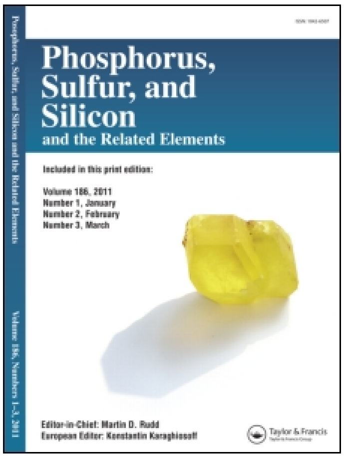

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Convenient Synthesis of Some Thiadiazolotriazinones Carrying 4-Methylthiobenzyl Moieties as Possible Antimicrobial Agents

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Convenient Synthesis of Some Thiadiazolotriazinones Carrying 4-Methylthiobenzyl Moieties as Possible Antimicrobial Agents

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*4-amino-6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(*4H*)-one was condensed with various substituted benzoic acids / aryloxyacetic acids in the presence of phosphorus oxychloride to afford a series of 7-(substituted aryl / aryloxymethyl)-3-(4-methylthiobenzyl)-4H-1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazin-4-ones in good yields. The structures of the newly synthesized compounds were confirmed on the basis of elemental analysis, IR, ^{1}H NMR, and mass spectral studies. All the newly synthesized compounds were tested for their antibacterial and antifungal activity against a variety of microorganisms.*

Keywords 1,3,4-Thiadiazole; 1,2,4-triazin-5(*4H*)-one; antibacterial; antifungal

INTRODUCTION

Various substituted 1,2,4-triazines and their derivatives are important biological agents, and a significant amount of research activity has been directed toward this class of compounds. Triazinones and their condensation products find important applications in medicinal and agricultural fields.[1–7] Some 1,2,4-triazinone derivatives are reported to possess antidiuretic, neurodepressant, and herbicidal properties.[8,9] A large number of 1,3,4-thiadiazoles are reported to possess antibacterial properties similar to those of well-known sulfonamide drugs.[10] Similarly, the 1,3,4-thiadiazole nucleus is associated

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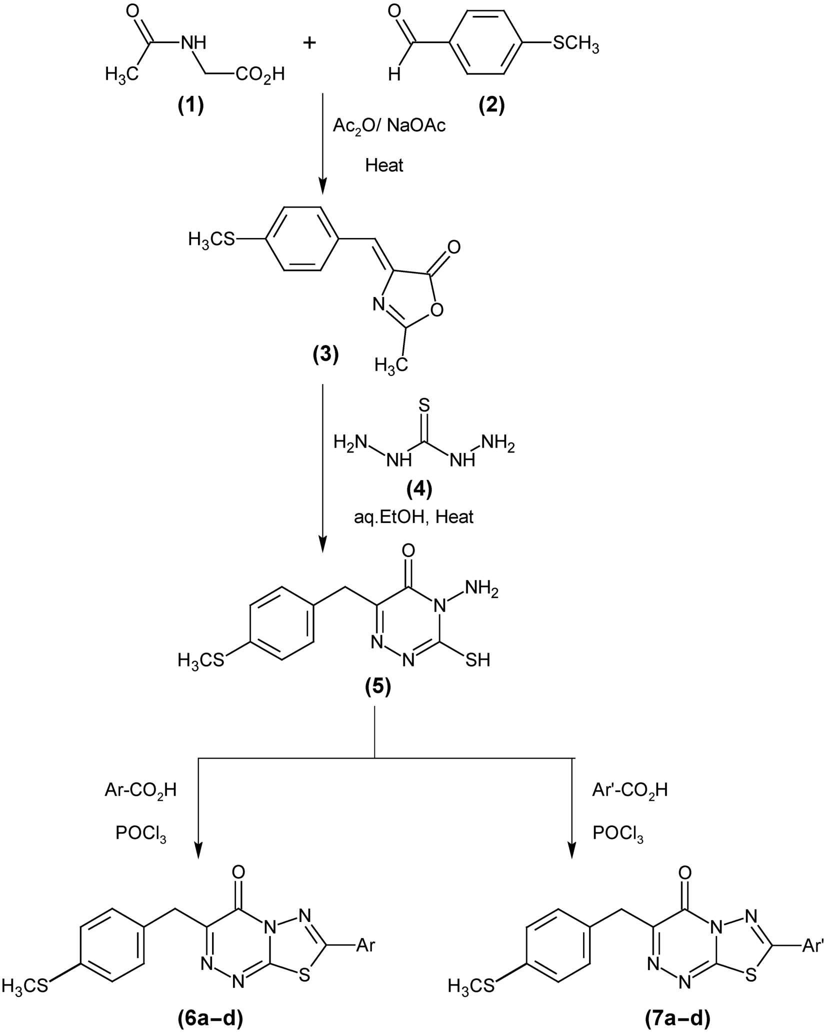
with a wide range of biological activities, namely anticancer, antimycobacterial, antitrypanosomal, antihypertensive, anesthetic, and cardiotonic activities.[11–16] It is also known that the thiadiazole nucleus, due to its pharmacophoric N-C-S linkage exhibits a large number of biological activities.[17] In view of the possible pharmacological activity of new heterocyclic systems containing 1,3,4-thiadiazoles, a series of [1,3,4]-thiadiazolo[2,3-*c*][1,2,4]-triazines were synthesized and reported in the literature.[18–20] As a part of our research program to find potent biologically active molecules,[21] we have herein synthesized some new 7-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-*4H*-1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazin-4-ones and studied their antibacterial and antifungal properties.

