Synthesis and Biological Evaluation of Some [1,2,4]Triazolo[3,4-*b*][1,3,4]Thiadiazoles and [1,2,4]Triazolo[3,4-*b*][1,3,4]Thiadiazines

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Abstract: The reaction of 2-(aryloxymethyl)benzoic acid hydrazide with carbon disulfide in the presence of potassium hydroxide followed by hydrazine hydrate afforded 4-amino-3{2-(aryloxymethyl)pheny}[1,2,4]triazole-5-thiol (**5a-d**). The cyclo-condensation of **5a-d** with various aromatic carboxylic acids in the presence of phosphorous oxychloride and with phenacyl bromides afforded two series of fused heterocycles namely; 6-substituted-3-{2-(aryloxymethyl)phenyl}-1,2,4-triazolo[3,4-*b*][1,3,4]thiadizoles (**6a-t**) and 6-substituted-3-{2-(aryloxymethyl)phenyl)}[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazines (**7a-p**) respectively. The structures of these newly synthesized compounds were established on the basis of their IR, ^{1}H-NMR ^{13}C-NMR and Mass spectral data. All the title compounds were screened for their antimicrobial and anti-inflammatory activities. Preliminary results indicated that some of them exhibit promising activities and they deserve more consideration as potential antimicrobial and anti-inflammatory agents.

Keywords: Triazolothiadiazoles, Triazolothiadiazines, N-Bridged condensed heterocycle, 2-(Aryloxymethyl)phenyl moiety, Antimicrobial activity, Anti-inflammatory activity.

INTRODUCTION

Various [1, 2, 4] triazolo[3,4-*b*][1,3,4]thiadiazoles and [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines possess a wide range of biological activities, such as antimicrobial [1-6], Anti-hypertensive [7], anti-inflammatory [8,9] and anticancer [10, 11] effects. The synthesis of triazoles fused to another heterocyclic ring has attracted particular attention due to their diverse applications as antibacterial, antidepressant, antiviral, anti-HIV-1, antitumor, anti-inflammatory agents, pesticides, herbicides, lubricant, analytical reagents, etc [12-14]. A number of triazoles fused to thiadiazines or thiadiazoles are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities [15-20]. The present study describes the synthesis of a series of 6-aryl-3-{2-(aryloxymethyl)phenyl}-[1, 2, 4]triazolo[3, 4-*b*] [1, 3, 4] thiadiazoles (**6a-6t**) and 6-aryl-3-{2-(aryloxymethyl) phenyl}-1, 2, 4-triazolo [3, 4-*b*][1, 3, 4]thiadiazines (**7a-7p**) from 5-substituted-4-amino-4*H*-[1, 2, 4] triazole-3-thioles (**5a-5d**) (Fig. **1**) and evaluation of their antimicrobial and anti-inflammatory activities.

RESULTS AND DISCUSSION

Chemistry

The reaction sequences employed for the synthesis of title compounds are shown in Scheme**1**. The key intermediate, the 2-(aryloxymethyl) benzoic acid ethylesters (**2a-2d**) were conveniently converted to 2-(aryloxymethyl) benzoic acid hydrazides (**3a-3d**) by refluxing them with hydrazine hydrate in propanol. The compound (**3a-3d**) on reaction with carbon disulphide in methanolic potassium hydroxide yielded corresponding potassium dithiocarbazates (**4a-4d**) in good yield. The required 4-amino-5-{2-(aryloxymethyl) phenyl}-4*H*-[1,2,4]triazole-3-thioles (**5a-5d**) were synthesized by refluxing (**4a-4d**) with aqueous hydrazine hydrate. Condensation of (**5a-5d**) with various aromatic carboxylic acids in boiling phosphorous oxychloride yielded 6-substituted-3-{2-(aryloxymethyl)phenyl}-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadizoles (**6a-6t**). 6-Substituted-3-{2-(aryloxymethyl) phenyl}-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazines (**7a-7p**) were synthesized by the cyclocondensation of various phenacyl bromides with (**5a-5d**) in refluxing ethanol.

The structural assignments to new compounds were based on their elemental analyses and spectral (IR, ^{1}H-NMR ^{13}C-NMR and MASS) data. The characterization data of all the new compounds are summarized in Table **1**. The formation of 2-(aryloxymethyl) benzoic acid hydrazide (**3d**) from 2-(aryloxymethyl) benzoic acid ethyl ester (**2d**) was confirmed by its elemental analyses and spectral data. Its spectrum showed absorption bands at 3291, 3224, 3070, 1688 and 1544 cm^{-1} due to $-NH_2$, -NH, Ar-CH_3, >C=O, and >C=C< groups respectively, while ^{1}H-NMR showed sharp singlets at δ 4.07 and δ 5.22, which correspond to -NH_2 and $-OCH_2$ protons respectively. Two doublets observed at δ 6.92 (J = 7.2 Hz) and δ 6.25 (J = 7.2 Hz) accounted for the four aromatic protons of ring A. The ring B protons appeared

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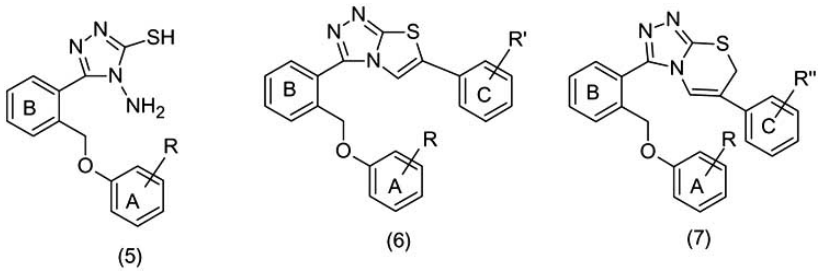
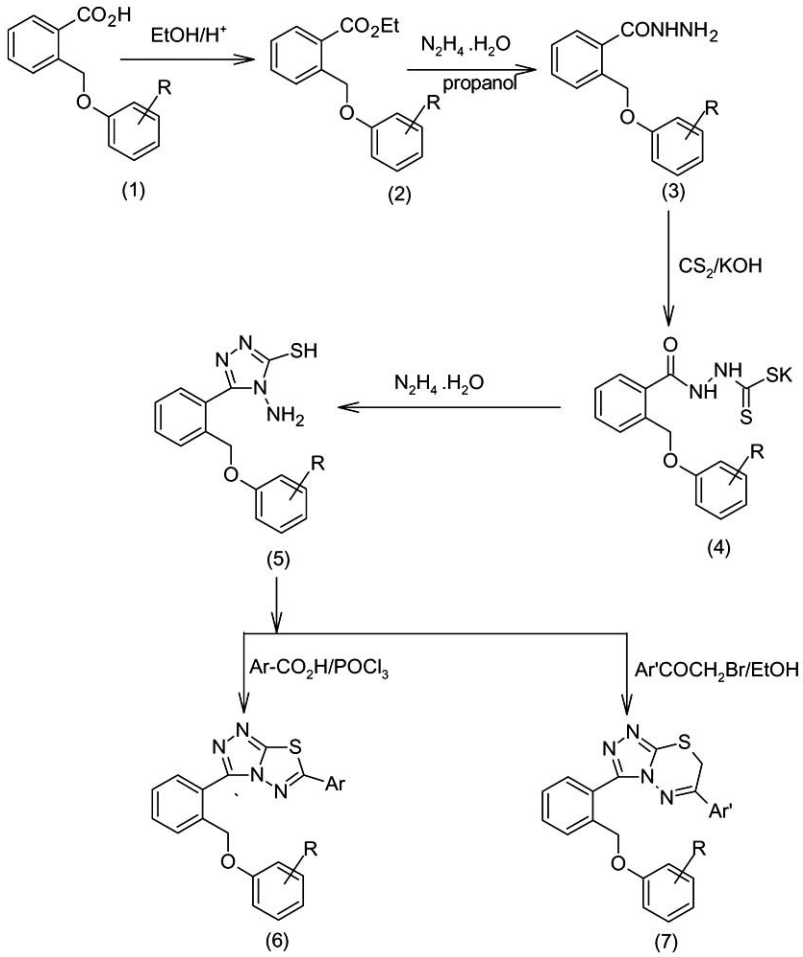


Fig. (1). General structures of 1,2,4-triazolo[3,4-*b*]1,3,4]thiadiazoles and [1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazines



	R	Ar	Ar′
1a-5a	2-CH_3	-	-
1b-5b	3-CH_3	-	-
1c-5c	4-CH_3	-	-
1d-5d	4-Cl	-	-
6a-t	2-CH_3, 3-CH_3, 4-CH_3, 4-Cl	2-Cl-pyridin-3-yl, 3,5-$(CH_3)_2$-C_6H_3 , 3,5-Cl_2-C_6H_3, 3,4,5-$(OCH_3)_3$-C_6H_2, 3-Br-C_6H_4,	-
7a-p	2-CH_3, 3-CH_3, 4-CH_3, 4-Cl	-	3-$CONH_2$-4-OH-C_6H_3, 5-Cl-2-SO_2NH_2-3-thienyl, 3-Br-C_6H_4, 4-NO_2-C_6H_4

Scheme 1.

as a multiplet in the region δ 7.48-7.58 integrating for four protons. The spectrum also showed a broad singlet at δ 7.40 for its NH protons. The potassium dithiocarbazate (**4a**) was directly used for the next step without characterization. Further, the cyclization of (**4a**) to 4-amino-3{2-(aryloxymethyl)}[1,2,4]triazole-5-thiole (**5a**) was confirmed by recording its IR spectrum, which showed absorption bands at 3313, 2667 and 1617 cm^{-1} due to its -NH_2, -SH, and C=N respectively. The disappearance of characteristic absorption band due to >C=O group of (**4a**) is clearly an indication of its smooth cyclization to afford (**5a**). Its ^{1}H-NMR spectrum showed two singlets at δ 2.16, δ 4.75 and δ 5.20 which

