}H NMR (presence of a singlet at δ 4.47–4.93 due to methylene proton N–CH_2–N), the ^{13}C NMR spectra of **7(a–e)** displayed peaks in the range of δ 68.76–68.82 for the carbon bonded with two nitrogen (N–CH_2–N) and the peaks in the range of δ 170–172 for the carbon in the triazole ring attached with sulphur (C=S). All these peaks were absent in the compounds **(5a–5e)** and the spectral data are in line with Barbuceanu et al. [25] which confirms the formation of the compounds **(7a–7e).**

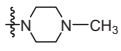
Anti-inflammatory activity

The results of anti-inflammatory activity are summarized in Table 1. Among the tested compounds, **6c, 6e, 6g and 6l** from triazole series and **7b**, **7e** from Mannich base series were found to be active and have significant activity (59.69, 59.69, 64.69, 79.84, 54.54, 79.69% and 52.55, 57.50, 72.52, 83.03, 60.06, 84.08% inhibition in paw oedema at 3 h and 5 h respectively) when compared to the standard drug ibuprofen (78.93 and 82.58% at 3 h and 5 h). Compounds **6l** and **7e** were exhibited more active than the standard drug ibuprofen.

From the biological data, the structure activity relationship (SAR) can be drawn as follows. Compounds having no substitution on the aromatic ring attached to the 1,2,4-triazollyl ring and the electron withdrawing group on the phenacyl aromatic ring are showing better activity. Substitution of electron donating groups on triazolyl attached aromatic ring decreases the activity but is comparable with the standard. Whereas when the same aromatic ring was substituted with electron withdrawing groups the activity significantly decreases. Compounds having bulkier substitutions like -Br and –NO_2 groups on the phenacyl aromatic ring are showing potential activity. Whereas the simple benzylation on the 3-mercapto 1,2,4-triazoles significantly diminishes the activity. These data suggests that the carbonyl functionality of the phenacyl group is playing an important role in the enzymatic interactions responsible for



Comp.	R_1	R_2
6a	4-Cl	
6b	4-Cl	
6c	4-Cl	
6d	4-Cl	
6e	4-NO_2	
6f	4-NO_2	
6g	4-OMe	
6h	4-OMe	
6i	4-OMe	
6j	4-OMe	
6k	H	
6l	H	
6m	H	
6n	H	
6o	4-F	
6p	4-F	
6q	4-Cl	
6r	4-F	
6s	4-F	

Comp.	R_1	R_3
7a	4-Cl	
7b	4-OMe	
7c	4-OMe	
7d	H	
7e	2-Me	

Reagents and conditions: (a) Dry acetone, anhydrous K_2CO_3, ethylchloroacetate, reflux 10 h; **(b)** $NH_2NH_2.H_2O$, Abs. alcohol, reflux 3 h; **(c)** Abs. alcohol, ArNCS, reflux 4–6 h; **(d)** Abs. alcohol, TEA, reflux 6–8 h **(e)** Abs. alcohol, Na metal, substituted phenacylbromide, reflux 4–6 h **(f)** Abs. alcohol, HCHO, amines, stirring at room temperature, 24 h.

Scheme 1. Synthetic route employed for the synthesis of target molecules.

Table 1. Results of anti-inflammatory activity of azole derivatives against carrageenan induced rat paw edema model.

Compounds	Change in paw edema volume (mL) After drug treatment		Antiinflamatory activity % Inhibition	
	3 h	5 h	3 h	5 h
6a	$0.319 \pm 0.067^{***}$	$0.321 \pm 0.075^{***}$	51.66	51.36
6b	$0.341 \pm 0.056^{***}$	$0.338 \pm 0.068^{***}$	48.33	49.24
6c	$0.266 \pm 0.071^{***}$	$0.316 \pm 0.090^{***}$	59.69	52.55
6d	0.566 ± 0.049^{ns}	0.450 ± 0.125^{ns}	14.24	32.43
6e	$0.266 \pm 0.042^{***}$	$0.283 \pm 0.040^{***}$	59.69	57.50
6f	0.583 ± 0.065^{ns}	0.600 ± 0.089^{ns}	11.66	09.00
6g	$0.233 \pm 0.049^{***}$	$0.183 \pm 0.040^{***}$	64.69	72.52
6h	$0.423 \pm 0.058^{**}$	0.413 ± 0.057^{ns}	35.90	37.98
6i	0.533 ± 0.042^{ns}	0.483 ± 0.118^{ns}	19.24	27.24
6j	$0.330 \pm 0.049^{***}$	$0.366 \pm 0.055^{**}$	50.00	45.04
6k	$0.300 \pm 0.036^{***}$	$0.283 \pm 0.040^{***}$	54.54	57.50
6l	$0.133 \pm 0.021^{***}$	$0.113 \pm 0.033^{***}$	79.84	83.03
6m	0.583 ± 0.060^{ns}	$0.383 \pm 0.047^{*}$	11.66	42.49
6n	$0.466 \pm 0.061^{*}$	0.466 ± 0.033^{ns}	28.64	29.39
6o	$0.300 \pm 0.051^{***}$	$0.366 \pm 0.076^{**}$	54.54	45.04
6p	$0.298 \pm 0.057^{***}$	$0.300 \pm 0.057^{***}$	54.69	54.95
6q	$0.295 \pm 0.046^{***}$	$0.279 \pm 0.036^{***}$	55.30	58.10
6r	$0.345 \pm 0.036^{**}$	$0.331 \pm 0.039^{**}$	47.72	50.30
6s	$0.350 \pm 0.042^{***}$	$0.233 \pm 0.091^{***}$	46.96	65.01
7a	$0.387 \pm 0.043^{**}$	$0.367 \pm 0.047^{**}$	41.36	44.89
7b	$0.300 \pm 0.066^{***}$	$0.266 \pm 0.088^{***}$	54.54	60.06
7c	$0.410 \pm 0.072^{***}$	0.436 ± 0.074^{ns}	37.87	34.53
7d	0.566 ± 0.042^{ns}	0.516 ± 0.119^{ns}	14.24	22.52
7e	$0.134 \pm 0.055^{***}$	$0.106 \pm 0.047^{***}$	79.69	84.08
Ibuprofen	$0.139 \pm 0.021^{***}$	$0.116 \pm 0.051^{***}$	78.93	82.58
Control	0.660 ± 0.042	0.666 ± 0.088	–	–

Data analyzed by one-way ANOVA followed by Dunnett's 't' test (n = 6).
* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$ significantly different from standard; ns, not significant.

anti-inflammatory activity. As aryl substituted 1,2,4-triazoles and some of the other azole derivatives (celocoxib) are reported to be selective inhibitors of COX-2 [26], the possible mode of action of above synthesized compounds might be similar to other azole drugs. Overall the compound **6l** substituted with nitro group at the *para* position on the aromatic ring of the phenacyl group is showing significant activity compared to standard drug ibuprofen.

From the Mannich base series, compounds having morpholine ring and the substitution of electron donating groups on

Table 2. Results of analgesic activity of the synthesized compounds.

Compounds	Mean value of tail flick latency (s) ± SEM			
	0 min	30 min	60 min	120 min
6c	1.93 ± 0.33	3.04 ± 0.284	2.84 ± 0.302	2.85 ± 0.372
6e	2.07 ± 0.368	3.81 ± 0.257	3.96 ± 0.377	3.08 ± 0.499
6l	2.16 ± 0.298	2.85 ± 0.367	3.91 ± 0.529	2.516 ± 0.323
6g	1.88 ± 0.324	3.23 ± 0.370	3.603 ± 0.341	2.56 ± 0.227
7b	2.08 ± 0.32	3.80 ± 0.233	3.225 ± 0.264	2.94 ± 0.232
7e	1.93 ± 0.303	3.86 ± 0.346	3.88 ± 0.243	2.85 ± 0.237
Control	2.03 ± 0.330	1.94 ± 0.236	2.02 ± 0.192	2.85 ± 0.237
Ibuprofen	1.98 ± 0.307	3.716 ± 0.388	3.956 ± 0.473	2.76 ± 0.212

Data analyzed by one-way ANOVA followed by Dunnett's 't' test (n = 6), $^{*}p < 0.05$, $^{**}p < 0.01$ & $^{***}p < 0.001$ significantly different from standard; ns, not significant.

the aromatic ring attached to triazollyl ring are showing better activity. Compound **7e** with methyl substitution at the *ortho* position and with morpholine ring is showing significant activity compared to the standard ibuprofen.

All these data were analyzed by one-way ANOVA test followed by Dunnet's test in carrageenan induced rat paw oedema model.

Gastric ulceration study

When compared with ibuprofen, compounds **6g**, **7b** and **7e** did not cause any gastric ulceration and disruption of gastric epithelial cells at 3 times the dose used for anti-inflammatory activity (Experimental). Hence gastric tolerance to these compounds was better than that of ibuprofen indicating that carboxylic group present in the ibuprofen is responsible for ulceration [27]. Stomach wall of ibuprofen treated group at low power (10x) photomicrograph showed damage of the mucosa and the submucosa. Stomach wall of the same section at high power (40x) photomicrograph showed desquamated epithelial cells in the lumen whereas in tested compounds treated **(6c), (6e)** and **(6l)** animals, surface epithelial damage was significant, slight submucosal damage was seen, however there was lesser damage in comparison to the ibuprofen. Stomach wall of **6g, 7b** and **7e** treated animal showed no damage of any layer. The results are shown in Table 3 and Figure 1.

