Synthesis of Some Novel 3-Alkyl/aryl-6-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles

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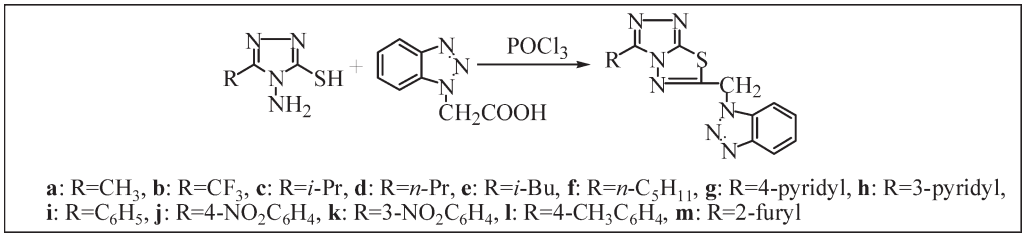

a: R=CH_3, **b**: R=CF_3, **c**: R=*i*-Pr, **d**: R=*n*-Pr, **e**: R=*i*-Bu, **f**: R=*n*-C_5H_{11}, **g**: R=4-pyridyl, **h**: R=3-pyridyl, **i**: R=C_6H_5, **j**: R=4-$NO_2C_6H_4$, **k**: R=3-$NO_2C_6H_4$, **l**: R=4-$CH_3C_6H_4$, **m**: R=2-furyl

A series of 3-alkyl/aryl substituted-6-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadizoles **4a–m** are prepared by the condensation of 3-alkyl/aryl substituted-4-amino-5-mercapto-1,2,4-triazoles **2a–m** with benzotriazole-1-yl acetic acid **3** through a single step reaction. The structures of all newly synthesized compounds are established on the basis of their IR, ^{1}H NMR, and elemental analyses data. Two selected compounds **4l** and **4m** are investigated for their analgesic and anti-inflammatory activities; they showed weak anti-inflammatory activity and no analgesic activity.

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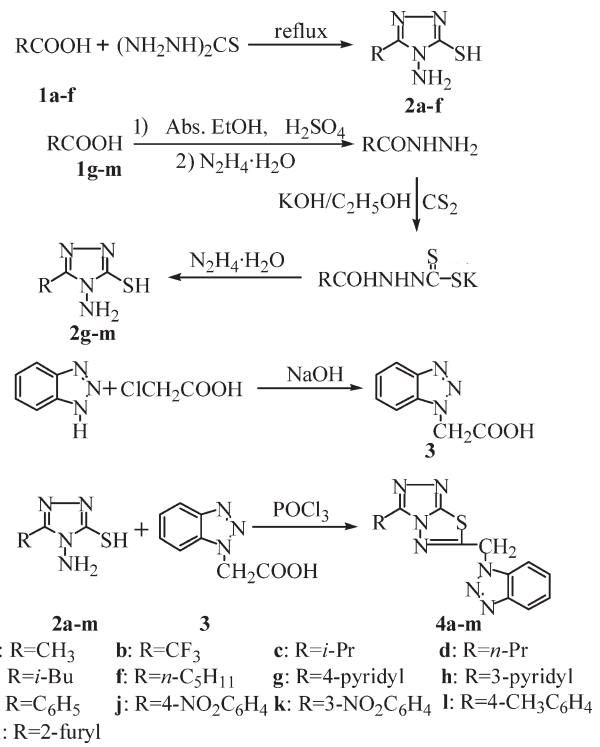
INTRODUCTION