Table 1. Physical Constants and Characterization Data of Compounds (6a-t) and (7a-p)

Compd	R	Ar	Mol. Formula	Mol. Mass	Yield (%)	MP (^{0}C)	Elemental Analysis, % Found (calculated)		
							C	H	N
6a	2-CH_3	2-Cl-pyridin-3-yl	$C_{22}H_{16}ClN_5OS$	433.9	65	140-142	60.84(60.90)	3.65(3.72)	16.10(16.14)
6b	3-CH_3	2-Cl-pyridin-3-yl	$C_{22}H_{16}ClN_5OS$	433.9	63	110-112	60.58(60.90)	3.68(3.72)	15.92 (16.14)
6c	4-CH_3	2-Cl-pyridin-3-yl	$C_{22}H_{16}ClN_5OS$	433.9	60	142-143	60.54(60.90)	3.62(3.72)	16.33 (16.14)
6d	4-Cl	2-Cl-pyridin-3-yl	$C_{21}H_{13}Cl_2N_5OS$	454.3	64	112-114	56.35(55.52)	2.74 (2.88)	15.37(15.41)
6e	2-CH_3	3,5-$(CH_3)_2C_6H_3$	$C_{25}H_{22}N_4OS$	426.5	66	120-122	70.32(70.40)	4.92 (5.20)	13.28 (13.14)
6f	3-CH_3	3,5$(CH_3)_2C_6H_3$	$C_{25}H_{22}N_4OS$	426.5	63	106-107	69.92(70.40)	5.20 (5.20)	13.10 (13.14)
6g	4-CH_3	3,5$(CH_3)_2C_6H_3$	$C_{25}H_{22}N_4OS$	426.5	68	112-115	70.22(70.40)	5.14 (5.20)	12.88 (13.14)
6h	4-Cl	3,5$(CH_3)_2C_6H_3$	$C_{24}H_{19}ClN_4OS$	446.9	66	129-131	64.40(64.49)	4.39(4.28)	12.38(12.54)
6i	2-CH_3	3,4,5-$(OCH_3)_3C_6H_2$	$C_{26}H_{24}N_4O_4S$	488.5	67	102-104	64.80 (63.92)	4.52(4.95)	11.28(11.47)
6j	3-CH_3	3,4,5-$(OCH_3)_3C_6H_2$	$C_{26}H_{24}N_4O_4S$	488.5	63	105-108	64.70 (63.92)	4.75(4.95)	12.18(11.47)
6k	4-CH_3	3,4,5-$(OCH_3)_3C_6H_2$	$C_{26}H_{24}N_4O_4S$	488.5	64	98-100	64.55 (63.92)	4.02(4.16)	11.38(11.47)
6l	4-Cl	3,4,5-$(OCH_3)_3C_6H_2$	$C_{25}H_{21}ClN_4O_4S$	508.9	66	120-122	58.85 (58.99)	4.11 (4.16)	11.14 (11.01)
6m	2-CH_3	3,5-Cl_2-C_6H_3	$C_{23}H_{16}Cl_2N_4OS$	467.3	63	118-119	59.20 (59.11)	3.40 (3.45)	12.12 (11.99)
6n	3-CH_3	3,5-Cl_2-C_6H_3	$C_{23}H_{16}Cl_2N_4OS$	467.3	67	185-187	59.10 (59.11)	3.40 (3.45)	12.00 (11.99)
6o	4-CH_3	3,5-Cl_2-C_6H_3	$C_{23}H_{16}Cl_2N_4OS$	467.3	65	220-221	59.32 (59.11)	3.42 (3.45)	12.23 (11.99)
6p	4-Cl	3,5-Cl_2-C_6H_3	$C_{22}H_{13}Cl_3N_4OS$	487.7	65	200-202	54.20 (54.17)	2.58 (2.69)	11.35 (11.49)
6q	2-CH_3	3-Br-C_6H_4	$C_{23}H_{17}BrN_4OS$	477.3	65	196-198	57.52 (57.87)	3.42 (3.59)	11.33 (11.74)
6r	3-CH_3	3-Br-C_6H_4	$C_{23}H_{17}BrN_4OS$	477.3	65	170-171	57.74 (57.87)	3.50 (3.59)	11.58 (11.74)
6s	4-CH_3	3-Br-C_6H_4	$C_{23}H_{17}BrN_4OS$	477.3	65	110-112	57.66 (57.87)	3.52 (3.59)	11.62 (11.74)
6t	4-Cl	3-Br-C_6H_4	$C_{22}H_{14}BrClN_4OS$	497.7	68	180-181	53.12 (53.08)	2.78 (2.83)	11.19 (11.25)
7a	2-CH_3	3-$CONH_2$-4-OH- C_6H_3	$C_{25}H_{21}N_5O_3S$	471.5	72	218-220	63.48 (63.68)	4.33 (4.49)	14.82 (14.85)
7b	3-CH_3	3-$CONH_2$-4-OH- C_6H_3	$C_{25}H_{21}N_5O_3S$	471.5	74	214-216	63.36 (63.68)	4.40 (4.49)	14.85 (14.85)
7c	4-CH_3	3-$CONH_2$-4-OH- C_6H_3	$C_{25}H_{21}N_5O_3S$	471.5	74	224-228	63.18 (63.68)	4.33 (4.49)	14.36 (14.85)
7d	4-Cl	3-$CONH_2$-4-OH- C_6H_3	$C_{24}H_{18}ClN_5O_3S$	491.9	76	238-240	58.54 (58.59)	3.52 (3.69)	14.11 (14.24)
7e	2-CH_3	5-Cl-2-SO_2NH_2-3-thienyl	$C_{22}H_{18}ClN_5O_3S_3$	532.0	68	224-226	49.46 (49.66)	3.32 (3.41)	12.88 (13.16)
7f	3-CH_3	5-Cl-2-SO_2NH_2-3-thienyl	$C_{22}H_{18}ClN_5O_3S_3$	532.0	70	170-172	49.23 (49.66)	3.52 (3.41)	13.05 (13.16)
7g	4-CH_3	5-Cl-2-SO_2NH_2-3-thienyl	$C_{22}H_{18}ClN_5O_3S_3$	532.0	67	150-154	49.72 (49.66)	3.32 (3.41)	12.92 (13.16)
7h	4-Cl	5-Cl-2-SO_2NH_2-3-thienyl	$C_{21}H_{15}Cl_2N_5O_3S_3$	552.4	70	192-195	45.38 (45.65)	2.72 (2.74)	12.42 (12.68)
7i	2-CH_3	3-Br-C_6H_4	$C_{24}H_{19}BrN_4OS$	491.4	68	170-172	58.24 (58.66)	3.75 (3.90)	11.27(11.40)
7j	3-CH_3	3-Br-C_6H_4	$C_{24}H_{19}BrN_4OS$	491.4	69	180-182	58.45 (58.66)	3.92 (3.90)	11.32(11.40)
7k	4-CH_3	3-Br-C_6H_4	$C_{24}H_{19}BrN_4OS$	491.4	69	194-197	58.62 (58.66)	3.85 (3.90)	11.42(11.40)
7l	4-Cl	3-Br-C_6H_4	$C_{23}H_{16}BrClN_4OS$	511.8	69	168-170	53.85 (53.97)	3.05 (3.15)	10.92(10.95)
7m	2-CH_3	4-NO_2-C_6H_4	$C_{24}H_{19}N_5O_3S$	457.5	72	174-176	63.24 (63.01)	3.85 (4.19)	15.27(15.31)
7n	3-CH_3	4-NO_2-C_6H_4	$C_{24}H_{19}N_5O_3S$	457.5	76	164-166	63.11 (63.01)	3.98 (4.19)	15.10(15.31)
7o	4-CH_3	4-NO_2-C_6H_4	$C_{24}H_{19}N_5O_3S$	457.5	76	178-180	59.95 (63.01)	4.20 (4.19)	15.22(15.31)
7p	4-Cl	4-NO_2-C_6H_4	$C_{23}H_{16}ClN_5O_3S$	477.9	78	188-190	57.55 (57.80)	3.10 (3.37)	14.48(14.65)

correspond to ring A-CH_3, -NH_2 and –OCH_2 protons respectively. A doublet at δ 6.76 (*J* = 7.5 Hz) and a triplet at δ 6.96 (*J* = 7.2 Hz) integrating for one proton each of ring A were also seen along with a multiplet in the region δ 7.07-7.12 integrating for remaining two protons. The four ring B protons appeared as a complex multiplet in the region δ 7.44-7.73. The sharp downfield singlet appearing at δ 11.8 was assigned to –SH proton. ^{13}C-NMR spectrum of (**5a**) showed signals at δ 16.17, 67.96 due to ring A-CH_3 and OCH_2 carbon atoms respectively. The other peaks seen in the spectrum were at δ 111.8 and 121.0 (C_2 and C_3 of ring A), 123.0 and 126.8 (C_6 and C_4 of ring A), 126.8 (C_4 of ring A), 127.8 (C_1 of ring B), 128.5 and 130.9 (C_2 and C_5 of ring B), 130.9 and 131.3 (C_4 and C_3 of ring B), 137.8 (C_6 of ring B), 150.3 (C_5 of triazole), 156.2 (C_1 of Ring A) and 166.91 (C_3 of triazole moiety) respectively. The peaks due to quaternary carbon atoms of the compound disappeared on DEPT experimentation.