Antimicrobial activity

All the newly synthesized compounds were tested against various microbial strains for their anti-microbial activity (Table 4). Some of the compounds from both the series have shown moderate to good activity against certain strains. Compound **6f** showed moderate activity against all the tested fungal strains whereas compounds **6g** and **6h** were active against *Aspergillus flavus*. Compounds **6g, 6j, 6o, 6q** and **7d** showed moderate activity against *Candida albicans*. From the results it can be depicted that the compounds containing phenacyl group are showing better activity than the simple benzylated ones.

Compounds **6l, 6p** and **6q** showed moderate activity against bacterial strain *Pseudomonas aeruginosa* whereas compounds **7a, 7b and 7e** from the Mannich base series showed good activity against *Staphylococcus aureus* (Gram possitive bacteria). By closely observing the results it can be concluded that most of the halogen substituted compounds (**6g, 6h, 6o, 6q, 6p**) displayed good activity against various fungal strains.

Table 3. Results of gastric ulcerogenic study.

Group	Surface epithelial damage	Submucosal damage	Deep mucosal damage	Muscular layer damage
6c	++	++	–	–
6e	++	++	–	–
6l	++	++	–	–
6g	–	–	–	–
7b	–	–	–	–
7e	–	–	–	–
Control	–	–	–	–
Ibuprofen	+++	+++	–	–

[–], No damage; [+], [++], [+++], indicates increasing degree of damage

Experimental

Chemistry

Melting points were determined on an Electro thermal apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker spectrometer (300, 400 MHz), chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Coupling constants *J* (H–H) are in Hertz (Hz). FT-IR spectra were recorded on a Bruker spectrometer, Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Elemental analyses were recorded on CHN analyzer model and found within range (±0.4%) of theoretical value.

*General procedure for the synthesis of ethyl-2-biphenyl-4-yloxy-acetate (**2**)*

To a mixture of *p*-hydroxybiphenyl (10 mM) and ethylchloroacetate (10 mM) in 50 mL of anhydrous acetone was added K_2CO_3 (36 mM). The suspension was refluxed for 20 h, after completion of reaction, monitored by TLC, the reaction mixture was filtered in hot condition, the filtrate was concentrated and finally the crude product was crystallized from methanol in cold condition.

Yield 75%; white flakes; m.p. 40–42°C; IR (KBr) cm^{-1}: 3055 (Ar-CH), 2921 (CH_2), 1674 (C=O), 1108 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54–7.53 (m, 4H), 7.42–7.36 (d, J = 7.6 Hz, 2H), 7.31–7.24 (m, 1H, Ar–H), 7.04–7.01 (d, J = 8.6 Hz, 2H), 5.29 (s, 2H, O–CH_2), 3.92 (q, J = 5.8 Hz, 2H), 1.85 (t, J = 5.6 Hz, 3H); FAB-MS (*m/z*): 256 (M^+), 257 (M+1); Anal. Calcd. for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found C, 74.68; H, 6.21.

*General procedure for synthesis of hydrazide (**3**)*

The ethanolic solution of ethyl-2-biphenyloxy-acetate (1) (10 mM) and of hydrazine monohydrate (10 mM) was refluxed for 4 h. After the completion of reaction, the reaction mixture was cooled and the white flakes so obtained were filtered and recrystallized from alcohol.

Yield 90%; white flakes; m.p. 165–167°C; IR (KBr) cm^{-1}: 3350 (N–H), 3055 (Ar–H), 2921 (CH_2), 1674 (C=O), 1108 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52–7.51 (m, 4 H, Ar–H), 7.43–7.37 (d, J = 7.8 Hz, 2H), 7.32–7.25 (m, 1H, Ar–H), 7.05–7.02 (d, J = 8.5 Hz, 2H), 6.29 (s, 1H, NH), 5.21 (s, 2H, O–CH_2), 3.2 (s, 2 H, NH_2); FAB-MS (*m/z*): 242 (M^+), 243 (M+1); Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.13; H, 5.84; N, 11.51%.

*General procedure for synthesis of thiosemicarbazides (**4a–4f**)*

To a solution of hydrazide (3) (1 mM) in absolute alcohol (50 mL) was added arylisothiocyanate (1 mM) and the reaction mixture

Table 4. Results of antimicrobial activity.

Compounds	Anti-fungal activity zone of inhibition (mm) 200 (100 μg)				Anti-bacterial activity zone of inhibition (mm) 200 (100 μg)			
	A.f	A.n	C.a	C.c	K.p	P.a	E.c	S.a
6a	19 ± 1.6 (8 ± .6)	14 ± 1.4 (2 ± .7)	8 ± .5 (6 ± .3)	6 ± .3 (–)	6 ± .3 (–)	10 ± 1.2 (6 ± .5)	9 ± .8 (–)	–
6b	12 ± 1.4 (6 ± .4)	10 ± 1.5 (6 ± .5)	8 ±1.6 (6 ± .6)	–	–	8 ± .8 (–)	8 ± .5 (–)	–
6c	12 ± 1.6 (6 ± .4)	10 ± 1.4 (6 ± .3)	8 ± 3 (6 ± .4)	6 ± .4 (–)	8 ± .5 (–)	12 ± 1.3 (6)	10 ± 1.1 (8 ± .5)	8 ± .4 (–)
6d	14 ± 1.2 (8 ± 1)	12 ± 1.4 (8 ± .4)	10 ± 1.4 (6)	8 ± .6 (6 ± 1.6)	10 ± .9 (8 ± .7)	12 ± 1.2 (6 ± .4)	8 ± .8 (–))	10 ± 1.2 (8 ± .6)
6e	10 ± 1.3 (8 ± 1.6)	12 ± 1.6 (10)	8 ± .9 (–)	8 ± 1.2 (6 ± .7)	14 ± 1.6(10 ± .6)	9 ± .8 (–)	10 ± 1.1 (–)	–
6f	**22 ± 1.8 (12 ± 1.6)**	**18 ± .8 (10 ± .4)**	**12 ± 1.1(8 ± .5)**	**10 ±.8 (8 ± .6)**	10 ± 1.2 (6 ± .4)	6 ± .5 (–)	–	–
6g	**22 ±1.9 (10 ± 1.2)**	14 ± .6 (7 ± .5)	10 ± 1.1(6 ± .4)	**10 ± .8 (6 ± .4)**	10 ± 1.6 (6 ± .6)	8 ± 1 (–)	–	–
6h	**20 ± 1.4 (10 ± 1.2)**	12 ±.9 (6 ± .5)	8 ±.8 (6 ± .4)	6 ± .5 (–)	11 ±1.2 (6 ± .4)	9 ± 1 (–)	–	–
6i	18 ±1.2 (8 ± 1.6)	10 ±.8 (8 ± .7)	10 ±.9 (6 ± .4)	8 ± 9 (6 ± 4)	12 ± 1.1 (6 ± .6)	–	10 ± 1.2 (–)	–
6j	18 ± 1.8 (10 ± 1.1)	12 ± 1.2 (6 ± .4)	8 ±.8 (6 ± .5)	**10 ±.9 (6 ± 5)**	14 ± 1.6 (10 ± 1.2)	11 ± .9 (–)	10 ± 1.2 (5 ± .4)	–
6k	18 ± 1.5 (10 ± .8)	6 ± .5 (–)	8 ±.6 (6 ± .4)	8 ± .8 (6 ± .6)	14 ± 1.2 (6 ± .6)	12 ± 1.2 (8 ± .6)	8 ± .7 (6 ± .6)	–
6l	18 ± 1.3 (10 ± 1.2)	10 ±1 (6 ± .5)	6 ± 1.6 (5 ± .4)	8 ± .6 (–)	8 ±.5 (6 ± .4)	**14 ± 1.7 (10 ± 1)**	10 ± 1.2 (6 ± .6)	8 ±.7 (–)
6m	18 ±1.6 (8 ± .6)	10 ±1.2(6 ± .4)	8 ± .9 (6 ± .5)	8 ±.9 (6 ± .6)	12 ±1.6 (8 ± .6)	12 ±1.2 (8 ± .7)	10 ± .9 (6 ± .6)	8 ±.6 (–)
6n	14 ±1.6 (10 ± .9)	8 ±.6 (–)	6 ± .5 (–)	9 ± 1.2 (6 ± .8)	10 ± 1.2 (6 ± .6)	8 ± .6(–)	–	–
60	18 ± 1.8 (10 ± 1.2)	12 ± 9 (6 ± .4)	6 ±.6 (–)	**10 ± .6 (6 ± .4)**	12 ±1.2 (8 ± .7)	12 ± 1.2 (9 ± .6)	10 ± 1.2 (–)	8 ± 1.2 (6 ± .6)
6p	14 ±1.2 (8 ± .7)	12 ± 1.1 (8 ± .4)	8 ± .8 (6 ± .3)	8 ± 1.2 (6 ± .4)	8 ± .6 (6 ± .3)	**14 ± 1.2 (10 ± .8)**	8 ± .6 (–)	6 ± .6 (–)
6q	12 ±1.2 (6 ± .4)	10 ± 1.2 (8 ± .6)	8 ± 6 (–)	**10 ±.8 (8 ± .6)**	8 ±.6 (6 ± .4)	**14 ± 1.2 (10 ± .7)**	8 ± .6 (–)	6 ±.4 (–)
6r	8 ±.6 (–)	10 ± .6 (6 ± .4)	8 ± 7 (–)	8 ± .8 (6 ± .4)	8 ± .6 (6 ± .4)	10 ± .8 (6 ± .6)	6 ± .3 (–)	6 ± .3 (–)
6s	14 ±1.2 (6 ± .3)	10 ± 1.2 (6 ± .6)	8 ± 6 (5 ± .3)	**9 ±.8 (7 ± .6)**	12 ± 1.2 (8 ± .6)	–	–	–
7a	8 ±.6 (6 ± .4)	8 ± .6 (–)	6 ± .4 (–)	9 ±.6 (6 ± .4)	8 ± .4 (–)	–	–	**12 ± 1.2 (6 ± .3)**
7b	6 ±.6 (–)	8 ± 7 (–)	10 ± .8 (8 ± .6)	–	9 ± .4 (–)	–	–	**12 ± 1.2 (6 ± .6)**
7c	6 ± .4 (–)	8 ± 8 (–)	10 ± 1.2(8 ± .5)	8 ± 1.2 (8 ± .6)	10 ±.9 (6 ± .4)	8 ± .6 (–)	–	10 ± 8 (8 ± .7)
7d	12 ± 1.6 (6 ± .4)	10 ± 1.6 ±1.6)	10 ± 1.6(6 ± .8)	**1 0 ± 1.6(8 ± 1.2)**	10 ± 1.6 (5 ± 1.6)	8 ± 1.6 (4)	8 ± 1.6 (6 ± 1.6)	10 ± 1.6 (6 ± 1.6)
7e	10 ± 1.2 (6 ± .4)	11 ± 1 (6 ± .3)	8 ± .6 (–)	8 ± .6 (6 ± .6)	10 ± 1.2 (6 ± .4)	8 ± .4 (–)	10 ± 1.2 (6 ± .6)	**12 ± .9 (6 ± .5)**
FLuconazole	22 ± 1.8 (12 ± .9)	16 ± 1 (10 ± .6)	14 ± 1.2(10 ± .)	**10 ± 1.2 (8 ± .7)**	Nt	Nt	Nt	nt
Amoxicillin	Nt	nt	nt	nt	16 ± 1 (12 ± .8)	14 ± .8 (10 ± .5)	12 ± .7 (8 ± .4)	12 ± .6 (6 ± .3)
Control	–	–	–	–	–	–	–	–