1,2,4-Triazoles and their heterocyclic derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities, such as antifungal [1], antibacterial [2], antihypertensive [3], antileishmanial [4], anti-inflammatory [5], antiviral [6] activities. A large number of thiadiazole-containing ring system exhibits antibacterial [7] and antituberculosis [8] properties. Moreover, a survey of literature reveal that [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole rings received much attention during recent years on account of their prominent utilization as antifungal [9], anti-inflammatory [10,11], antiviral, analgesic [12], anthelmintic [13], anti-HIV-1 [14], and antibacterial agents [15]. A triazolo thiadiazole system may be viewed as a cyclic analogue of two very important components thiosemicarbazide [16] and biguanide [17], which often display diverse biological activities. On the other hand, benzotriazole derivatives are of wide interest because of their diverse biological activity and potential clinical applications such as anti-inflammatory, antiviral, inactivator of Severe acute respiratory syndrome (SARS) protease and so on [18]. Therefore, it is envisaged that chemical entities with both [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles and benzotriazole would generate new interesting biological activities. In continuation of our ongoing research program aimed at developing new biologically active nitrogen and sulphur containing heterocycles, here we report the reaction of 3-alkyl/aryl substituted-4-amino-5-mercapto-1,2,4-triazoles **2a–m** with benzotriazole-1-yl acetic acid **3** in the presence of phosphorous oxychloride to give 3-alkyl/aryl-6-((1*H*-benzo[*d*][1,2,3]-triazol-1-yl)methyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadizoles **4a–m** (Scheme 1). In view of the reported biological activities of triazolo thiadiazoles, two selected compounds **4l** and **4m** are studied for their anti-inflammatory and analgesic activities.

RESULTS AND DISCUSSION

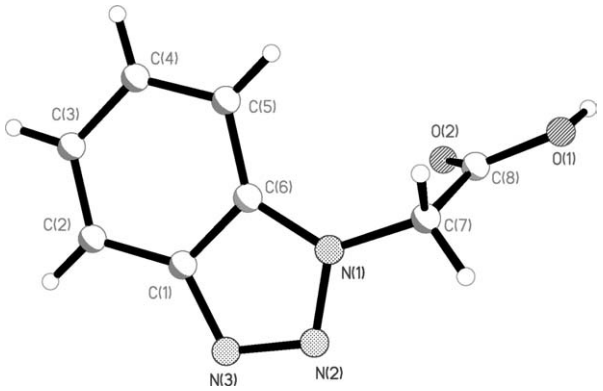
The reaction sequences employed for synthesis of title compounds are shown in Scheme 1. The 3-alkyl-4-amino-5-mercapto-1,2,4-triazoles **2a-f** are synthesized by reacting alkyl acid with thiocarbohydrazide [19]. The required aromatic hydrazides are prepared by esterification of aromatic acid **1g-m** followed by treatment with hydrazine hydrate in absolute ethanol. The aromatic hydrazides are allowed to react with carbon disulphide in the presence of potassium hydroxide in ethanol to afford the corresponding intermediate potassium dithiocarbazinate. This salt undergoes ring closure with an excess of 80% hydrazine hydrate to give 4-amino-3-aryl-5-mercapto-1,2,4-triazoles **2g-m** [20]. Benzotriazole-1-yl acetic acid **3** is prepared by treating benzotriazole with chloroacetic acid in sodium hydroxide solution

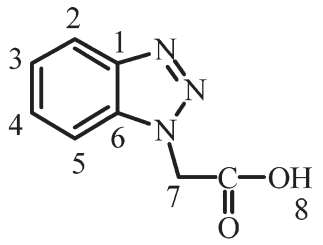
Scheme 1



[21]. Condensation of 3-alkyl/aryl substituted-4-amino-5-mercapto-1,2,4-triazoles **2a–m** with benzotriazole-1-yl acetic acid **3** in presence of boiling phosphorous oxychloride yield 3-alkyl/aryl substituted-6-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadizoles **4a–m** in 53–98% yields. Phosphorous oxychloride is necessary for this condensation, which activate the carbonyl group of acids and increase its electrophilicity to enhance the addition of 1,2,4-triazoles to the target compounds.

The structural elucidation of **3** is completed by analysis of gHSQC and gHMBC data. We can see that $^{3}J_{H7\text{-}C6}$ (Fig. 1). In addition, we get the single crystal of compound **3**. So compound **3** is benzotriazol-1-yl-acetic acid but not benzotriazol-2-yl-acetic acid.





NMR data for compound **3** (DMSO-d_6).

Position	δ	
	δ_H	δ_C
1	–	145.7
2	8.07(d)	119.7
3	7.42 (t)	124.6
4	7.58 (t)	128.1
5	7.84 (d)	111.4
6	–	134.2
7	5.66 (s)	169.3
8	13.43 (brs)	–

Figure 1. Benzotriazole-1-yl acetic acid.