The structural elucidation of title compound (**6p**) is based on its IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral studies. IR spectrum of (**6p**) showed absorption bands at 3055, 1612, 1284, 1074 and 756 cm^{-1} for its aromatic C-H, C=N, C=C, C-S and C-Cl stretching vibrations respectively. In its ^{1}H-NMR spectrum, peaks due to -OCH_2 protons appeared at δ 5.55. The peaks due to ring A protons appeared as two doublets at δ 6.86 (*J* = 8.9 Hz) and 7.17 (*J* = 8.9 Hz) integrating for two protons each. Three protons of ring C appeared at δ 7.54-7.61 as a complex multiplet, while ring B protons appeared as a complex multiplet in the region δ 7.76-7.77 integrating for two protons and the remaining two protons appeared as two distinct doublets at δ 7.83 (*J* = 7.2 Hz) and δ 8.15 (*J* = 7.2 Hz). ^{13}C-NMR spectrum of (**6p**) showed signals at δ 68.42 due to OCH_2 carbon atom. Peaks also appeared at δ 116.3 (C_2 and C_6 of ring A), 122.7 (C_3 of ring C), 125.4 (C_2 and C_6 of ring C), 125.8 (C_5 of ring C), 127.9 (C_4 of ring C), 128.1 (C_3 and C_5 of ring A), 128.9 (C_2 of ring B), 129.3 (C_5 of ring B), 130.8 (C_4 of ring B), 131.9 (C_1 of ring C), 132.6 (C_3 of ring B), 136.5 (C_4 of ring A), 136.8 (C_1 of ring B), 146.4 (C_6 of ring B), 153.7 (C_3 and C_5 of triazole), 157.6 (C_1 of ring A) and 163.9 (C_7 of thiadiazole ring) respectively. The signals due to quaternary carbon atoms disappeared on DEPT experimentation. The absence of characteristic IR absorption bands due to –NH_2 and -SH groups of (**5d**) clearly confirmed condensation with acid to afford (**6p**). Further, LCMS of (**6p**) showed the molecular ion peak as a base peak at m/z 488, which is consistent with its molecular formula, $C_{22}H_{13}Cl_3N_4OS$.

The buildup of N-bridged condensed heterocycle (**7o**) from (**5c**) is evidenced by its IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data. IR spectrum of (**7o**) indicated the presence of aromatic C-H, C=N, C-S stretching bands at 3055, 1616 and 1068 cm^{-1}, respectively. The signals observed at δ 2.07, 3.67 and 5.25 as singlets in its ^{1}H-NMR spectrum confirmed the presence of ring A-CH_3, -CH_2 (thiadiazine ring) and -OCH_2 protons respectively. Ring A protons appeared as two distinct doublets at δ 6.70 (*J* = 8.1 Hz) and 7.05 (*J* = 8.1 Hz) integrating for two protons each. Two triplets at 7.48 (*J* = 7.2 Hz) and 7.58 (*J* = 7.2 Hz) and also two distinct doublets at δ 7.68 (*J* = 7.2 Hz) and 7.73 (*J* = 7.2 Hz) for one proton each of ring B were also seen in the spectrum. Ring C protons appeared as two distinct doublets at δ 7.89 (*J* = 8.8 Hz) and 8.29 (*J* = 8.8 Hz) integrating for two protons each. ^{13}C-NMR spectrum of (**7o**) showed signals at δ 16.01, 23.08, 67.58 due to ring A-CH_3, CH_2 and OCH_2 carbon atoms. The other signals observed in the spectrum were δ 110.8 (C_2 and C_6 of ring A), 120.7 (C_2 and C_6 of ring C), 123.8 (C_4 of ring A), 124.2 (C_2 of ring B), 126.7 (C_4 of ring B), 126.8 (C_1 of ring C), 127.7 (C_5 of ring B), 128.4 (C_3 and C_5 of ring C), 130.8 (C_3 and C_5 of ring A), 131.3 (C_3 of ring B), 137.5 (C_1 of ring B), 139.1 (C_6 of ring B), 140.9 (C_5 of triazole), 149.5 (C_3 of triazole), 151.6 (C_4 of ring C), 153.1(C_1 of ring A) and 156.4 (C_7 of thiadiazine) respectively. Further, LCMS of (**7o**) showed the molecular ion peak as a base peak at m/z 457 which is in agreement with its molecular formula, $C_{24}H_{19}N_5O_3S_3$. Characterization data of (**6a-6t**) and (**7a-7p**) are given in Table **1.**

Antimicrobial Studies

The antimicrobial activity was determined using disc diffusion method [21] by measuring zone of inhibition in mm. All the compounds, (**6a-6t**) and (**7a-7p**) were screened *in-vitro* at a concentration of 10 μg / disc for their antibacterial activity against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus subtilis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa)*. Antifungal evaluation was also carried out against *Candida albicans* and *Aspergillus niger* at a concentration of 10μg / disc. Standard antibacterial drug ciprofloxacin (10μg / disc) and antifungal drug fluconazole (10μg / disc) were also tested under similar conditions against these organisms. All synthesized compounds exhibited significant antibacterial activities and moderate antifungal activities. Each experiment was done in triplicate and the average reading was taken. The antibacterial activity was classified as highly active (≥26 mm), moderately active (11-25 mm) and least active (<11 mm). The results of antibacterial and antifungal activities are expressed in terms of zone of inhibition and presented in Table **2**.

The investigation of antibacterial and antifungal screening data revealed that all the tested compounds (**6a-6t**) and (**7a-7p**) showed moderate to good activity. The compounds containing p-chlorophenoxy methylphenyl and 2-chloronicotinyl (**6d**), 3,4,5-trimethoxyphenyl (**6l**), 3,5-dichlorophenyl (**6p**)**,** 2-hydroxy-4-carboxamidophenyl (**7d**), and 5-chloro-2-sulfonamido phenyl (**7h**) moieties attached to the thiadiazole and thiadiazine nuclei showed comparatively good activity against all the bacterial strains. However, the compounds **6a, 6b, 6c, 6h, 6i, 6j, 6k, 6m, 6n, 6o, 7a, 7b, 7c, 7e, 7f, 7g** and **7p** exhibited moderate activity compared to that of standard against all bacterial strains.

The compounds having p-chlorophenoxy methylphenyl and 3,4,5-trimethoxy- (**6l**), 3,5-dichloro- (**6p**) and 2-hydoxy-4-carboxamido-phenyl (**7h**) groups attached to the thiadiazole and thiadiazine ring showed comparatively good growth inhibition of all the fungal strains. On the other hand, compounds **6d, 6h, 6i, 6j, 6k, 6m, 6n, 6o, 7d, 7e, 7f, 7g** and **7p** exhibited moderate activity compared to that of standard against *C. albicans and A. niger*. It has also been observed that the thiadiazole derivatives are found to be more active than thiadiazines. More extensive study is also warranted to determine additional physicochemical and biological parameters to have a deeper insight into structure-activity rela-

Table 2. Antibacterial Activity Data of Compounds (6a-t) and (7a-p)

Compd	Zone of Inhition in mm					
	Antibacterial Activity				Antifungal Activity	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
6a	18	18	20	21	09	10
6b	22	22	20	22	10	11
6c	20	20	19	20	10	08
6d	28	26	28	24	27	25
6e	11	10	10	11	11	10
6f	09	08	08	09	10	08
6g	10	12	10	09	08	09
6h	23	22	20	21	20	18
6i	18	19	18	17	18	19
6j	22	20	21	20	21	20
6k	20	18	20	18	19	20
6l	30	28	30	25	26	24
6m	23	20	23	21	21	20
6n	23	22	23	20	18	19
6o	22	18	22	18	20	18
6p	28	28	26	24	26	24
6q	08	10	10	11	09	10
6r	10	12	08	08	10	11
6s	11	10	09	10	08	09
6t	10	09	10	12	10	10
7a	23	23	20	18	09	08
7b	19	20	22	20	10	11
7c	20	21	18	18	10	08
7d	28	26	26	25	18	20
7e	22	22	20	20	20	19
7f	20	20	18	18	19	20
7g	18	20	19	18	22	20
7h	27	26	28	26	25	25
7i	08	11	10	09	08	10
7j	10	12	08	08	10	11
7k	11	10	09	10	08	09
7l	09	08	10	10	11	09
7m	10	10	09	08	06	08
7n	10	12	08	08	10	11
7o	11	10	09	10	08	09
7p	22	20	22	18	20	18
Ciprofloxacin	26	26	28	25	-	-
Flucanazol	-	-	-	-	26	25

tionship and to optimize the effectiveness of this series of molecules.

Anti-Inflammatory Activity

The *in vivo* anti-inflammatory activity of all the newly synthesized compounds was evaluated by carrageenan-induced rat paw oedema method [22]. Wistar albino rats of either sex weighing 180-250g were used for the experiment. The compounds were tested at 10mg / kg oral dose and were compared with the standard drug Diclofenac Sodium at the same oral dose. The results were expressed as % inhibition of oedema over the untreated control group (Table **3**).