A.n.: *Aspergillus niger* [MTCC 8189], A.f.: *Aspergillus flavus* [MTTC 277], K.p.: *Klebsiella pneumoniae* [ATCC 700603] P.a.: *Pseudomonas aeruginosa* [ATCC-27853], *Escherichia coli* [ATCC 25922], *Staphylococcus aureus* [ATCC 25923], DMSO [dimethylsulfoxide], Flu.: fluconazole, Amp.: ampicillin, [nt] not tested, [–] no zone of inhibition.

was refluxed for 4–6 h. After the completion of reaction, monitored by TLC, the reaction mixture was cooled to room temperature and the white crystals so formed were filtered and crystallized from alcohol to yield the pure hydrazide (**4a–4g**).

*1-[2-(4-Biphenyloxy)acetyl]-4(4-chlorophenyl)thiosemicarbazide (**4a**)* Yield 74%; white crystalss; m.p. 183–185°C; IR (KBr) cm^{-1}: 3322 (N–H), 2958 (Ar–H), 1714 (C=O), 1195 (C=S), 1108 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 (brs, 1H, CONH), 9.70 (brs, 1H, CSNH), 9.21 (brs, 1H, ArNH), 7.52–7.47 (m, 6H), 7.43–7.36 (m, 4H), 7.30 (t, J = 5.7 Hz, 1H), 6.94 (d, J = 6.4 Hz, 2H), 4.69 (s, 2H, O–CH_2); FAB-MS (*m/z*): 411 (M^+), 412 (M+1); Anal. Calcd. for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20. Found: C, 61.41; H, 4.48; N, 10.18%.

*1-[2-(4-Biphenyloxy)acetyl]-4(4-nitrophenyl)thiosemicarbazide (**4b**)* Yield 69%; light yellow crystalss; m.p. 198–200°C; IR (KBr) cm^{-1}: 3246 (N–H), 2958 (CH_2), 1693 (C=O), 1208 (C=S), 1113 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.58 (brs, 1H, CONH), 8.81 (brs, 1H, CSNH), 8.02 (brs, 1H, ArNH), 8.38 (d, J = 8.6 Hz, 2H), 7.94 (d, J = 8.6 Hz, 2H), 7.52–7.26 (m, 7H), 6.94 (d, J = 6.6 Hz, 2H), 4.69 (s, 2H, O–CH_2). FAB-MS (*m/z*): 422 (M^+), 423 (M+1); Anal. Calcd. for $C_{21}H_{18}N_4O_4S$: C, 59.70; H, 4.29; N, 13.26. Found: C, 59.92; H, 4.32; N, 13.20.

*1-[2-(4-Biphenyloxy)acetyl]-4(4-methoxyphenyl)thiosemicarbazide (**4c**)* Yield 72%; white crystalss; m.p. 176–178°C; IR (KBr) cm^{-1}: 3246 (N–H), 2953 (CH_2), 1702 (C=O), 1205 (C=S), 1109 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.21 (brs, 1H, CONH), 9.12 (brs, 1H, CSNH), 8.92 (brs, 1H, ArNH), 7.61–7.47 (m, 6H), 7.46 (t, J = 5.7 Hz, 2H), 7.33 (t, J = 7.5 Hz, 1H), 7.08–7.01 (m, 4H), 4.68 (s, 2H, O–CH_2), 3.88 (s, 3H, O–CH_3); FAB-MS (*m/z*): 407 (M^+), 408 (M+1); Anal. Calcd. for $C_{22}H_{21}N_3O_3S$: C, 64.85; H, 5.19; N, 10.31. Found: C, 65.08; H, 5.22; N, 10.26.

*1-[2-(4-Biphenyloxy)acetyl]-4-phenylthiosemicarbazide (**4d**)* Yield 77%; white crystals; m.p. 138–140°C; IR (KBr) cm^{-1}: 3324 (N–H), 2945 (CH_2), 1701 (C=O), 1212 (C=S), 1109 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.14 (brs, 1H, CONH), 9.21 (brs, 1H, CSNH), 8.92 (brs, 1H, ArNH), 7.54–7.44 (m, 4H), 7.42–7.25 (m, 8H), 7.03 (d, J = 8.6 Hz, 2H), 4.68 (s, 2H, O–CH_2); FAB-MS (*m/z*): 377 (M^+), 378 (M+1); Anal. Calcd. for $C_{21}H_{19}N_3O_2S$: C, 66.82; H, 5.07; N, 11.13. Found: C, 66.48; H, 5.15; N 11.25.

*1-[2-(4-Biphenyloxy)acetyl]-4(4-fluorophenyl)thiosemicarbazide (**4e**)* Yield 76%; white crystals; m.p. 186–188°C; IR (KBr) cm^{-1}: 3244 (N–H), 3070 (Ar–H), 2950 (CH_2), 1683 (C=O), 1202 (C=S), 1106 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.56 (brs, 1H, CONH), 8.96 (brs, 1H, CSNH), 8.67 (brs, 1H, ArNH), 7.88 (d, J = 6.4 Hz, 2H), 7.51–7.46 (m, 4H), 7.42–7.37 (m, 4H), 7.31–7.19 (m, 3H), 4.66 (s, 2H, O–CH_2); FAB-MS (*m/z*): 395 (M^+), 396 (M+1); Anal. Calcd. for $C_{21}H_{18}FN_3O_2S$: C, 63.78; H, 4.59; N, 10.63. Found: C, 63.98; H, 4.53; N, 10.58.

*1-[2-(4-Biphenyloxy)acetyl]-4(2-methylphenyl)thiosemicarbazide (**4f**)* Yield 71%; white crystals; m.p. 138–140°C; IR (KBr) cm^{-1}: 3320 (N–H), 2948 (CH_2), 1704 (C=O), 1215 (C=S), 1254 (C=C), 1204 (C=C), 1102

(C–O); ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.12 (brs, 1H, CONH), 9.21 (brs, 1H, CSNH), 8.92 (brs, 1H, ArNH), 7.56–7.30 (m, 11H), 6.86 (2H, d, J = 6.4 Hz), 4.67 (s, 2H, O–CH_2), 2.38 (s, 3 H, CH_3); Maldi-MS (*m/z*): 391 (M^+), 392 (M+1); Anal. Calcd. for $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.07; N, 10.73. Found: C, 67.85; H, 5.11; N 10.68.

*General method for synthesis of 5-[(biphenyl-4-yloxy)-methyl]4-aryl-3-mercapto-(4H)-1,2,4-triazole (**5a–5f**)*

To a solution of thiosemicarbazide (**4a–4f**) (5 mM) in absolute alcohol (50 mL) was added triethyl amine (1 mL) and the reaction mixture was refluxed for 4–8 h. After the completion of reaction, monitored by TLC, the reaction mixture was cooled to room temperature and concentrated under reduced pressure (25 mL), the concentrated solution was poured on crushed ice, the precipitate obtained was filtered off and washed with cold water, dried and crystallized in ethanol to yield the pure 3-mercapto-1,2,4-triazoles (**5a–5f**).

*5-[(Biphenyl-4-yloxy)methyl]4-(4-chlorophenyl)-3-mercapto-(4H)-1,2,4-triazole (**5a**)* Yield 85%; white crystals; m.p. 228–230°C; IR (KBr) cm^{-1}: 3056 (Ar–H), 2922 (CH_2), 2690 (S–H), 1606 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.71 (brs, 1H, SH), 7.57–7.54 (t, J = 8.4 Hz, 2H), 7.54–7.52 (d, J = 6.9 Hz, 2H), 7.44–7.39 (m, 6H), 7.32–7.29 (t, J = 8.1 Hz, 1H), 7.06–7.03 (d, J = 8.7 Hz, 2H), 4.52 (s, 2H, O–CH_2). Maldi-MS (*m/z*): 393 (M^+), 394 (M+1); Anal. Calcd. for $C_{21}H_{16}ClN_3OS$: C, 64.03; H, 4.09; N, 10.67. Found: C, 64.08; H, 4.12; N, 10.63%.