The structure assignments to new compounds **4a–m** are based on their elemental analyses and spectral data (IR, ^{1}H NMR). The IR spectra of the cyclized products **4a–m** show a characteristic absorption at 1445–1592 cm^{-1} attributed to benzene ring of benzotriazole stretching. The band in the range of 1610–1614 cm^{-1} indicate the absorption of C=N. In the ^{1}H NMR spectra of compounds **4a-m**, the absorption bands characteristic of the $-CO_2H$ and $-NH_2$ groups are absent, thereby indicating the involvement of the amino group of triazole and the carboxylic group of benzotriazole-1-yl acetic acid in the condensation reaction. Moreover, we discover CH_2 resonance appear singlets at 6.08–6.79 ppm. Thus, it is further confirmed the involvement of these functional groups in the cyclization of triazoles to triazolo thiadiazoles.

The inhibition ratio of compounds **4l** and **4m** are 8 and 22%, they show weak anti-inflammatory activity (Table 1). **4l** and **4m** are further tested for their analgesic activity; unfortunately the results are not very good, as they show no analgesic activity (Table 2).

Table 1

Anti-inflammatory activity tests.

	Inhibition ratio
4l	8%
4m	22%

Table 2

Analgesic activity tests.

	20 min	40 min	60 min
4l	0	0	12.76%
4m	10.07%	0	0

EXPERIMENTAL

The IR spectra are obtained as potassium bromide pellets with a FTS-40 spectrometer (BIO-RAD, U.S.A). The one-dimensional (^{1}H) and two-dimensional (gHSQC, gHMBC) NMR spectra are obtained on a Varian Inova-400 (400 MHz) spectrometer using $CDCl_3$ or DMSO-d_6 as solvent (shown in detail in data part) and tetramethylsilane as an internal standard, chemical shifts are given in ppm. Elemental analyses (C, H, N) are performed on a Perkin-Elmer Analyzer 2400. Melting points are determined using a Büchi B-540 instrument and are uncorrected (Tables 3–6).

Table 3

Crystal data and summary of data collection and structure refinment.

Compound	$C_8H_7N_3O_2$
Color/shape	Colorless/chip
Formula weight	177.17
Temperature (°C)	293(2)
Crystal system	Monoclinic
Space group	P2(1)/c
Cell constants	
a (Å)	13.234(2)
b (Å)	4.4889(7)
c (Å)	15.142(2)
α (°)	90.00
β (°)	113.135(3)
γ (°)	90.00
Volume (Å^3)	827.2(2)
Formula units/units cell	4
D_{calc} (g cm^{-3})	1.423
F (000)	368.0
Absorption coefficient, m μ^{-1}	0.106
Diffractometer/Scan	Enraf-Noius CAD4, ω/2θ
Radiation, graphite	λ = 0.71073 Å
Monochromator Mo Kα	
Reflections for cell measurement and θ range (°)	27.48, 3.35–27.48
Index ranges	$-17 \le h \le 17$; $-5 \le k \le 5$; $-19 \le l \le 19$
Reflection observed [$1 > 2\sigma(I)$]	1775 [R(int) = 0.0249]
Maximum value of θ(°)	27.48
Computing	Data collection CAD4 Cell refinement CAD4 Data reduction PCSDP
Structure solution	SHELXL-97
Structure refinement	SHELXL-97
Data/restrains/parameters	1775/0/123
Goodness-of-fit on F^2	1.071
Final R indices	$R_1 = 0.0406$; $wR_2 = 0.1115$
Largest diff. peak and hole (e Å^{-3})	0.208 and −0.186

Table 4

The fractional coordinates and mean temperature factors with estimated standard deviations.