The tested compounds showed anti-inflammatory activity ranging from 40.40 to 76.42 %, whereas standard drug Diclofenac Na showed 75.93 % inhibition, after 3 h. The anti-inflammatory activity of 1,2,4-triazolo[3,4-*b*][1,3,4] thiadizoles (**6a-6t**) is in the range of 40.40-76.42 %. The triazolothiadizole derivative having 4-chlorophenoxy-methyphenyl and 2-chloropyridin-3-yl groups (**6d**) attached

Table 3. Antiinflammatory Activity Data of Compounds (6a-t) and (7a-p)

Compound	Dose (mg/kg Body Weight, p.o)	Increase in Paw Volume in ml MEAN ± SEM)	% Inhibition of Paw Oedema
6a	10	0.0528±0.0017	67.42
6b	10	0.0458±0.0028	72.21
6c	10	0.0484±0.0023	70.12
6d	10	0.0382±0.0032	76.42
6e	10	0.0833±0.0038	48.61
6f	10	0.0743±0.0026	54.12
6g	10	0.0768±0.0024	52.58
6h	10	0.0588±0.0021	63.70
6i	10	0.0966±0.0040	40.40
6j	10	0.0896±0.0035	46.68
6k	10	0.0899±0.0027	44.51
6l	10	0.0597±0.0023	63.15
6m	10	0.0567±0.0031	65.00
6n	10	0.0538±0.0027	66.80
6o	10	0.0564±0.0029	65.21
6p	10	0.0533±0.0017	67.12
6q	10	0.0584±0.0031	63.98
6r	10	0.0581±0.0260	64.12
6s	10	0.0585±0.0021	63.92
6t	10	0.0568±0.0025	64.92
7a	10	0.0964±0.0027	40.52
7b	10	0.0925±0.0033	42.91
7c	10	0.0945±0.0026	41.68
7d	10	0.0633±0.0016	60.90
7e	10	0.0565±0.0020	65.12
7f	10	0.0523±0.0084	67.70
7g	10	0.0508±0.0071	68.65
7h	10	0.0496±0.0056	69.04
7i	10	0.0698±0.0041	56.90
7j	10	0.0624±0.0036	61.51
7k	10	0.0606±0.0029	62.58
7l	10	0.0641±0.0031	60.42
7m	10	0.0578±0.0019	64.30
7n	10	0.0497±0.0023	69.31
7o	10	0.0504±0.0031	68.92
7p	10	0.0472±0.0017	70.88
Control	0.1ml/kg	-	-
Standard	10	0.0390±0.0026	75.93

Diclofenac Na is used as the standard: N=6 in each group.
CMC- Carboxy methyl cellulose as a suspending agent.

to 3rd and 6th positions respectively presented highest anti-inflammatory activity (76.42%) better than the standard drug Diclofenac Sodium. Other five compounds **6a**, **6b**, **6c**, **6n** and **6p** showed very significant activity. They contained 2-Me-, 3-Me-, 4-Me- and 4-Cl-phenoxymethyphenyl group on C-3 and 2-chloropyridin-3-yl and 3,5-dichlorophenyl groups at C-6 position. On the other hand, the remaining compounds exhibited moderate to good inhibition.

The anti-inflammatory activity of [1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazines **(7a-7p)** is in the range of 40.52-70.88 %. Compounds **7e**, **7f**, **7g**, **7h**, **7m**, **7n**, **7o** and **7p** showed significant anti-inflammatory activity. However, the remaining triazolothiadiazines possessed moderate to good activity. It was observed that the triazolothiadiazine derivatives having 5-chloro-2-sulfanamido-3-thienyl and 4-nitrophenyl groups at C-6 position possess high activity.

EXPERIMENTAL PROTOCOLS

Chemistry

Melting points were determined by the open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 4100 type A spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian 400 MHz NMR spectrometer/Perkin-Elmer EM300 MHz spectrometer using TMS as an internal standard. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 spectrophotometer / Data system using Argon/Xenon (6KV,10mA)FAB gas, at 70 eV. Mass spectra were recorded on LC–MS-Aglilent 1100 series with MSD (Ion trap) using 0.1% aqueous TFA in acetonitrile system on C18-BDS column for 10 min duration. Elemental analysis was carried out using Flash EA 1112 Series, CHNSO Analyzer (Thermo). The progress of the reactionwas monitored by thin layer chromatography (TLC) on pre-coated silica gel G plates. Solvents and reagents were purchased from the commercial vendors in the appropriate grade and were used without purification.

General Procedure for the Preparation of 2-(Aryloxymethyl) Benzoic Acid [23] (1a-1d)

Phthalide (15.0g, 112 mmol), substituted phenols (23.6g, 214 mmol) and sodium methoxide (47.6g, 220 mmol) were suspended in 150 mL of n-butanol and stirred at 140° C for 15 h. The reaction mixture was cooled, 400 ml of water were added, and the mixture was acidified to pH-4 at 20° C with concentrated hydrochloric acid. The product obtained was filtered washed with water and dried. The crude product was recrystallized from methanol / water.

2-(2-Methyl Phenoxy Methyl)Benzoic Acid (1a)

Colourless solid (75 %); mp 150-152° C; IR (KBr, ν cm^{-1}): 3450 (O-H), 3056 (ArC-H), 2941 (C-H), 1694 (C=O), 1245 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm) : 2.21 (s, 3H, Ring A-CH_3), 5.46 (s, 2H, - OCH_2), 6.71-6.79 (m, 3H, Ring A-H), 7.107 (t, 1H, Ring A-H), 7.30 (d, 1H, Ring B-H, *J* = 8.8 Hz),7.48 (d, 1H, Ring B-H, *J* = 8.9 Hz), 7.86 (t, 1H, Ring B-H, *J* = 8.9 Hz), 8.12 (t, 1H, Ring B-H, *J* = 8.9 Hz).

2-(3-Methyl Phenoxy Methyl)Benzoic Acid (1b)

Colourless solid (74 %); mp 146-148° C; IR (KBr, ν cm^{-1}): 3445 (O-H), 3058 (ArC-H), 2992 (C-H), 1689 (C=O), 1255 (C-O).

2-(4-Methyl Phenoxy Methyl)Benzoic Acid (1c)

Colourless solid (78 %); mp 126-128° C; IR (KBr, ν cm^{-1}): 3455 (O-H), 3078 (ArC-H), 2970 (C-H), 1685 (C=O), 1256 (C-O); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 2.38 (s, 3H, Ring A-CH_3), 5.36 (s, 2H, - OCH_2), 6.75 (d, 2H, Ring A-H, *J* = 8.3 Hz), 6.85 (d, 2H, Ring A-H, *J* = 8.3 Hz), 7.07-7.50 (m, 2H, Ring B-H), 7.65- 8.18 (m, 2H, Ring B-H).

2-(4-chloro phenoxy methyl)benzoic acid (1d)

Colourless solid (80 %); mp 167-169° C; IR (KBr, ν cm^{-1}): 3440 (O-H), 3096 (ArC-H), 2988 (C-H), 1694 (C=O), 1236 (C-O), 748 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 5.53 (s, 2H, -OCH_2), 6.93 (d, 2H, Ring A-H, *J* = 8.9 Hz), 7.26 (d, 2H, Ring A-H, *J* = 8.9 Hz), 7.44 (t, 1H, Ring B-H, *J* = 7.5 Hz), 7.63 (t, 1H, Ring B-H, *J* = 7.5 Hz), 7.77 (d, 1H, Ring B-H, *J* = 7.2 Hz), 8.18 (d, 1H, Ring B-H, *J* = 7.2 Hz).

General Procedure for the Preparation of Ethyl 2-(Aryloxymethyl) Benzoates (2a-2d)

The above esters were prepared by refluxing 2-(aryloxymethyl) benzoic acids **(1a-d)** in excess absolute ethanol in the presence of few drops of conc. sulfuric acid as per the general method employed for the esterification [24]. The resulting esters had been judged to be pure by TLC.

Ethyl 2-(2-Methyl Phenoxy Methyl)Benzoate (2a)

Colourless solid (82 %); mp 54-56° C; IR (KBr, ν cm^{-1}): 3072 (ArC-H), 2980 (C-H), 1716 (C=O), 1247 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm) : 1.39 (t, 3H, -CH_3 ester, *J* = 7.2 Hz), 2.36 (s, 3H, Ring A-CH_3), 4.36 (q, 2H, -CH_2 ester, *J* = 7.2 Hz), 5.51 (s, 2H, -OCH_2), 6.80-6.87 (m, 3H, Ring A-H), 7.17 (t, 1H, Ring A-H, *J* = 7.8 Hz), 7.39 (d, 1H, Ring B-H, *J* = 8.7 Hz), 7.58 (d, 1H, Ring B-H, *J* = 7.8 Hz), 7.81 (t, 1H, Ring B-H, *J* = 7.5 Hz), 8.06 (t, 1H, Ring B-H, *J* = 7.5 Hz).

Ethyl 2-(3-Methyl Phenoxy Methyl)Benzoate (2b)

Colourless solid (85 %); mp 68-70° C; IR (KBr, ν cm^{-1}): 3082 (ArC-H), 2972 (C-H), 1715 (C=O), 1264 (C-O); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.42 (t, 3H, -CH_3 ester, *J* = 7.2 Hz), 2.42 (s, 3H, Ring A-CH_3), 4.42 (q, 2H, -CH_2 ester, *J*=7.2 Hz), 5.58 (s, 2H, - OCH_2), 6.86-7.17 (m, 4H, Ring A-H), 7.23-7.52 (m, 2H, Ring B-H), 7.67 (d, 1H, Ring B-H, *J* = 7.8 Hz), 7.90 (d, 1H, Ring B-H).