*5-[(Biphenyl-4-yloxy)methyl]4-(4-nitrophenyl)-3-mercapto-(4H)-1,2,4-triazole (**5b**)* Yield 89%; light yellow crystals; m.p. 221–222°C; IR (KBr) cm^{-1}: 2912 (CH_2), 2690 (S–H), 1604 (C=N), 1102 (C–O). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (brs, 1H, S-H), 8.39 (d, J = 8.6 Hz, 2H,), 7.65 (d, J = 8.8 Hz, 2H), 7.52–7.32 (m, 6H), 7.28 (t, J = 6.9 Hz, 1H), 6.96 (d, J = 6.6 Hz, 2H), 4.81 (s, 2H, O–CH_2). Maldi-MS (*m/z*): 404 (M^+), 405 (M+1). Anal. Calcd. for $C_{21}H_{16}N_4O_3S$: C, 62.36; H, 3.99; N, 13.85. Found: C, 62.39; H, 4.01; N, 12.79%.

*5-[(Biphenyl-4-yloxy)methyl]4-(4-methoxyphenyl)-3-mercapto-(4H)-1,2,4-triazole (**5c**)* Yield 68%; white crystals; m.p. 168–170°C; IR (KBr) cm^{-1}: 3058 (Ar–H), 2688 (S–H), 1603 (C=N), 1107 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.71 (brs, 1H, SH), 7.52 (d, J = 5.4 Hz, 2H), 7.49 (d, J = 6.9 Hz, 2H), 7.42 (d, J = 5.7 Hz, 2H), 7.36–7.30 (m, 3H), 7.01 (d, J = 6.6 Hz, 2H), 6.91 (d, J = 6.6 Hz, 2H), 4.93 (s, 2H, O-CH_2), 3.88 (s, 3H, O–CH_3); Maldi-MS (*m/z*): 389 (M^+), 390 (M+1); Anal. Calcd. for $C_{22}H_{19}N_3O_2S$: C, 67.84; H, 4.92; N, 10.79. Found: C 67.76; H 4.89; N 10.75.

*5-[(Biphenyl-4-yloxy)methyl]4-(phenyl)-3-mercapto-(4H)-1,2,4-triazole (**5d**)* Yield 78%; white crystals; m.p. 234–236°C; IR (KBr) cm^{-1}: 2912 (CH_2), 2690 (S–H), 1607 (C=N), 1110 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.57 (brs, 1H, S–H), 7.57–7.52 (m, 4H), 7.44–7.25 (m, 8H), 7.04 (2H, d, J = 8.7 Hz), 4.69 (s, 2H, O–CH_2); Maldi- MS (*m/z*): 359 (M^+), 360 (M+1); Anal. Calcd. for $C_{21}H_{17}N_3OS$: C, 70.17; H, 4.77; N, 11.69. Found: C, 70.09; H, 4.64; N, 11.58.

*5-[(Biphenyl-4-yloxy)methyl]4-(4-fluorophenyl)-3-mercapto-(4H)-1,2,4-triazole (**5e**)* Yield 76%; white crystals; m.p. 186–188°C; IR (KBr) cm^{-1}: 3049 (Ar–H), 2929 (CH_2), 2680 (S–H), 1603, 1514 (C=N), 1105 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (brs, 1H, SH), 7.51–7.46 (m, 4H), 7.41–7.37 (m, 4H), 7.28 (t, J = 5.4 Hz, 1H), 7.21 (t, J = 8.3 Hz, 2H), 6.94 (d, J = 6.3 Hz, 2H), 4.73 (s, 2H, O–CH_2); Maldi-MS (*m/z*): 377 (M^+), 378 (M+1); Anal. Calcd. for $C_{21}H_{16}FN_3OS$: C, 66.83; H, 4.27; N, 11.13. Found: C, 66.92; H, 4.25; N, 11.06.

*5-[(Biphenyl-4-yloxy)methyl]4-(2-methylphenyl)-3-mercapto-(4H)-1,2,4-triazole (**5f**)* Yield 72%; white crystals; m.p. 186–188°C; IR (cm^{-1}): 2934 (CH_2), 2686 (S–H), 1604, 1594 (C=N), 1108 (C–O–C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (brs, 1H, S–H), 7.54–7.51 (m, 4H), 7.43 (m, 7H), 6.89 (2H, d, J = 6.6 Hz), 4.68 (s, 2H, O–CH_2), 2.54 (3H, s, CH_3); Maldi-MS (*m/z*): 373 (M^+), 374 (M+1). Anal. Calcd. for $C_{22}H_{19}N_3OS$: C 70.75; H 5.13; N 11.25. Found: C 70.82; H 5.10; N 11.30%.

*General method for synthesis of 1(aryl)-2-[5-{(biphenyl-4-yloxy)methyl}4-aryl-3-mercapto-(4H)-1,2,4-triazol-3-ylthio)] ethanone/ethane (**6a–6s**)*

To a solution of 3-mercapto-1,2,4-triazole (**5a–5e**, 0.5 mM) in absolute ethanol (50 mL) was added sodium metal (0.5 mM) and different substituted phenacyl bromide (0.5 mM) and the reaction mixture was refluxed for 1–6 h; after completion of reaction (monitored by TLC), the reaction mixture was concentrated under reduced pressure (25 mL), the concentrated solution was cooled and the crystals obtained were filtered, washed with water, dried and recrystallized from dichloromethane (DCM) and methanol to yield the pure compound (**6a–6s**).

*2-{5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(phenyl)ethanone (**6a**)* Yield 68%; white crystals; m.p. 192–194°C; IR (KBr) cm^{-1}: 2924 (CH_2), 1680 (C=O), 1584 (C=N), 1107 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.88 (d, J = 6.2 Hz, 2H), 7.58–7.60 (d, J = 7.8 Hz, 2H), 7.50–7.45 (m, 6H, Ar–H), 7.39–7.28 (m, 6H, Ar–H), 6.94–6.92 (d, J = 6.4 Hz, 2H), 5.08 (s, 2H, O–CH_2), 4.51 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.85, 156.34, 148.47, 145.75, 136.46, 135.18, 132.76, 131.05, 130.27, 129.67, 129.18, 128.98, 128.99, 127.85, 128.67, 127.24, 126.88, 114.98, 60.07, 36.96; Maldi-MS (*m/z*): 512 (M^+), 513 (M+1); Anal. Calcd. for $C_{29}H_{22}ClN_3O_2S$: C, 68.03; H, 4.33; N, 8.21. Found: C, 68.09; H, 4.30; N, 8.19.

*2-{5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-methoxyphenyl) ethanone (**6b**)* Yield 75%; white crystals; m.p. 178–180°C; IR (KBr) cm^{-1}: 2912 (CH_2), 1681 (C=O), 1607 (C=N), 1093 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.84 (d, J = 6.4 Hz, 2H), 7.62–7.60 (d, J = 6.4 Hz, 2H), 7.46–7.51 (m, 6H), 7.33–7.27 (m, 3H), 6.96–6.91 (dd, J = 6.6 Hz, 4H), 5.01 (s, 2H, O–CH_2), 4.53 (s, 2H, S–CH_2), 3.83 (s, 3H, O–CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.94, 156.78, 156.34, 148.47, 145.75, 135.16, 134.78, 131.05, 130.26, 130.16, 129.57, 128.96, 126.88, 129.86, 128.95, 127.85, 127.24, 117.56, 114.98, 60.07, 36.96; Maldi-MS (*m/z*): 542 (M^+), 543 (M+1); Anal. Calcd. for $C_{30}H_{24}ClN_3O_3S$: C, 66.47; H, 4.46; N, 7.75. Found: C, 66.50; H, 4.47; N, 7.72.

*2-{5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-bromophenyl)ethanone (**6c**)* Yield 68%; white crystals; m.p. 154–156°C; IR (cm^{-1}): 2912 (CH_2), 1683 (C=O), 1607, 1584 (C=N), 1093 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 6 Hz, 2H), 7.62 (d, J = 6 Hz, 2H), 7.51–7.46 (m, 6H), 7.39 (t, J = 5.7 Hz, 2H), 7.34 (d, J = 6.3 Hz, 2H), 7.29 (t, J = 5.7 Hz, 1H), 6.94 (d, J = 6.3 Hz, 2H), 5.12 (s, 2H, O-CH_2), 4.92 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.64, 156.95, 148.24, 145.25, 142.00, 135.17, 134.76, 130.85, 130.26, 130.06, 129.35, 129.27, 128.87, 128.82, 128.76, 127.65, 126.92, 126.88, 115.18, 59.96, 35.87; Maldi-MS (*m/z*): 590 (M^+), 591 (M+1); Anal. Calcd. for $C_{29}H_{21}ClBrN_3O_2S$: C, 58.94; H, 3.58; N, 7.11. Found: C, 59.02 H, 3.59; N, 7.08.