	x	y	z	U_{eq}
O1	0.39231 (10)	0.5275 (3)	0.16463 (9)	0.0530 (4)
O2	0.26751 (15)	0.2779 (4)	0.19909 (10)	0.0896 (6)
N1	0.34289 (11)	0.5060 (3)	0.38224 (9)	0.0421 (4)
N2	0.38836 (12)	0.3101 (4)	0.45424 (9)	0.0472 (4)
N3	0.32632 (12)	0.2965 (4)	0.50331 (10)	0.0484 (4)
C1	0.23907 (14)	0.4873 (4)	0.46255 (11)	0.0435 (4)
C2	0.15091 (16)	0.5552 (5)	0.48832 (15)	0.0580 (5)
H2B	0.1442	0.4699	0.5418	0.070
C3	0.07567 (18)	0.7523 (5)	0.43111 (17)	0.0670 (6)
H3B	0.0155	0.8006	0.4455	0.080
C4	0.08574 (16)	0.8857 (5)	0.35093 (16)	0.0635 (6)
H4A	0.0321	1.0196	0.3141	0.076
C5	0.17192 (15)	0.8249 (4)	0.32535 (13)	0.0525 (5)
H5A	0.1788	0.9136	0.2725	0.063
C6	0.24864 (13)	0.6217 (4)	0.38365 (11)	0.0404 (4)
C7	0.39868 (14)	0.5775 (5)	0.31981 (11)	0.0472 (4)
H7A	0.4739	0.5066	0.3489	0.057
H7B	0.4009	0.7922	0.3136	0.057
C8	0.34422 (15)	0.4428 (4)	0.22160 (12)	0.0456 (4)
H1	0.360 (2)	0.419 (6)	0.1049 (19)	0.094 (8)

Benzotriazole-1-yl acetic acid (3). Add 4.8 g (40 mmol) benzotriazole, 3.8 g (40 mmol) chloroacetic acid, 3.2 g (80 mmol) sodium hydroxide, and 100 mL water into a round bottom flask, reflux slowly for 3 h, filter immediately after reflux. The filtrate is cooled at room temperature and acidified with dilute hydrochloric acid till no deposit appear. It is filtered and washed thoroughly with cold water, dried, and recrystallized from butanol, Yield 78%, mp 216–218°.

gHMBC: (DMSO-d_6): $^3J_{H3\text{-}C1}$, $^3J_{H5\text{-}C1}$, $^3J_{H4\text{-}C2}$, $^3J_{H5\text{-}C3}$, $^3J_{H2\text{-}C4}$, $^3J_{H3\text{-}C5}$, $^3J_{H4\text{-}C6}$, $^3J_{H2\text{-}C6}$, $^3J_{H7\text{-}C6}$. Anal. Calcd. for $C_8H_7N_3O_2$ (177.16): C, 54.24; H, 3.98; N, 23.72. found: C, 54.43; H, 3.92; N, 23.61.

General procedure for preparation synthesis of 3-alkyl/aryl substituted-6-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (4a–m). An equimolar mixture of respective triazole **2a–m** (1 mmol) and benzotriazole-1-acetic acid (1 mmol) in phosphorus oxychlorid (8 mL) are refluxed for 7 h. Excess of phosphorus oxychlorid is removed under reduced pressure. The resulting reaction mass is cooled and gradually poured onto crushed ice with stirring, the result mixture is allowed to stand overnight and the solid separated out is filtered. Finally, the filter cake is washed thoroughly with water till the pH of the filtrate is raised to 7, dried, and recrystallized from a mixture of DMF and ethanol.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-methyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4a). This compound was obtained as white needle solid, 87% yield, mp 141–142°; IR (KBr): 3090, 2986, 2933, 1608, 1592, 1536, 1494, 1464, 1290 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.75 (s, 3H), 6.13 (s, 2H), 7.45–8.16 (m, 4H). Anal. Calcd. for $C_{11}H_9N_7S$ (271.30): C, 48.70; H, 3.34; N, 36.14. Found: C, 48.82; H, 3.28; N, 36.27.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-(trifluoromethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4b). This compound was obtained as white solid, 93% yield, mp 148–151°; IR (KBr): 3094, 2937, 1613, 1592, 1544, 1524, 1497, 1295 cm^{-1}; ^{1}H

Table 5

Selected bond lengths.

O1	C8	1.3136 (19)
O1	H1	0.97 (3)
O2	C8	1.193 (2)
N1	N2	1.3448 (19)
N1	C6	1.359 (2)
N1	C7	1.4457 (19)
N2	N3	1.3074 (19)
N3	C1	1.374 (2)
C1	C6	1.388 (2)
C1	C2	1.402 (2)
C2	C3	1.359 (3)
C2	H2B	0.9300
C3	C4	1.406 (3)
C3	H3B	0.9300
C4	C5	1.368 (3)
C4	H4A	0.9300
C5	C6	1.392 (2)
C5	H5A	0.9300
C7	C8	1.501 (2)
C7	H7A	0.9700
C7	H7B	0.9700