Ethyl 2-(4-Methyl Phenoxy Methyl)Benzoate (2c)

Colourless solid (87 %); mp 52-54° C; IR (KBr, ν cm^{-1}): 3094 (ArC-H), 2975 (C-H), 1712 (C=O), 1231 (C-O); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.32 (t, 3H, -CH_3 ester, *J* = 7.2 Hz), 2.32 (s, 3H, Ring A-CH_3), 4.46 (q, 2H, -CH_2 ester, *J* = 7.2 Hz), 5.56 (s, 2H, - OCH_2), 6.74 (d, 2H, Ring A-H, *J* = 7.5 Hz), 7.05-7.12 (d, 2H, Ring A-H, *J* = 7.5 Hz), 7.39 (d, 1H, Ring B-H, *J* = 8.7 Hz), 7.49 (d, 1H, Ring B-H, *J* = 7.8 Hz), 7.78 (t, 1H, Ring B-H, *J*= 7.5 Hz), 8.12-8.18 (t, 1H, Ring B-H, *J* = 7.5 Hz).

Ethyl 2-(4-Chloro Phenoxy Methyl)Benzoate (2d)

Colourless solid (89 %); mp 60-62° C; IR (KBr, ν cm^{-1}): 3092 (ArC-H), 3004 (C-H), 1708 (C=O), 1284 (C-O), 748 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.42 (t, 3H, -CH_3 ester, *J* = 7.2 Hz), 4.52 (q, 2H, -CH_2 ester, *J* = 7.2 Hz), 5.62 (s, 2H, -OCH_2), 6.67 (d, 2H, Ring A-H, *J* = 8.9 Hz), 7.16 (d, 2H, Ring A-H, *J* = 8.9 Hz), 7.33-7.62 (m, 2H, Ring B-H), 7.72 (d, 1H, Ring B-H, *J* = 7.8 Hz), 7.92 (d, 1H, Ring B-H, *J* = 7.8 Hz).

General Procedure for the Preparation of 2-(Aryloxymethyl) Benzoyl Hydrazide [20] (3a-3d)

Ethyl 2-(aryloxymethyl)benzoates **(2a-d)** (0.1 mol), hydrazine hydrate (0.15 mol) and 20 mL of n-propanol were refluxed on an oil bath for 10 h. The excess solvent was

then distilled off under reduced pressure and the concentrated solution was quenched to ice cold water. The solid separated was filtered, washed and dried. The crude product was purified by recrystallization from ethanol.

2-(2-Methyl Phenoxy Methyl) Benzoyl Hydrazide (3a)

Colourless solid (89 %); mp 118-120° C; IR (KBr, ν cm^{-1}): 3286 (NH_2/NH), 3012 (ArC-H), 1644 (C=O), 1252 (C-O) ; ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 2.30 (s, 3H, Ring A-CH_3), 4.18 (s, 2H, -NH_2), 5.31 (s, 2H, -OCH_2), 6.93 (m, 3H, Ring A-H), 6.36 (t, 1H, Ring A-H, *J* = 7.8 Hz), 7.48-7.56 (m, 4H, Ring B-H), 7.48 (br.s, 1H, NH).

2-(3-Methyl Phenoxy Methyl) Benzoyl Hydrazide (3b)

Colourless solid (92 %); mp 108-110° C; IR (KBr, ν cm^{-1}): 3292 (NH_2/NH), 3016 (ArC-H), 1638 (C=O), 1256 (C-O); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 2.36 (s, 3H, Ar-CH_3), 4.16 (s, 2H, -NH_2), 5.26 (s, 2H, -OCH_2), 6.76-6.80 (m, 4H, Ring A-H), 7.48-7.52 (m, 4H, Ring B-H), 7.48 (br.s, 1H, NH).

2-(4-Methyl Phenoxy Methyl) Benzoyl Hydrazide (3c)

Colourless solid (90 %); mp 112-114° C; IR (KBr, ν cm^{-1}): 3288 (NH_2/NH), 3020 (ArC-H), 1646 (C=O), 1250 (C-O); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 2.12 (s, 3H, Ar-CH_3), 4.11 (s, 2H, -NH_2), 5.34 (s, 2H, -OCH_2), 6.35 (d, 2H, Ring A-H, *J* = 7.2 Hz), 6.92 (d, 2H, Ring A-H, *J* = 7.2 Hz), 7.48-7.58 (m, 4H, Ring B-H), 7.55 (br.s, 1H, NH).

2-(4-Chloro Phenoxy Methyl) Benzoyl Hydrazide (3d)

Colourless solid (92 %); mp 122-125° C; IR (KBr, ν cm^{-1}): 3291 (NH_2/NH), 3014 (ArC-H), 1638 (C=O), 1256 (C-O), 746 (C-Cl); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm) : 4.07 (s, 2H, -NH_2), 5.22 (s, 2H, -OCH_2), 6.92 (d, 2H, Ring A-H, *J* = 7.2 Hz), 6.25 (d, 2H, Ring A-H, *J* = 7.2 Hz), 7.59-7.48 (m, 4H, Ring B-H), 7.40 (br.s, 1H, NH).

Procedure for the Preparation of 4-Amino-5-[2-(Aryloxymethyl)]-4H-[1,2,4]-Triazole-3-Thiol (5a-5d)

2-(Aryloxymethyl)benzoic acid hydrazide (**3a-3d**) (0.0835 mol) was treated with a solution of potassium hydroxide (0.125 mol) in methanol (50 mL) at 0-5^0 C under stirring. Then carbon disulfide (0.125 mol) was added slowly and the reaction mixture was stirred over night at room temperature. The separated product, potassium dithiocarbazate **(4a-4d)**, was filtered, washed with chilled methanol and finally dried. It was directly used for next step without purification.

The above potassium dithiocarbazate was treated with a mixture of water (8 mL) and hydrazine hydrate (2 mmol) and was refluxed for 11-12 h. The reaction mixture turned to green with evolution of hydrogen sulfide and finally it became homogeneous. It was then diluted with little cold water and acidified with concentrated hydrochloric acid. The white precipitate formed was filtered, washed with cold water and recrystallized from aqueous ethanol.

4-Amino-5-[2-(2-Methyl Phenoxy Methyl)]-4H-[1,2,4]-Triazole-3-Thiol (5a)

Colourless solid (78 %); mp 146-148° C; IR (KBr, ν cm^{-1}): 3308, 3244 (NH_2), 3030 (ArC-H), 2557 (SH), 1617 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.16 (s, 3H, Ar-CH_3), 4.70 (s, 2H, NH_2), 5.25 (s, 2H, OCH_2), 6.76 (d, 1H, Ring A-H, *J* = 8.1 Hz), 6.86 (t, 1H, Ring A-H, *J* = 7.4 Hz), 7.07-7.13 (m, 2H, Ring A-H), 7.48 (t, 1H, Ring B-H, *J* = 7.2 Hz), 7.57-7.61 (m, 2H, Ring B-H), 7.72 (d, 1H, Ring B-H, *J* = 8.2 Hz), 11.18 (s, 1H, SH); *Anal*. calculated for $C_{16}H_{16}N_4OS$: C, 61.52; H, 5.16; N, 17.93; found: C, 61.48; H, 5.12; N, 17.82.

4-Amino-5-[2-(3-Methyl Phenoxy Methyl)]-4H-[1,2,4]-Triazole-3-Thiol (5b)

Colourless solid (72 %); mp 160-162° C; IR (KBr, ν cm^{-1}): 3314, 3228 (NH_2), 3032 (ArC-H), 2548 (SH), 1621 (C=N); *Anal*. calculated for $C_{16}H_{16}N_4OS$: C, 61.52; H, 5.16; N, 17.93; found: C, 61.50; H, 5.10; N, 17.78.

4-Amino-5-[2-(4-Methyl Phenoxy Methyl)]-4H-[1,2,4]-Triazole-3-Thiol (5c)

Colourless solid (70 %); mp 124-126° C; IR (KBr, ν cm^{-1}): 3310, 3240 (NH_2), 3027 (ArC-H), 2550 (SH), 1614 (C=N); *Anal*. calculated for $C_{16}H_{16}N_4OS$: C, 61.52; H, 5.16; N, 17.93; found: C, 61.42; H, 5.15; N, 17.90.

4-Amino-5-[2-(4-Chloro Phenoxy Methyl)]-4H-[1,2,4]-Triazole-3-Thiol (5d)

Colourless solid (75 %); mp 168-170° C; IR (KBr, ν cm^{-1}): 3314, 3250 (NH_2), 3038 (ArC-H), 2554 (SH), 1611 (C=N), 748 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 4.48 (s, 2H, NH_2), 5.16 (s, 2H, OCH_2), 6.85 (d, 2H, Ring A-H, *J* = 7.8 Hz), 7.24 (d, 2H, Ring A-H, *J* = 7.8 Hz), 7.45 (d, 1H, Ring B-H, *J* = 7.8 Hz), 7.62 (t, 1H, Ring B-H, *J* = 7.1 Hz), 7.84 (t, 1H, Ring B-H, *J* = 7.1 Hz), 8.04 (d, 1H, Ring B-H, *J* = 7.8 Hz), 11.17 (s, 1H, SH); *Anal c*alculated for $C_{15}H_{13}ClN_4OS$: C, 54.13; H, 3.94; N, 16.83; found: C, 54.05; H, 4.10; N, 16.92.