*2-{5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-nitrophenyl)ethanone (**6d**)* Yield 72%; light yellow crystals; m.p. 183–184°C; IR (KBr) cm^{-1}: 2905 (C–H), 1684 (C=O), 1605, 1525 (C=N), 1320 (N=O), 1093 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 6.6 Hz, 2H), 8.20 (d, J = 6.6 Hz, 2H), 7.53–7.47 (m, 6H), 7.41 (t, J = 5.7Hz; 2H), 7.37 (d, J = 6.6 Hz, 2H), 7.31 (t, J = 5.7 Hz, 1H), 6.95 (d, J = 6.3 Hz, 2H), 5.10 (s, 2H, O–CH_2), 4.91 (s, 2H, S–CH_2);

^{13}C NMR (100 MHz, DMSO-d_6) δ 191.65, 156.81, 150.00, 147.00, 140.41, 139.72, 136, 135.17, 131.12, 130.92, 130.28, 128.78, 128.31, 126.97, 126.79, 124.09, 115.10, 59.87, 36.75; Maldi-MS (*m/z*) 557 (M^+), 558 (M+1); Anal. Calcd. for $C_{29}H_{21}ClN_4O_4S$: C, 62.53; H, 3.80; N, 10.06. Found: C, 62.62; H, 3.82; N, 9.96.

2-{5-[(4-Biphenyloxy)methyl]-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(phenyl)ethanone (***6e***) Yield 66%; light yellow crystals; m.p. 168–170°C; IR (KBr) cm^{-1}: 2907 (CH_2), 1686 (C=O), 1515, 1524 (C=N), 1093 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 8.7 Hz, 2H), 7.98 (d, J = 9 Hz, 2H), 7.66 (d, J = 8.9 Hz, 2H), 7.52–7.28 (m, 10H), 6.96 (d, J = 6.6 Hz, 2H), 5.14 (s, 2H, O–CH_2), 4.91 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.24, 156.88, 150.12, 147.34, 141.46, 138.56, 135.86, 135.76, 130.68, 130.27, 128.65, 128.32, 128.31, 126.79, 124.09, 122.34, 115.17, 59.86, 35.79; MS (*m/z*): 522 (M^+), 523 (M+1); Anal. Calcd. for $C_{29}H_{22}N_4O_4S$: C, 66.65; H, 4.24; N, 10.72. Found: C, 66.72; H, 4.26; N, 10.68.

2-{5-[(4-Biphenyloxy)methyl]-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-phenylphenyl)ethanone (***6f***) Yield 70%, light yellow crystals, m.p. 175–177°C; IR (KBr) cm^{-1}: 2907 (C–H), 1671 (C=O), 1601, 1558 (C=N), 1093 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, J = 8.4 Hz, 2H), 8.10–8.07 (d, J = 7.8 Hz, 2H), 7.73–7.63 (m, 6H), 7.52–7.39 (m, 8H), 7.31 (t, J = 5.7 Hz, 1H), 6.93 (d, J = 7.8 Hz, 2H), 5.15 (s, 2H, O–CH_2), 5.00 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.34, 191.28, 156.98, 150.14, 147.33, 141.47, 138.86, 138.57, 135.84, 135.38, 135.12, 130.73, 130.70, 130.69, 130.29, 128.37, 128.32, 127.34, 126.87, 126.80, 124.09, 122.36, 115.16, 61.09, 37.06; Maldi-MS (*m/z*): 598 (M^+) 599 (M+1); Anal. Calcd. for $C_{35}H_{26}N_4O_4S$: C, 70.22; H, 4.38; N, 9.36. Found: C, 70.14; H, 4.35; N, 9.34.

2-{5-[(4-Biphenyloxy)methyl]-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-bromophenyl)ethanone (***6g***) Yield 70%; white crystals; m.p. 156–158°C; IR (KBr) cm^{-1}: 2907 (C–H), 1686 (C=O), 1605, 1536 (C=N), 1105 (C–O–C); ^{1}H NMR ($CDCl_3$) δ 7.53–7.48 (m, 4H), 7.51–7.37 (m, 8H), 7.28 (t, J = 5.5 Hz, 1H), 7.24–7.18 (m, 2H), 7.01 (d, J = 6.7 Hz, 2H), 6.90 (d, J = 6.6 Hz, 2H), 5.15 (s, 2H, O–CH_2), 4.85 (s, 2H, S–CH_2), 3.88 (s, 3H, O–CH_3); ^{13}C NMR ($CDCl_3$) δ 191.45, 160.65, 156.84, 150.25, 147.34, 140.34, 135.89, 134.81, 132.24, 130.65, 129.08, 128.96, 128.84, 128.74, 128.56, 127.22, 126.98, 126.80, 122.48, 114.94, 60.09, 37.58; Maldi-MS (*m/z*): 586 (M^+), 587 (M+1); Anal. Calcd. for $C_{30}H_{24}BrN_3O_3S$: C, 61.44; H, 4.12; N, 7.16. Found: C, 61.48; H, 4.09; N, 7.13.

2-{5-[(4-Biphenyloxy)methyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-chlorophenyl)ethanone (***6h***) Yield 79%; white crystals; m.p. 154–156°C; IR (KBr) cm^{-1}: 2907 (C–H_2), 1655 (C=O), 1605 (C=N), 1239 (C=C), 1093 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.4 Hz, 2H), 7.52–7.46 (m, 4H), 7.40 (t, J = 7.5 Hz, 2H), 7.32–7.25 (m, 5H), 7.00 (d, J = 9 Hz, 2H), 6.96 (d, J = 8.4 Hz, 2H), 5.12 (s, 2H, O–CH_2), 4.91 (s, 2H,S–CH_2), 3.85 (s, 3H,O–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.24, 160.69, 156.84, 150.15, 147.35, 140.21, 137.87, 135.87, 134.81, 132.24, 130.73, 129.03, 128.99, 128.57, 128.08, 127.12, 126.81, 115.27, 114.94, 60.12, 38.03, 55.35; Maldi-MS (*m/z*): 542 (M^+), 543 (M+1); Anal. Calcd. for $C_{30}H_{24}ClN_3O_3S$: C, 66.47; H, 4.46; N, 7.75. Found: C, 66.53; H, 4.44; N, 7.69.

2-{5-[(4-Biphenyloxy)methyl]-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio}-1-(phenyl)ethanone (***6i***) Yield 82%, white crystals, m.p. 150–152°C; IR (KBr) cm^{-1}: 2907 (CH_2), 1686 (C=O), 1541 (C=N), 1102 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.4 Hz, 2H,), 7.46–7.40 (m, 6H), 7.40 (t, J = 7.5 Hz, 2H), 7.32–7.25 (m, 6H), 6.96 (d, J = 8.4 Hz, 2H), 5.10 (s, 2H, O–CH_2), 4.92 (s, 2H, S–CH_2), 3.85 (s, 3H, O–CH_3); ^{13}C NMR ($CDCl_3$) δ 190.20, 160.69, 156.81, 150.17, 147.37, 137.26, 135.87, 134.83, 133.20, 132.27, 130.76, 129.05, 128.92, 128.56, 128.09, 127.12, 126.79, 115.12, 115.12, 114.94, 60.24, 56.14, 36.13; Maldi-MS (*m/z*) 507 (M^+), 508 (M+1); Anal. Calcd. for $C_{30}H_{25}N_3O_3S$: C, 70.98; H, 4.96; N, 8.28. Found: C, 71.02; H, 4.95; N, 8.25.

2-{5-[(4-Biphenyloxy)methyl]-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-methoxyphenyl)ethanone (***6j***) Yield 78%; white crystals; m.p. 157–159°C; IR (KBr) cm^{-1}: 2907 (CH_2), 1687 (C=O), 1586 (C=N), 1087 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 9.0 Hz, 2H), 7.67 (d, J = 8.9 Hz, 2H), 7.52–7.46 (m, 4H), 7.41 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 5.8 Hz, 1H), 7.08–6.88 (m, 6 H), 5.15 (s, 2H, O–CH_2), 4.92 (s, 2H, S–CH_2), 3.87 (s, 6H, O–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.94, 164.56, 156.82, 160.68, 150.19, 147.38, 135.14, 134.54, 130.96, 129.96, 129.07, 128.92, 128.56, 127.13, 127.10, 126.80, 115.13, 114.94, 114.92, 60.25, 55.63, 37.10; Maldi-MS (*m/z*): 537 (M^+), 538 (M+1); Anal. Calcd. for $C_{31}H_{27}N_3O_4S$: C, 69.25; H, 5.06; N, 7.82. Found: C, 69.30; H, 5.02; N, 7.79.

2-{5-[(4-Biphenyloxy)methyl]-4-(phenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-bromophenyl) ethanone (***6k***) Yield 72%; white crystals; m.p. 164–166°C; IR (cm^{-1}): 2907 (CH_2), 1686 (C=O), 1570 (C=N), 1093 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 6.0 Hz, 2H), 7.56 (d, J = 6.3 Hz, 2H), 7.41–7.34 (m, 11H), 7.23 (t, J = 5.4 Hz, 1H), 6.87 (d, J = 6.3 Hz, 2H), 5.02 (s, 2H, O–CH_2), 4.97 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.19, 156.99, 150.17, 147.37, 135.14, 134.91, 132.52, 132.24, 130.07, 129.98, 128.78, 128.26, 126.95, 126.91, 126.81, 126.79, 115.18, 63.35, 41.01; Maldi-MS (*m/z*): 556 (M^+), 557 (M+1); Anal. Calcd. for $C_{29}H_{22}BrN_3O_2S$: C, 62.59; H, 3.98; N, 7.55. Found: C, 62.64; H, 4.01; N, 7.52.