NMR ($CDCl_3$): δ 6.21 (s, 2H), 7.47–8.17 (m, 4H). Anal. Calcd. for $C_{11}H_6N_7F_3S$ (325.27): C, 40.62; H, 1.86; N, 30.14. Found: C, 40.79; H, 1.91; N, 30.32.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-isopropyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4c). This compound was obtained as brown solid, 66% yield, mp 238–240°; IR (KBr): 3077, 2962, 1612, 1565, 1549, 1467, 1445, 1260 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.23 (d, 6H), 2.67 (m, 1H), 6.50 (s, 2H), 7.45–8.10 (m, 4H). Anal. Calcd. for $C_{13}H_{13}N_7S$ (299.35): C, 52.16; H, 4.38; N, 32.75. Found: C, 52.27; H, 4.42; N, 32.58.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-propyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4d). This compound was obtained as gray solid, 82% yield, mp 130–132°; IR (KBr): 3085, 2971, 1610, 1584, 1572, 1493, 1468, 1263 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.21 (t, 3H), 1.86 (m, 2H), 2.42 (t, 2H), 6.53 (s, 2H), 7.43–8.12 (m, 4H). Anal. Calcd. for $C_{13}H_{13}N_7S$ (299.35): C, 52.16; H, 4.38; N, 32.75. Found: C, 51.94; H, 4.33; N, 32.59.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-isobutyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4e). This compound was obtained as light yellow solid, 58% yield, mp 129–131°; IR (KBr): 3088, 2988, 2937, 1613, 1590, 1570, 1483, 1448, 1293 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.88 (d, 6H), 2.11 (m, 1H), 2.84 (d, 2H), 6.55 (s, 2H), 7.46–8.14 (m, 4H). Anal. Calcd. for $C_{14}H_{15}N_7S$ (313.38): C, 53.66; H, 4.82; N, 31.29. Found: C, 53.83; H, 4.89; N, 31.38.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-pentyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4f). This compound was obtained as pink solid, 94% yield, mp 105–108°; IR: 3080, 2991, 2939, 1611, 1585, 1567, 1484, 1455, 1298 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.93 (t, 3H), 1.33–1.90 (m, 6H), 3.10 (t, 2H), 6.08 (s, 2H), 7.45–8.16 (m, 4H). Anal. Calcd. for $C_{15}H_{17}N_7S$ (327.41): C, 55.03; H, 5.23; N, 29.95. Found: C, 55.19; H, 5.19; N, 30.13.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4g). This compound was obtained as brown solid, 53% yield, mp 208–211°; IR (KBr): 3072, 2954, 1613, 1581, 1568, 1475, 1459, 1274 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.25 (s, 2H), 7.49–8.86 (m, 8H). Anal. Calcd. for $C_{15}H_{10}N_8S$ (334.36): C, 53.88; H, 3.01; N, 33.51. Found: C, 53.76; H, 3.07; N, 33.62.

6-((*1H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-(pyridin-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4h). This compound was obtained as brown solid, 59% yield, mp 149–152°; IR (KBr): 3071, 2938, 1612, 1586, 1566, 1471, 1464, 1268 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.36 (s, 2H), 7.53–8.88 (m, 8H). Anal. Calcd. for $C_{15}H_{10}N_8S$ (334.36): C, 53.88; H, 3.01; N, 33.51. Found: C, 54.01; H, 3.08; N, 33.69.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-phenyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4i). This compound was obtained as brown solid, 98% yield, mp 205–207°; IR (KBr): 3084, 2953, 1614, 1585, 1550, 1471, 1463, 1265 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.63 (s, 2H), 7.49–8.16 (m, 9H). Anal. Calcd. for $C_{16}H_{11}N_7S$ (333.37): C, 57.65; H, 3.33; N, 29.41. Found: C, 57.81; H, 3.27; N, 29.27.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-(4-nitrophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4j). This compound was obtained as muddy color solid, 98% yield, mp 172–175°; IR (KBr): 3089, 2973, 1611, 1582, 1574, 1462, 1453, 1283 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.75 (s, 2H), 7.55–8.25 (m, 8H). Anal. Calcd. for $C_{16}H_{10}N_8O_2S$ (378.37): C, 50.79; H, 2.66; N, 29.61. Found: C, 50.93; H, 2.71; N, 29.47.