General Procedure for the Preparation of 2-Aryl-6-(2-{Aryloxymethl}Phenyl-[1,2,4]-Triazolo[3,4-*b*]-[1,3,4]-Thiadiazoles (6a-6t)

A mixture of 4-amino-5-[2-(aryloxymethyl)]-4*H*-[1,2,4]-triazole-3-thiol **(5a-d)** (1 mmol) and (un)substituted benzoic acid (1.1mmol) in phosphorous oxychloride (5 ml) was refluxed for 6-7 h. The reaction mixture was slowly quenched into crushed ice with stirring and neutralized with solid sodium bicarbonate. The solid separated after standing the mixture overnight was filtered, washed with cold water, dried and recrystallized from appropriate solvent to afford the title compounds. Characterization data of compounds (**6a-t**) are summarized in Table **1**.

2-(2-Chloropyridin-3-yl)-6-{2-[(2-Methylphenoxy)Methyl]Phenyl}-[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6a)

IR (KBr, ν cm^{-1}): 3038 (aromatic C-H), 1616 (C=N), 1071 (C-S), 890 (C-Cl); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.31 (3H, s, Ring A-CH_3), 5.69 (s, 2H, O-CH_2), 6.85 (t, 1H, Ring A-H, *J* = 7.3 Hz), 6.94 (d, 1H, Ring A-H, *J* = 8.6 Hz), 7.12-7.76 (m, 2H, Ring A-H), 7.46 (t, 1H, Ring C-H, *J* = 7.5 Hz), 7.57 (d, 2H, Ring C-H, *J* = 7.8 Hz), 7.86 (d, 1H, Ring B-H, *J* = 7.8 Hz), 8.30 (d, 1H, Ring B-H, *J* = 7.6 Hz), 8.91-8.94 (2H, m, Ring B-H); ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm): 29.7 (RingA-CH_3), 68.4 (OCH_2), 111.7 (C_6

of Ring A), 119.9 (C_2 of Ring A) ,120.5 (C_4 of Ring C), 122.7 (C_3 of Ring A), 125.8 (C_2 of Ring C), 126.9 (C_3 of Ring B), 127.3 / 127.4 (C_4 / C_5 of Ring B), 130.3 (C_2 of Ring B) , 130.6 (C_5 of Ring A), 131.0 (C_5 of Ring C), 138.1 (C_1 & C_6 of Ring B), 139.9 (C_4 of Ring A), 151.7 / 154.6 (C_3 / C_6 of Ring C), 154.8 (C_3 of triazole), 155.4 (C_5 of triazole), 156.8 (C_1 of Ring A), 164.5 (C_7 of thiadiazole). DEPT: 29.7 (CH_3), 68.38 (CH_2), 111.7, 120.5, 122.7, 126.9, 127.3, 127.4, 130.3, 130.6, 131.0, 139.9, 154.6; LCMS: m / z 434 (M^+ , 100%).

2-(2-Chloropyridin-3-yl)-6-{2-[(3-Methylphenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6b)

IR (KBr, ν cm^{-1}): 3042 (aromatic C-H), 1621 (C=N), 1068 (C-S), 887 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.36 (3H, s, Ring A-CH_3), 5.52 (s, 2H, O-CH_2), 6.80-7.23 (m, 3H, Ring A-H), 7.68 (s, 1H, Ring A-H), 7.74 (t, 1H, Ring C-H, *J* = 7.6 Hz), 7.87 (d, 2H, Ring C-H, *J* = 7.8 Hz), 7.88 (d, 1H, Ring B-H, *J* = 7.8 Hz), 8.12 (d, 1H, Ring B-H, *J* = 7.6 Hz), 8.31-8.34 (2H, m, Ring B-H).

2-(2-Chloropyridin-3-yl)-6-{2-[(4-Methylphenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6c)

IR (KBr, ν cm^{-1}): 3038 (ArC-H), 1622 (C=N), 1075 (C-S), 892 (C-Cl); LCMS: m/z 434 (M^+ 100%).

2-(2-Chloropyridin-3-yl)-6-{2-[(4-Chlorophenoxy)Methyl]phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6d)

IR (KBr, ν cm^{-1}): 3044 (ArC-H), 1628 (C=N), 1070 (C-S), 882 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 5.64 (s, 2H, O-CH_2), 6.85 (d, 2H, Ring A-H, *J* = 7.8 Hz), 7.24 (d, 2H, Ring A-H, *J* = 7.8 Hz), 7.54 (t, 1H, Ring C-H, *J* = 7.6 Hz), 7.67 (d, 2H, Ring C-H, *J* = 7.8 Hz), 7.92 (d, 2H, Ring B-H, *J* = 7.4 Hz), 8.32 (d, 2H, Ring B-H, *J* = 7.4 Hz).

2-(3,5-Dimethylphenyl)-6-{2-[(2-Methylphenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6e)

IR (KBr, ν cm^{-1}): 3042 (ArC-H), 1621 (C=N), 1081 (C-S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.13 (3H, s, Ring A-CH_3), 2.27 (s, 6H, Ring C-$[CH_3]_2$), 5.59 (s, 2H, OCH_2), 6.81 (s, 1H, Ring C-H), 6.85 (s, 1H, Ring C-H), 7.06 (s, 1H, Ring C-H), 7.25-7.26 (m, 2H, Ring B-H), 7.50-7.57 (m, 2H, Ring B-H), 7.60-7.62 (m, 2H, Ring A-H), 7.92 (d, 1H, Ring A-H, *J* = 8.5 Hz), 8.19 (d, 1H, Ring A-H, *J* = 8.5 Hz) ; LCMS: m / z 427 (M^+ +1, 100%).

2-(3,5-Dimethylphenyl)-6-{2-[(3-Methylphenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6f)

IR (KBr, ν cm^{-1}): 3062 (ArC-H), 1632 (C=N), 1069 (C-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.17 (3H, s, Ring A-CH_3), 2.37 (s, 6H, Ring C-$[CH_3]_2$), 5.42 (s,2H, OCH_2), 6.71-6.75 (m, 2H, Ring B-H), 6.99-7.01 (m, 2H, Ring B-H), 7.56-7.65 (m, 2H, Ring A-H), 7.31 (s, 1H, Ring C-H), 7.75 (m, 1H, Ring C-H), 8.03 (m, 1H, Ring C-H).

2-(3,5-Dimethylphenyl)-6-{2-[(4-Methylphenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6g)

IR (KBr, ν cm^{-1}): 3072 (ArC-H), 1630 (C=N), 1068 (C-S); LCMS: m / z 427 (M^+ +1, 100%).

2-(3,5-Dimethylphenyl)-6-{2-[(4-Chlorophenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6h)

IR (KBr, ν cm^{-1}): 3039 (ArC-H), 1618 (C=N), 1071 (C-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.42 (s, 6H, Ring C-$[CH_3]_2$), 5.59 (s,2H, OCH_2), 6.71 (s, 1H, Ring C-H), 6.75 (s, 1H, Ring C-H), 6.94 (s, 1H, Ring C-H), 7.16 (d, 2H, Ring B-H, *J* = 7.8 Hz), 7.36 (d, 2H, Ring B-H, *J* = 7.8 Hz), 7.52-7.56 (m, 2H, Ring A-H), 7.63 (d, 1H, Ring A-H, *J* = 8.5 Hz), 8.12 (d, 1H, Ring A-H, *J* = 8.5 Hz); LCMS: m / z 448 (M^+ +1, 100%).

2-(3,4,5-Trimethoxylphenyl)-6-{2-[(2-Methylphenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b][1,3,4]Thiadiazole (6i)

IR (KBr, ν cm^{-1}): 3047 (ArC-H), 1624 (C=N), 1077 (C-S); LCMS: m / z 488 (M^+, 100%).

2-(3,4,5-Trimethoxylphenyl)-6-{2-[(3-Methylphenoxy)Methyl]Phenyl} [1,2,4] Triazolo [5,1-b][1,3,4]Thiadiazole (6j)

IR (KBr, ν cm^{-1}): 3047 (ArC-H), 1624 (C=N), 1069 (C-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.07 (s, 3H, Ring A-CH_3), 3.76 (s, 3H , Ring C-CH_3), 3.78-3.99 (m, 6H, Ring C-$[CH_3]_2$), 5.42 (s, 2H, OCH_2), 6.66-6.76 (m, 3H, Ring A-H), 7.14 (t, 1H, Ring A-H, *J* = 8.1 Hz), 7.21-7.29 (m, 2H, Ring C-H), 7.63-7.71 (m, 2H, Ring B-H), 7.92 (t, 1H, Ring B-H, *J* = 7.8 Hz), 8.09 (t, 1H, Ring B-H, *J* = 7.8 Hz); LCMS: m / z 488 (M^+, 100%).

2-(3,4,5-TRIMETHOXYLPHENYL)-6-{2-[(4-Chlorophenoxy)Methyl]Phenyl} [1,2,4]Triazolo[5,1-b][1,3,4]Thiadiazole (6l)

IR (KBr, ν cm^{-1}): 3047 (ArC-H), 1624 (C=N), 1074 (C-S), 887 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.64 (s, 3H, Ring C-OCH_3), 3.68 (s, 3H, Ring C-OCH_3), 3.70 (s, 3H, Ring C-OCH_3), 5.51 (s, 2H, OCH_2), 6.42 (d, 1H, Ring A-H, *J* = 8.1 Hz), 6.56 (d, 1H, Ring A-H, *J* = 8.3 Hz), 7.11-7.19 (m, 2H, Ring C-H), 7.33-7.41 (m, 2H, Ring B-H), 7.68 (t, 1H, Ring B-H, *J* = 7.8 Hz), 7.96 (t,1H, Ring B-H, *J* = 7.8 Hz); LCMS: m/z 510 (M^+, 100%).