2-{5-[(4-Biphenyloxy)methyl]-4-(phenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-nitrophenyl)ethanone (***6l***) Yield 67%; light yellow crystals; m.p. 170–172°C; IR (cm^{-1}): 2907 (CH_2), 1684 (C=O), 1541 (C=N), 1107 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 6.6Hz, 2H), 8.22 (d, J = 6.6 Hz, 2H), 7.56–7.54 (m, 3H), 7.51 (d, J = 6.3Hz, 2H), 7.47 (d, J = 6.6 Hz, 2H), 7.42–7.39 (m, 4H), 7.30 (t, J = 5.4 Hz, 1H), 6.95 (d, J = 6.3 Hz, 2H), 5.10 (s, 2H, O–CH_2), 4.92 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.88, 156.95, 152.22, 150.73, 140.46, 139.75, 134.97, 132.43, 130.53, 130.03, 129.72, 128.78, 128.27, 126.92, 126.78, 124.09, 115.17, 59.92, 39.86; TOF-MS (*m/z*): 522 (M^+), 523 (M+1); Anal. Calcd. for $C_{29}H_{22}N_4O_4S$: C, 66.65; H, 4.24; N, 10.72. Found: C, 66.69; H, 4.23; N, 10.6.

3-(4-Nitrophenylethylthio)-5-[(4-biphenyloxy)methyl]-4-phenyl-4H-1,2,4-triazole (***6m***) Yield 74%; light yellow crystals; m.p. 138–140°C; IR (KBr) cm^{-1}: 2907 (CH_2), 1525 (C=N), 1128 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 6.0 Hz, 2H), 7.56–7.47 (m, 8H), 7.41 (d, J = 7.2 Hz, 2H), 7.30 (t, J = 5.4 Hz, 1H), 7.23 (d, J = 6.6 Hz, 2H), 6.94 (d, J = 5.7 Hz, 2H), 5.09 (s, 2H, O–CH_2), 4.53 (s, 2H, S–CH_2), 3.74 (t, J = 8.4 Hz, 2H), 3.12 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.90, 148.45, 147.43, 144.24, 140.45, 135.92, 130.43, 130.11, 130.45, 129.45, 129.45, 128.77, 126.88, 123.82, 115.17, 59.87, 35.87; Maldi-MS (*m/z*): 508 (M^+), 509 (M+1); Anal. Calcd. for $C_{29}H_{24}N_4O_3S$: C, 68.49; H, 4.76; N, 11.02. Found: C, 68.55; H, 4.74; N, 11.05.

3-(Phenylethylthio)-5-[(4-biphenyloxy)methyl]-4-phenyl-4H-1,2,4-triazole (***6n***) Yield 70%; m.p. 138–140°C; IR (KBr) cm^{-1}: 2907 (CH_2), 1541, 1523 (C=N), 1107 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.49 (6H, m), 7.40 (2H, t, J = 7.2 Hz), 7.34–7.31 (m, 3H), 7.25–7.18 (m, 6H), 6.96 (2H, d, J = 9Hz), 5.10 (2H, s, OCH_2), 3.50 (2H, t, J = 7.8 Hz, S–CH_2), 3.08 (2H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.88, 148.79, 148.34, 145.56, 142.43, 134.91, 130.45, 130.43, 130.34, 130.04,

129.98, 128.79, 128.76, 128.77, 126.88, 124.24, 123.89, 115.16, 59.84, 36.74, 35.86; Maldi-MS *(m/z)*: 522 (M^+), 523 (M+1); Anal. Calcd. for $C_{29}H_{22}N_4O_4S$: C, 66.65; H, 4.24; N, 10.72. Found: C, 66.60; H, 4.23; N, 10.69.

*2-{5-[(4-Biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-bromophenyl)ethanone (**6o**)* Yield 72%; white crystals; m.p. 160–162°C; IR (cm^{-1}): 2907 (CH_2), 1684 (C=O), 1605 (C=N), 1108 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92–7.90 (m, 17 H, Ar–H), 5.08 (s, 2H, O–CH_2), 4.92 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.34, 162.98, 156.87, 145.69, 145.58, 137.24, 134.89, 131.29, 130.87, 130.76, 130.76, 130.46, 130.41, 130.09, 128.78, 126.87, 126.79, 126.23, 115.15, 114.97, 59.85, 36.79; Maldi-MS *(m/z):* 574 (M^+), 575 (M+1); Anal. Calcd. for $C_{29}H_{21}BrFN_3O_2S$: C, 60.63; H, 3.68; N, 7.31. Found: C, 60.67; H, 3.69; N, 7.32.

*2-{5-[(4-Phenylphenoxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(phenyl)ethanone (**6p**)* Yield 74%; white crystals; m.p. 156–158°C; IR (cm^{-1}): 2933 (CH_2), 1698 (C=O), 1515 (C=N), 1105 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 6.3 Hz, 2H), 7.63 (d, J = 6 Hz, 2H), 7.51–7.46 (m, 5H), 7.41–7.37 (m, 4H), 7.29 (t, J = 5.4 Hz, 1H), 7.41 (t, J = 8.4Hz, 2H), 6.93 (d, J = 6.3Hz, 2H), 5.08 (s, 2H, O–CH_2), 4.92 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.36, 162.97, 156.78, 145.63, 145.27, 137.15, 136.15, 134.34, 133.89, 130.88, 130.87, 130.67, 130.45, 130.12, 130.10, 129.89, 128.77, 126.25, 115.13, 114.79, 58.97, 37.03; Maldi-MS (*m/z*): 495 (M^+), 496 (M+1); Anal. Calcd. for $C_{29}H_{22}ClFN_3O_2S$: C, 70.29; H, 4.47; N, 8.48. Found: C, 70.28; H, 4.43; N, 8.50.

*2-{5-[(4-Biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazol-3-ylthio}-1-(4-methoxyphenyl) ethanone (**6q**)* Yield 85%, white crystals, m.p. 172–174°C; IR (cm^{-1}): 2907 (CH_2), 1686 (C=O), 1605 (C=N), 1093 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87–7.89 (m, 18 H), 5.14 (s, O–CH_2, 2H), 4.91 (s, S–CH_2, 2H), 3.87 (s, 3H, O–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.05, 162.97, 161.67, 156.87, 146.00, 145.27, 133.91, 130.89, 130.26, 130.15, 130.12, 130.11, 129.87, 128.00, 126.26, 115.14, 114.96, 114.78, 114.15, 58.98, 37.47, 55.87; Maldi-MS (*m/z*): 525 (M^+), 526 (M+1); Anal. Calcd. for $C_{30}H_{24}FN_3O_3S$: C, 68.56; H, 4.60; N, 7.99. Found: C, 68.60; H, 4.58; N, 8.01.

*3-(Phenylethylthio)-5-[(4-biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazole (**6r**)* Yield 62%; white crystals; m.p. 144–146°C; IR (KBr) cm^{-1}: 2907 (C–H), 1520, 1517 (C=N), 1108 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52–7.49 (m, 6H), 7.40 (t, J = 7.8 Hz, 2H), 7.34–7.18 (m, 9H), 6.96 (d, J = 9.0 Hz, 2H), 5.08 (s, 2H, O–CH_2), 3.50 (t, J = 7.5 Hz, 2H, S–CH_2), 3.08 (t, J = 8.1 Hz, 2H,); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.69, 156.98, 149.00, 147.18, 145.14, 135.14, 134.79, 130.98, 130.45, 130.25, 130.08, 130.03, 129.85, 129.85, 129.17, 127.99, 123.07, 114.78, 114.56, 59.12, 37.49, 55.58; Maldi-MS (*m/z*): 481 (M^+), 482 (M+1); Anal. Calcd. for $C_{29}H_{24}FN_3OS$: C, 72.33; H, 5.02; N, 8.73. Found: C, 72.29; H, 5.04; N, 8.69.

*3-(4-Nitrophenylmethylthio)-5[{(4-biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazole (**6s**)* Yield 68%; light yellow crystals; m.p. 168–172°C; IR (KBr) cm^{-1}: 2916 (C–H), 1524 (C=N), 1106 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (2H, d, J = 8.4 Hz), 7.49–7.40 (m, 6H), 7.33 (t, J = 7.6 Hz, 2H), 7.23 (t, J = 5.7 Hz, 1H), 7.16–7.08 (m, 4H), 6.87 (d, J = 8.4 Hz, 2H), 5.01 (s, 2H, O–CH_2), 4.45 (s, 2H, S–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.97, 156.89, 155.76, 146.57, 146.23, 137.73, 134.91, 131.27, 130.89, 130.48, 130.27, 130.12, 129.89, 128.79, 126.89, 126.27, 123.27, 115.14, 115.14, 114.97, 59.89, 36.86; Maldi-MS (*m/z*): 512 (M^+), 513 (M+1); Anal. Calcd. for $C_{28}H_{21}FN_4O_3S$: C, 65.61; H, 4.13; N, 10.93. Found: C, 65.67; H, 4.09; N, 10.90.

*General procedure for synthesis of 4-(substituted phenyl)-3-(amine-4-ylmethylthio)-5-(4-biphenyloxymethyl)-4H-1,2,4-triazole (**7a–7e**)*

To 50 mL absolute alcohol was added appropriate triazole (**5b**, **5f** and **5g**) (5 mM), formaldehyde (5 mM) and appropriate amine (5 mM), the reaction mixture was stirred for 24 h, and allowed to stand overnight in refrigerator. The precipitate so obtained was filtered, washed with cold alcohol and recrystallized from alcohol.