Table 6

Selected bond angles (°).

C8	O1	H1	108.6 (15)
N2	N1	C6	110.99 (13)
N2	N1	C7	119.63 (14)
C6	N1	C7	129.29 (15)
N3	N2	N1	108.07 (14)
N2	N3	C1	108.69 (14)
N3	C1	C6	108.22 (14)
N3	C1	C2	130.92 (17)
C6	C1	C2	120.85 (18)
C3	C2	C1	116.46 (19)
C3	C2	H2B	121.8
C1	C2	H2B	121.8
C2	C3	C4	122.24 (19)
C2	C3	H3B	118.9
C4	C3	H3B	118.9
C5	C4	C3	122.1 (2)
C5	C4	H4A	118.9
C3	C4	H4A	118.9
C4	C5	C6	115.62 (18)
C4	C5	H5A	122.2
C6	C5	H5A	122.2
N1	C6	C1	104.02 (15)
N1	C6	C5	133.28 (15)
C1	C6	C5	122.70 (16)
N1	C7	C8	112.86 (14)
N1	C7	H7A	109.0
C8	C7	H7A	109.0
N1	C7	H7B	109.0
C8	C7	H7B	109.0
H7A	C7	H7B	107.8
O2	C8	O1	124.73 (17)
O2	C8	C7	123.80 (15)
O1	C8	C7	111.46 (15)

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-(3-nitrophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4k). This compound was obtained as light blue solid, 82% yield, mp 148–151°; IR (KBr): 3084, 2970, 1614, 1578, 1563, 1471, 1458, 1263 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.79 (s, 2H), 7.61–8.22 (m, 8H). Anal. Calcd. for $C_{16}H_{10}N_8O_2S$ (378.37): C, 50.79; H, 2.66; N, 29.61. Found: C, 50.62; H, 2.59; N, 29.73.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-*p*-tolyl-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazole (4l). This compound was obtained as white solid, 98% yield, mp 207–210°; IR (KBr): 3071, 2940, 1610, 1588, 1544, 1471, 1462, 1271 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.38 (s, 3H), 6.62 (s, 2H), 7.36–8.16(m, 8H). Anal. Calcd. $C_{17}H_{13}N_7S$ (347.40): C, 58.78; H, 3.77; N, 28.22. Found: C, 58.61; H, 3.83; N, 28.35.

6-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-3-(furan-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (4m). This compound was obtained as white solid, 64% yield, mp 189–192°; IR (KBr): 3082, 2963, 1612, 1587, 1550, 1482, 1448, 1282 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.62 (s, 2H), 6.76–8.15 (m, 7H). Anal. Calcd. for $C_{14}H_9N_7OS$ (323.33): C, 52.01; H, 2.81; N, 30.32. Found: C, 52.22; H, 2.75; N, 30.45.

Anti-inflammatory activity. This activity is performed by the following procedure of Winter *et al*. [22] on groups of six animals each. A freshly prepared suspension of carrageen in (1.0% w/v, 0.1 mL) is injected in the planter region of right hind paw of each rat. One group is kept as control and the animals of the other group are pretreated with the test drugs suspended in 1.0% CMC given orally 1 h before the carrageenin treatment. The volume is measured before and after 4 h of carrageenin treatment with the help of pleythysmometer. The percent anti-inflammatory activity is calculated according to the formula given below:

$$\%\text{Anti-inflammatory activity} = (V_c - V_t/V_c) \times 100$$

where V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats. Data are expressed as mean ± S.E.M., Student's *t*-test is applied to determine the significance of the difference between the control group and rats treated with the test compounds.

Analgesic activity. This activity is performed by Eddy's hot plate technique [23], Mice (Swiss strain) of either sex weighing between 25 and 35 g are used for the experiment. In this method heat is used as a source of pain. Animals are individually placed on a hot plate, maintained at constant temperature (55°C) and the reaction of animals, such as paw licking or jumping response (whichever appears first) is taken as the end point. A cut-off time of 15 s is taken as maximum analgesic response to avoid injury to the paws. The tested compounds and diclofenac sodium (standard) at a dose of 30 mg/kg body weight in 1% gum acacia are given as suspension orally to animals and observe the reaction time of animals on the hot plate at 20, 40, and 60 min after the compound administration.

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