2-(3,5-Dichlorophenyl)-6-{2-[(2-Methylphenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6m)

IR (KBr, ν cm^{-1}): 3040 (ArC-H), 1590 (C=N), 1071 (C-S), 893 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.27 (s, 3H, Ring A-CH_3), 5.52 (2H, s -OCH_2), 6.69-6.78 (m, 3H, Ring C-H), 7.09 (t, 1H, Ring A-H, *J* = 8.4 Hz), 7.25 (t, 1H, Ring A-H, *J* = 8.1 Hz), 7.52-7.62 (m, 2H, Ring A-H), 7.75-7.78 (m, 2H, Ring B-H), 7.86 (d, 1H, Ring B-H, *J* = 8.4 Hz), 8.09 (d, 1H, Ring B-H, *J* = 8.4 Hz); MS FAB^+ (m / z): (468, M^++ 1).

2-(3,5-Dichlorophenyl)-6-{2-[(3-methylphenoxy)methyl]phenyl}[1,2,4]triazolo[5,1-b] [1,3,4]thiadiazole (6n)

IR (KBr, ν cm^{-1}): 3044 (ArC-H), 1581 (C=N), 1076 (C-S), 887 (C-Cl).

2-(3,5-Dichlorophenyl)-6-{2-[(3-Methylphenoxy)Methyl]Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6o)

IR (KBr, ν cm^{-1}): 3054 (ArC-H), 1593 (C=N), 1066 (C-S), 892 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.24 (s, 3H, Ring A-CH_3), 5.56 (2H, s -OCH_2), 6.74 (d, 2H, Ring A-H, *J* = 8.2 Hz), 7.06 (d, 2H, Ring A-H, *J* = 8.1 Hz), 7.42-7.49 (m, 3H, Ring C-H), 7.50-7.61 (m, 2H, Ring B-H), 7.86 (d, 1H, Ring B-H, *J* = 8.6 Hz), 8.04 (d, 1H, Ring B-H, *J* = 8.6 Hz); MS FAB^+ (m / z): (468,M^++ 1).

2-(3-Bromophenyl)-6-{2-[(2-Methylphenoxy)Methyl] Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4]Thiadiazole (6q)

IR (KBr, ν cm^{-1}): 3028 (ArC-H), 1587 (C=N), 1081 (C-S), 584 (C-Br); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.25 (s, 3H, Ring A-CH_3), 5.54 (2H, s -OCH_2), 6.72-7.18 (m, 4H, Ring A-H), 7.36 (d, 1H, Ring B-H, *J* = 7.2 Hz), 7.77-7.82 (m, 2H, Ring B-H), 7.92 (d, 1H, Ring B-H, *J* = 7.2 Hz), 7.98-8.05 (m, 2H, Ring C-H), 8.23 (s,1H, Ring C-H), 8.44 (d, 1H, Ring C-H, *J* = 7.4 Hz); MS FAB$^+$ (m / z): (478, M^++ 1).

2-(3-Bromophenyl)-6-{2-[(3-Methylphenoxy)Methyl] Phenyl}[1,2,4]Triazolo[5,1-b] [1,3,4] Thiadiazole (6r)

IR (KBr, ν cm^{-1}): 3032 (ArC-H), 1590 (C=N), 1069 (C-S), 577 (C-Br); MS FAB$^+$ (m / z): (478, M^++ 1).

2-(3-Bromophenyl)-6-{2-[(4-Chlorophenoxy)Methyl] Phenyl}[1,2,4]Triazolo[5,1-b][1,3,4] Thiadiazole (6t)

IR (KBr, ν cm^{-1}): 3046 (ArC-H), 1628 (C=N), 1072 (C-S), 890 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 5.57 (s, 2H, OCH_2), 6.88 (d, 2H, Ring A-H, *J* = 8.4 Hz), 7.18 (d, 2H, Ring A-H, *J* = 8.3 Hz), 7.42 (d, 1H, Ring B-H, *J* = 7.2 Hz), 7.54-7.62 (m, H, Ring B-H), 7.75 (d, 1H, Ring B-H, *J* = 7.2 Hz), 7.80-7.85 (m, 2H, Ring C-H), 8.07 (s, 1H, Ring C-H), 8.18 (d, 1H, Ring C-H, *J* = 7.6 Hz); LCMS: m / z 498 (M^+, 100%).

General Procedure for the Preparation of 6-aryl-3-({2-Aryloxymethyl}Phenyl)-7*H*-1,2,4-Triazolo[3,4-*b*][1,3,4] Thiadiazines (7a-7p)

A mixture 4-amino-5-({2-aryloxymethyl}phenyl)-4H-1,2,4-triazole-3-thiol (**5a-c**) (1 mmol) and substituted phenacyl bromide (1.2 mmol) in 10 mL of absolute ethanol was refluxed for 3-4 h. The reaction mixture was slowly quenched onto crushed ice with stirring and neutralized with solid sodium bicarbonate. The solid separated after standing overnight was filtered, washed with cold water, dried and recrystallised from absolute ethanol to afford the title compounds.

6-(3-Carboxamido-4-Hydroxyphenyl)-3-[2-(2-Methyl-phenoxymethyl)-Phenyl]-7H-[1,2,4]Triazolo[3,4-b][1,3,4] Thiadiazine (7a)

IR (KBr, ν cm^{-1}): 3380, 3046 (NH_2), 3024 (ArC-H), 1632 (C=N), 1064 (C-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.12 (s, 3H, Ring A-CH_3), 3.88 (2H, s, SCH_2), 5.30 (2H, s, OCH_2), 6.73 (d, 1H, Ring A-H, *J* = 8.1 Hz), 6.81 (t, 1H, Ring A-H, *J* = 7.3 Hz), 6.97-7.04 (m, 2H, Ring A-H), 6.64 (br.s, 1H, Ring C-H), 7.09 (d, 1H, Ring C-H, *J* = 8.1 Hz), 7.92-7.95 (m, 1H, Ring C-H), 7.48 (t, 1H, Ring B-H, *J* = 7.3 Hz), 7.56 (t, 1H, Ring B-H, *J* = 7.3 Hz), 7.69 (d, 1H, Ring B-H, *J* = 7.8 Hz), 7.76 (d, 1H, Ring B-H, *J* = 7.8 Hz), 8.28 (Br.s, 2H, Ring C-$CONH_2$), 13.46 (1 H, s, Ring C-OH); LCMS: m / z 471 (M^+, 100%).

6-(3-Carboxamido-4-hydroxyphenyl)-3-[2-(3-methyl-phenoxymethyl) phenyl]-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazine (7b)

IR (KBr, ν cm^{-1}): 3382, 3036 (NH_2), 3026 (ArC-H), 1634 (C=N), 1072 (C-S).

6-(3-Carboxamido-4-Hydroxyphenyl)-3-[2-(4-Chloro-phenoxymethyl)Phenyl]-7H-[1,2,4]Triazolo[3,4-b][1,3,4] Thiadiazine (7d)

IR (KBr, ν cm^{-1}): 3386, 3045 (NH_2), 3028 (aromatic C-H), 1636 (C=N), 1071 (C-S), 892 (C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.91 (2H, s, SCH_2), 5.38 (2H, s, OCH_2), 6.75 (t, 1H, Ring C-H, *J* = 7.3 Hz), 6.91 (d, 1H, Ring A-H, *J* = 8.9 Hz), 7.15 (d, 1H, Ring C-H, *J* = 8.3 Hz), 7.75 (d, 1H, Ring C-H, *J* = 5.1 Hz), 7.80 (d, 2H, Ring A-H, *J* = 8.9 Hz), 7.48-7.59 (m, 2H, Ring B-H), 7.61-7.72 (m, 2H, Ring B-H), 8.05 (s, 2H, Ring C-$CONH_2$),12.86 (s, 1H, Ring C-OH); LCMS: m / z 493 (M^+, 100%).

6-(3-(5-Chloro-2-Sulfonamido)Thienyl)-3-[2-(2-Methyl-phenoxymethyl)Phenyl]-7H-[1,2,4]Triazolo[3,4-b][1,3,4] Thiadiazine (7g)

IR (KBr, ν cm^{-1}): 3395, 3054 (NH_2), 3030 (ArC-H), 1631 (C=N), 1070 (C-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.23 (s, 3H, Ring A-CH_3), 3.68 (s, 2H, SCH_2), 4.82 (s, 2H, SO_2NH_2), 5.02 (s, 2H, -OCH_2), 6.57 (d, 2H, Ring A-H, *J* = 8.6 Hz), 6.94 (d, 2H, Ring A-H, *J* = 8.6 Hz), 6.98 (s, 1H, Ring C-H), 7.48-7.64 (m, 4H, Ring B-H); ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm): 20.4 (Ring A-CH_3), 25.3 (SCH_2), 67.6 (OCH_2), 114.2 (C_2 & C_6 of Ring A), 124.3 (C_3 of Ring C), 127.7 (C_2 of Ring B), 128.8 (C_5 of Ring B), 129.9 & 129.5 (C_4 & C_3 of Ring B), 130.5 (C_4 of Ring A), 131.5 (C_4 of Ring C), 131.5 (C_3 & C_5 of Ring A), 133.6 (C_1 of Ring B), 135.6 (C_5 of Ring C), 137.3 (C_6 of Ring B), 141.3 (C_5 of triazole),142.6 (C_3 of triazole), 148.1 (C_2 of Ring C), 152.6 (C_1 of Ring A), 155.9 (C_7 of thiadiazine). LCMS: m / z 533 (M^++1, 100%).