*5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (**7a**)* Yield 58%; white crystals; m.p. 180–182°C; IR (KBr) cm^{-1}: 2909 (C–H), 1608 (C=N), 1381 (C=S), 1115 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86–7.29 (m, 11H), 6.89 (d, J = 8.7 Hz, 2H), 5.18 (s, 2H, O–CH_2), 4.93 (s, 2H, N–CH_2–N), 3.72 (t, J = 3.9 Hz, 4H), 2.86 (t, J = 4.5 Hz 4H, N-$(CH_2)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.72, 156.41, 146.12, 140.22, 136.29, 135.51, 132.08, 129.95, 129.26, 128.34, 129.64, 127.03, 126.76, 115.11, 69.86, 66.81, 59.99, 50.74; Maldi-MS (*m/z*): 493 (M^+), 495 (M+2); Anal. Calcd. for $C_{26}H_{25}N_4O_2S$: C, 63.34; H, 5.11; N, 11.36. Found: C, 63.52; H, 5.18; N, 11.41.

*5-[(4-Biphenyloxy)methyl]-(4-methoxyphenyl)-3-(N-methyl-piperazine-4-ylmethylthio)-4H-1,2,4-triazole (**7b**)* Yield 54%; white crystals; m.p. 184–186°C; IR (cm^{-1}): 2907 (C–H), 1557 (C=N), 1326 (C=S), 1109 (C–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.60–7.36 (m, 9H), 7.11–6.96 (m, 4H), 5.07 (s, 2H O–CH_2), 4.49 (s, 2H, N–CH_2–N), 3.81 (s, 3H, O–CH_3), 2.71 (t, J = 7.8 Hz 4H), 2.51 (t, J = 9 Hz, 4H), 2.25 (s, 3H, N–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.82, 163.49, 155.64, 156.68, 149.66, 135.69, 130.29, 129.64, 129.61, 128.69, 127.89, 127.62, 126.92, 115.45, 68.82, 68.45, 56.24, 49.26, 44.14, 57.15; Maldi-MS (*m/z*) 501 (M^+), 502 (M+1); Anal. Calcd. for $C_{28}H_{31}N_5O_2S$: C, 67.04; H, 6.23; N, 13.96. Found: C, 67.01; H, 6.21; N, 13.93.

*5-[(4-Biphenyloxy)methyl]-4-(4-methoxyphenyl)-2-[(pyrrolidin-1-yl)methyl]-2H-1,2,4-triazole-3(4H)-thione (**7c**)* Yield 80%; white crystals; m.p. 174–176°C; IR (KBr) cm^{-1}: 2907 (C–H), 1607, 1516 (C=N), 1316 (C=S), 1104 (C–O–C); ^{1}H NMR (DMSO-d_6) δ 6.91–7.54 (m, 14H, Ar–H), 5.22 (s, 2H, O–CH_2), 4.47 (s, 2H, N–CH_2–N), 3.82 (s, 3H, O–CH_3), 2.24 (t, J = 8 Hz, 4H, N–CH_2), 1.53 (t, J = 7.5 Hz, 4H, CH_2–CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.86, 163.54, 155.76, 149.68, 135.65, 133.89, 130.27, 129.67, 129.58, 128.89, 128.69, 127.79, 126.89, 126.75, 115.42, 68.76, 68.45, 52.42, 26.41; Maldi-MS (*m/z*): 472 (M^+), 473 (M+1); Anal. Calcd. for $C_{27}H_{28}N_4O_2S$: C, 68.62; H, 5.97; N, 11.85. Found: C, 68.68; H, 5.99; N, 11.87.

*5-[(4-Biphenyloxy)methyl]-4-(phenyl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (**7d**)* Yield 64%; white crystals; m.p. 132–134°C; IR (cm^{-1}): 2912 (CH_2), 1607 (C=N), 1324 (C=S), 1103 (C–O–C); ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.54–7.40 (m, 12H), 6.93 (d, J = 8.8 Hz, 2H), 5.22 (s, 2H, O–CH_2), 4.97 (s, 2H, N–CH_2–N), 4.76 (t, J = 6.7 Hz, 4H), 2.90 (t, J = 7.0 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.56, 163.49, 155.76, 149.68, 135.65, 133.89, 130.27, 129.67, 129.58, 128.89, 128.69, 127.79, 126.89, 115.42, 115.42, 64.86, 68.76, 68.45, 64.86, 49.23; Maldi-MS (*m/z*): 458 (M^+), 459 (M+1); Anal. Calcd. for $C_{26}H_{26}N_4O_2S$: C, 68.10; H, 5.71; N, 12.22. Found: C, 68.07; H, 5.69; N, 12.20.

*5-[(4-Biphenyloxy)methyl]-4-(2-methylphenyl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (**7e**)* Yield 52%; white crystals; m.p. 126–128°C; IR (cm^{-1}): 2917 (C–H), 1516 (C=N), 1324 (C=S), 1108 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58–7.30 (m, 11H), 6.90 (d, J = 8.7 Hz, 2H), 5.23 (s, 2H, O–CH_2), 4.93 (s, 2H, N–CH_2–N), 3.57 (t, J = 6.7 Hz, 4H), 2.72 (t, J = 6.9 Hz, 4H), 2.50 (s, 3H, Ar–CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.58, 163.51,

155.86, 149.66, 135.66, 130.26, 136.84, 134.43, 129.63, 129.57, 128.86, 128.81, 126.88, 126.98, 126.97, 115.43, 68.79, 68.56, 64.87, 49.24; Maldi-MS (*m/z*) 472 (M^+), 473 (M+1); Anal. Calcd. for $C_{27}H_{28}N_4O_2S$: C, 68.62; H, 5.97; N, 11.85. Found: C, 68.65; H, 5.95; N, 11.83.

Biological screening

Albino Wistar rats of either sex (150–200 g) were obtained from Central Animal House, Hamdard University, New Delhi. The animals were kept in cages at room temperature and fed with food and water *ad libitum*. Fourteen hours before the start of the experiment the animals were sent to lab and fed only with water *ad libitum*. The experiments were performed in accordance with the rules of Institutional Animals Ethics Committee (registration number 173-CPCSEA). The animals were divided into six groups with six animals in each group. Bacterial and fungal cultures were obtained from Microbiology lab Batra Hospital, New Delhi, India and the screening tests were carried out at the Microbiology lab, Majidia hospital, Jamia Hamdard, New Delhi, India.

Anti-inflammatory activity

All the synthesized compounds were evaluated for their anti-inflammatory activity against carrageenan-induced acute paw oedema in albino rats (Wistar strain) weighing 150–200 g [28–29]. The animals were weighed and divided into control, standard, and test groups and each group contained six rats. The first group of rats was treated with 0.1 mL of 0.5% CMC suspension orally (control), second group was administered with a dose of 10 mg/kg of the suspension of ibuprofen (standard) and the test group was treated with equimolar dose of the suspension of test compounds relative to standard drug. After 30 min the animals were injected with 0.1 mL of 1% carrageenan in normal saline subcutaneously to the sub-plantar region of right hind paw. The paw volume was measured at 0 h, 1 h, 3 h and 5 h, by using plethysmometer. The amount of oedema in the drug-treated groups was compared in relation to the control group with the corresponding time intervals. The percent oedema inhibition was calculated from the mean effect in the control and treated animals according to the following equation: Percentage inhibition = 100 (1 – Vt/Vc), where Vt = mean increase in paw volume of test and Vc = mean increase in paw volume of control group of rats.

Analgesic activity

The analgesic activity was determined using tail flick method in albino rats (Wistar strain) weighing 150–200 g [30]. The animals were weighed and divided into control, standard, and test groups and each group contained six rats. The first group of rats was treated with 0.1 mL of 0.5% CMC suspension orally (control), second group was administered with a dose of 10 mg/kg of the suspension of ibuprofen (standard) and the test group was treated with equimolar dose of the suspension of test compounds relative to standard drug. After administration of drug reaction time was measured at 0 min, 30 min, 60 min and 120 min by immersing the tail in hot water (55 ± 0.5°C). Analgesic activity was expressed as reaction time of tail flicking, compounds having analgesic activity will show more reaction time than the control (Table 2).

Histopathological studies

For the histopathological study, rats were sacrificed under light anesthesia after 4 h of the doses (3 times to the dose used for anti-inflammatory) and their stomach specimens were removed and put into 10% formalin solution. A longitudinal section of stomach along the greater curvature, which included the ulcer base and both sides of the ulcer margin, was taken and fixed in 10% formalin for 24 h at 4°C and embedded in white solid paraffin. Morphological examination was performed with haematoxylin and eosin staining to analyze histological changes and examined under electron microscope. The results are given in Table 3 and Figure 1.

In vitro antimicrobial activity studies

All bacterial and yeast strains were obtained from the Batra Hospital, New Delhi, India. and Department of Biochemistry, Jamia Hamdnd, New Delhi, India as follows: *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Aspergillus niger* MTCC 8189, *Aspergillus flavus* MTCC 277. All the newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare chemicals of stock solution of 2 mg/1 mL.

Agar-well diffusion method

Simple susceptibility screening test using agar-well diffusion method [31] was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately to 10^6 colony forming unit (cfu)/mL. They were ''flood-inoculated'' onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) and then dried. For *Candida albicans*, *Candida krusei*, *Aspergillus niger* and *Aspergillus flavus*, SDA were used, and for *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* Macconcy agar was used and for *Escherichia coli* and *Staphylococcus aureus* Muller Hinton agar was used. Six-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 200 μg and 100 μg of the test compounds were delivered into the wells. The plates were incubated for 18 h at 35°C for bacteria and 48 h. for fungal strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (200, 100 μg) and fluconazole (200, 100 μg) were used as standard drugs. Dimethyl sulfoxide was used as solvent controls.

Statistical analysis

Statistical analysis was performed, followed by *t* test, to compare different groups. *P* values less than 0.05 were considered to be statistically significant.

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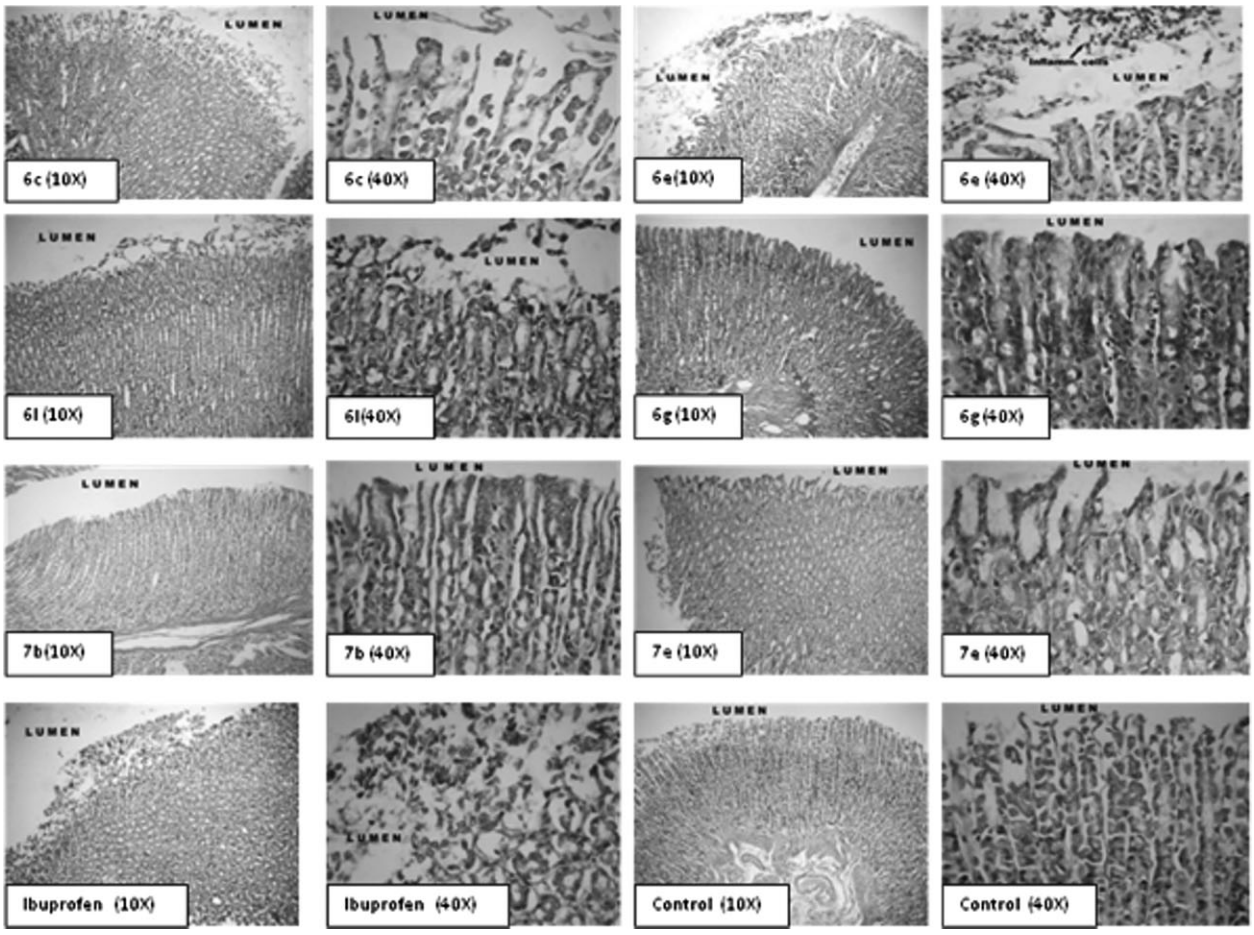

Figure 1. Haemtoxylin and eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.

References

[1] J. B. Polya, in *Comprehensive Heterocyclic Chemistry*, (Eds: A. R. Katritzky, C. W. Rees, K. T. Potts), Pergamon Press, Oxford **1984**, *5*, 733.

[2] M. M. Ghorab, E. l-Sharief, Y. A. Ammar, S. I. Mohamed, *Phosphorus, Sulfur & Silicon Relat. Elem.* **2001**, *173*, 223–233.

[3] W. Zhongyi, S. Haoxin, S. Haijian, *J. Heterocycl. Chem.* **2001**, *38*, 355–357.

[4] E. Palaska, G. Sahin, P. Kelicen, N. T. Durlu, G. Altinok, *Il Farmaco.* **2002**, *57*, 101–107.

[5] L. Labanauskas, V. Kalcas, E. Udrenaite, P. Gaidelis, A. Brukstus, V. Dauksas, *Pharmazie.* **2001**, *56*, 617–619.

[6] T. Gulhan, Z. A. Kaplancikli, A. Ozdemir, P. Chevallet, H. B. Kandilci, B. Gumusel, *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 586–590.

[7] S. Sharma, S. Ganga, R. Abdul, M. Zahin, *Arch. Pharm. Chem. Life Sci.* **2008**, *341*, 714–720.

[8] X. Y. Sun, C. X. Wei, K. Y. Chai, H. R. Piao, Z. S. Quan, *Arch. Pharm. Chem. Life Sci.* **2008**, *341*, 288–293.

[9] A. I. Alsarra, O. M. Ahmed, K. F. Alanazi, H. E. Tahir, M. A. Alsheikh, H. S. Neau, *Int. J. Med. Sci.* **2010**, *7*, 232–239.

[10] G. R. Jorge, T. L. David, *Geriat. Nephrol. Urol.* **1997**, *7*, 51–57.

[11] H. H. Tan, W. M. C. Ong, S. H. Lai, W. C. Chow, Sing. *Med. J.* **2007**, *48*, 582–585.

[12] H. Bayrak, A. Demirbas, S. A. Karaoglu, N. Demirbas, *Eur. J. Med. Chem.* **2009**, *44*, 1057–1066.

[13] K. Zamani, K. Faghihi, M. R. Sangi, J. Zolgharnein, *Turk. J. Chem.* **2003**, *27*, 119–125.

[14] S. G. Umut, G. K. Nesrin, G. Ozgur, K. Yavuz, K. Ekrem, I. Samil, A. Goknur, O. Meral, *Bioorg. Med. Chem.* **2007**, *15*, 5738–5751.

[15] A. A. Kadi, N. R. El-Brollosy, O. A. Al-Deeb, E. E. Habib, I. M. Tarek, A. A. El-Emam, *Eur. J. Med. Chem.* **2007**, *42*, 235–242.

[16] L. Navidpour, H. Shafaroodi, K. Abdi, M. Amini, M. H. Ghahremani, A. R. Dehpour, A. Shafiee, *Bioorg. Med. Chem.* **2006**, *14*, 2507–2517.

[17] G. Mazzone, F. Bonina, R. Arrigo-Reina, G. Blandino, *Farmaco. Ed. Sci.* **1981**, *36*, 181–196.

[18] T. Somorai, G. Szilagyi, E. Bozo, G. Nagy, Hung. Teljes HU. 34457 A2 850328. **1986** [*Chem. Abstr.* **1986**, *105*, 97474].

[19] G. Mekuskiene, P. Vainilavicius, P. Gaidelis, *Pharmazie.* **1998**, *53*, 94–96.

[20] T. Gulhan, Z. A. Kaplancikli, A. Ozdemir, P. Chevallet, H. B. Kandilci, B. Gumusel, *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 586–590.

[21] D. M. Springer, B. Y. Luh, J. J. Bronson, K. E. McElhone, M. M. Mansuri, K. R. Gregor, D. O. Nettleton, P. L. Stanley, K. M. Tramposch, *Bioorg. Med. Chem.* **2000**, *8*, 1087–1109.

[22] P. K. Halen, K. K. Chagti, R. Giridhar, M. R. Yadav, *Chem. Biol. Drug. Des.* **2007**, *70*, 450–455.

[23] M. R. Yadav, D. M. Brahamkshatriya, S. T. Shirude, A. Parmar, R. Balaraman, R. Giridhar, *Bioorg. Med. Chem.* **2006**, *14*, 8701–8706.

[24] H. Kumar, S. A. Javed, S. A. Khan, M. Amir, *Eur. J. Med. Chem.* **2008**, *43*, 2688–2698.

[25] S. F. Barbuceanu, G. L. Almajan, I. Saramet, C. Draghici, R. Socoteanu, F. Barbuceanu, *J. Serb. Chem. Soc.* **2009**, *74*, 1041–1049.

[26] B. Jiang, Y. Zeng, M. J. Li, J. Y. Xu, Y. N. Zhang, Q. J. Wang, N. Y. Sun, T. Lu, X. M. Wu, *Arch. Pharm.* **2010**, *343*, 500–508.

[27] S. V. Bhandari, K. G. Bothara, M. K. Raut, A. A. Patil, A. P. Sarkate, V. J. Mokale, *Bioorg. Med. Chem.* **2008**, *16*, 1822–1831.

[28] C. A. Winter, E. A. Risley, G. W. Nuss, *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544–547.

[29] S. H. Ferreira, *J. Pharma. Pharmaco.* **1979**, *31*, 648.

[30] D. M. Kar, S. K. Sahu, P. K. Misro, *Indian.Drug.* **2003**, *40*, 261–266.

[31] C. Perez, M. Pauli, P. Bazerque, *Acta. Biol. Med. Exp.* **1990**, *15*, 113–115.