6-(3-(5-Chloro-2-Sulfonamido)Thienyl)-3-[2-(4-Chloro-phenoxymethyl)Phenyl]-7H-[1,2,4]Triazolo[3,4-b][1,3,4] Thiadiazine (7h)

IR (KBr, ν cm^{-1}): 3390, 3049 (NH_2), 3033 (ArC-H), 1626 (C=N), 1065 (C-S), 894 (C-Cl); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 3.60 (s, 2H, SCH_2), 4.75 (s, 2H, SO_2NH_2), 5.22 (s, 2H, -OCH_2), 6.42 (d, 2H, Ring A-H, *J* = 8.4 Hz), 6.84 (d, 2H, Ring A-H, *J* = 8.4 Hz), 6.78 (s, 1H, Ring C-H), 7.48-7.52 (m, 2H, Ring B-H), 7.56-7.68 (m, 2H, Ring B-H); LCMS: m / z 553 (M^+, 100%).

6-(3-Bromophenyl)-3-[2-(2-Methylphenoxymethyl) Phenyl]-7H-[1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazine (7i)

IR (KBr, ν cm^{-1}): 3048 (ArC-H), 1587 (C=N), 1062 (C-S), 585 (C-Br); LCMS: m / z 492 (M^+, 100%).

6-(3-Bromophenyl)-3-[2-(4-Methylphenoxymethyl) Phenyl]-7H-[1,2,4]Triazolo[3,4-b] [1,3,4] Thiadiazine (7k)

IR (KBr, ν cm^{-1}): 3045 (ArC-H), 1594 (C=N), 1065 (C-S), 575 (C-Br); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.24 (3H, s, Ring A-CH_3), 4.01 (s, 2H, SCH_2), 5.23 (s, 2H, OCH_2), 6.43 (d, 2H, Ring A-H, *J* = 8.6 Hz), 7.01 (d, 2H, Ring A-H, *J* = 8.4 Hz), 7.37 (d, 1H, Ring C-H, *J* = 7.8 Hz), 7.52 (t, 1H, Ring C-H, *J* = 7.1 Hz), 7.61-7.65 (m, 2H, Ring C-H), 7.67-7.69 (m, 2H, Ring B-H), 7.75 (d, 1H, Ring B-H, *J* = 8.2 Hz), 7.82 (d, 1H, Ring B-H, *J* = 8.3 Hz); LCMS: m / z 492 (M^+, 100%).

6-(3-Bromophenyl)-3-[2-(4-Chlorophenoxymethyl) Phenyl]-7H-[1,2,4]Triazolo[3,4-b] [1,3,4] Thiadiazine (7l)

IR (KBr, ν cm^{-1}): 3042 (ArC-H), 1588 (C=N), 1068 (C-S) , 884 (C-Cl), 584 (C-Br); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.27 (3H, s, Ring A-CH_3), 4.23 (s, 2H, SCH_2), 5.38 (s, 2H, OCH_2), 6.54 (d, 2H, Ring A-H, *J* = 8.2 Hz), 7.12 (d, 2H, Ring A-H, *J* = 8.2 Hz), 7.41 (d, 1H, Ring C-H, *J* = 8.2 Hz), 7.65 (t, 1H, Ring C-H, *J* = 7.1 Hz), 7.82-7.88 (m, 2H, Ring C-H), 7.97-8.02 (m, 2H, Ring B-H), 8.16 (d, 1H, Ring B-H, *J* = 8.4 Hz), 8.28 (d, 1H, Ring B-H, *J* = 8.4 Hz); LCMS: m / z 512 (M^+, 100%).

3-{2-[(2-Methylphenoxy)Methyl]Phenyl}-6-(4-Nitrophenyl)-7H-[1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazine(7m)

IR (KBr, ν cm^{-1}): 3041 (ArC-H), 1596 (C=N), 1070 (C-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.34 (3H, s, Ring A-CH_3), 3.84 (s, 2H, SCH_2), 5.27 (s, 2H, OCH_2), 6.42-6.57 (m, 3H, Ring A-H), 6.58-6.61 (m, 1H, Ring A-H), 7.12 (t, 2H, Ring B-H, *J* = 7.3 Hz), 7.22-7.34 (m, 2H, Ring B-H), 8.05 (d, 2H, Ring C-H, *J* = 8.2 Hz), 8.21 (d, 2H, Ring C-H, *J* = 8.2 Hz); LCMS: m / z 457 (M^+, 100%).

3-{2-[(3-Methylphenoxy)Methyl]Phenyl}-6-(4-Nitrophenyl)-7H-[1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazine (7n)

IR (KBr, ν cm^{-1}): 3052 (ArC-H), 1586 (C=N), 1067 (C-S) ; LCMS: m / z 457 (M^+, 100%).

3-{2-[(4-Chlorophenoxy)Methyl]Phenyl}-6-(4-Nitrophenyl)-7H-[1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazine(7p)

IR (KBr, ν cm^{-1}): 3048 (ArC-H), 1581 (C=N), 1066 (C-S) , 887 (C-Cl); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 3.74 (s, 2H, SCH_2), 5.22 (s, 2H, OCH_2), 6.62 (d, 2H, Ring A-H, *J* = 8.2 Hz), 6.98 (d, 2H, Ring A-H, *J* = 8.2 Hz), 7.32 (t, 2H, Ring B-H, *J* = 7.3 Hz), 7.38-7.46 (m, 2H, Ring B-H), 7.82 (d, 2H, Ring C-H, *J* = 8.4 Hz), 8.18 (d, 2H, Ring C-H, *J* = 8.4 Hz); LCMS: m / z 479 (M^++1, 100%).

PHARMACOLOGY

Antimicrobial Activity (Determination of Zone of Inhibition)

The newly synthesized compounds (**6a-6t**) and (**7a-7p**) were screened for their antimicrobial activity against a total of four bacterial and two fungal organisms by disc diffusion method [21]. Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi that were incubated without agitation for 24 h at 37° C and 25° C respectively. The cultures were diluted with fresh Mueller-Hinton and Sabouraud dextrose broth to achieve optical densities corresponding to $2.0x10^6$ colony forming units (CFU / mL) for bacteria and $2.0x10^5$ spore / mL for fungal strains.

In-vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 mL of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The 10 µg / disc of each of all the test samples were loaded on 6 mm sterile discs. The loaded disc was placed on the surface of the corresponding medium the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37° C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate. Standard antibacterial drug ciprofloxacin (10µg / disc) and antifungal drug fluconazole (10µg / disc) were also tested under similar conditions against these organisms.

Anti-Inflammatory Activity

The anti-inflammatory activity of sixteen newly synthesized compounds was determined by carrageenan induced paw oedema method [22]. Wistar albino rats of either sex weighing 180-250g were used for the experiment. They were housed in the clean polypropylene cages and kept under room temperature (25° C), relative humidity (60-70 %) in 12 h of light-dark cycle. The animals were given standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during experimental hours.

The animals were divided into 38 groups with each group containing 6 animals. A mark was made on the hind paw (left) just below the tibia-tarsal junction, so that every time the paw was dipped in the mercury column up to fixed mark to ensure constant paw volume. The initial paw volume of each rat was noted by plethysmometrically. First group received 0.6% Na CMC and the second group received Diclofenac Sodium at a dose of 10mg / kg body weight intramuscularly. The 3rd to 38th groups were administered with the test compounds at a dose 10mg / kg (suspended in 0.6% CNC given p.o). Thirty minutes after the treatment of test compounds, 0.1 mL of 1% (w / v) carrageenan was injected in the sub plantar region of the left hind paw. The right paw served as a reference to non-inflamed paw for comparison. The initial paw volume was measured within 30 seconds of the injection. The relative increase in paw volume was measured in control, standard and test compounds at 3 h after the carregeenan injection. The difference between the two readings was taken as the volume of oedema, the percentage inhibition by the drugs was calculated using the formula,

Percentage of oedema inhibition=$100 - (V_{test} / V_{control}) \times 100$,

Where $V_{control}$ = volume of paw oedema in control group;

V_{test} = volume of paw oedema in the test compounds in treated group.

The results were expressed as % inhibition of oedema over the untreated control group. The results of anti-inflammatory studies are given in Table **3**.

CONCLUSION

Several 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadizoles and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines were successfully synthesized in 60-70% yields and were characterized by ^{1}H NMR, ^{13}C NMR, Mass spectrometry and IR studies. All the newly synthesized compounds were screened for antibacterial and antifungal properties. The antimicrobial activity study re-

vealed that all the compounds tested showed moderate to good antibacterial and antifungal activities against pathogenic strains. Structure and biological activity relationship of title compounds showed that the presence of 4-chloro phenoxymethylphenyl and 2-chloropyridin-3-yl, 3,4,5-trimethoxyphenyl, 3,5-dichlorophenyl, 2-hydoxy-4-carboxamidophenyl, 5-chloro-2-sulfonamidophenyl groups attached to position-2 / 3 and 6 of the thiadiazole and thiadiazine rings of the title compounds are responsible for good antimicrobial activity. The screening data showed that the newly prepared compounds have shown promising antibacterial and antifungal activities against the screened organisms. The investigation of anti-inflammatory activity revealed that many compounds showed significant anti-inflammatory properties. Few of them, particularly compounds containing 2-Me-, 3-Me-, 4-Me- and 4-Cl-phenoxymethylphenyl group on C-3 and 2-chloropyridin-3-yl and 3,5-dichlorophenyl groups at C-6 position showed good anti-inflammatory activity which is comparable to that of the standard. Therefore, it was concluded that there exists ample scope for further study in this class of compounds.

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