RESULTS AND DISCUSSION

In the present investigation, some new 7-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-4*H*-1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazin-4-ones (**6a–d**) and (**7a–d**) were prepared by reacting 4-amino-6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(*4H*)-one (**5**) with substituted benzoic acids/aryloxyacetic acids in the presence of phosphorus oxychloride (*Scheme 1*). 4-amino-6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(4*H*)-one (**5**) was prepared by reacting 4-(4-methylthiobenzylidene)-2-methyl-oxazol-5-one (**3**) with thiocarbohydrazide (**4**) in refluxing aqueous ethanol. Some of the newly synthesized compounds were well characterized by their elemental analysis, IR, ^{1}H NMR, and mass spectral studies.

The IR spectrum of oxazolone (**3**) showed characteristic absorption bands at 1694 cm^{-1} and 1598 cm^{-1}, which are due to the C=O and C=N functional groups, respectively. The ^{1}H NMR ($CDCl_3$) spectrum of (**3**) showed two singlets at δ 2.40 and δ 2.52, which are due to the CH_3 and SCH_3 protons, respectively. Another singlet at δ 7.08 is due to the olefinic proton. The four protons of the 4-methylthiophenyl ring resonated as a pair of doublets centred at δ 7.76 and δ 7.99 with a coupling constant of $J = 8.36$ Hz each. The mass spectrum of (**3**) showed a protonated molecular ion peak ($M^+ + 1$) at m/z 234 along with the molecular ion peak (M^+) at m/z 233 in accordance with its molecular formula $C_{12}H_{11}NO_2S$.

The IR spectrum of triazinone (**5**) showed characteristic absorption bands at 3290 cm^{-1} and 3161 cm^{-1}, which are due to the free amino group present. Absorption bands at 1160 cm^{-1} and 1527 cm^{-1} are due to C=O and C=N functional groups, respectively. The ^{1}H NMR (DMSO-d_6) spectrum of (**5**) showed two singlets at δ 2.43 and δ 3.86, which are due to SCH_3 and CH_2 protons. Another set of singlets at δ 6.47 and δ 13.86 that



Ar = C_6H_5, 4-$OCH_3C_6H_4$, 4-ClC_6H_4, 2,4-$Cl_2C_6H_3$

Ar' = $C_6H_5OCH_2$, 4-$CH_3C_6H_4OCH_2$, 4-$ClC_6H_4OCH_2$, 2,4-$Cl_2C_6H_3OCH_2$

SCHEME 1

are exchangeable with D_2O are due to NH_2 and SH protons. The four protons of 4-methylthiophenyl ring resonated as a multiplet at δ 7.17–7.22. The ^{13}C NMR (DMSO-d_6) spectrum of (**5**) showed peaks at 15, 35, 126, 130, 133, 136, 147, 148, and 168, which account for nine different carbon atoms present in the molecule. The mass spectrum of (**5**) showed the presence of a molecular ion peak (M^+) at m/z 280 in accordance with its molecular formula $C_{11}H_{12}N_4OS_2$, thereby confirming its formation.

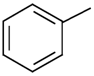
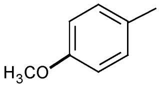
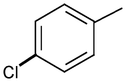
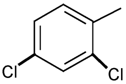
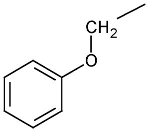
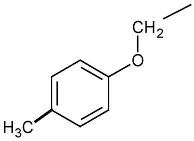
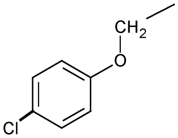
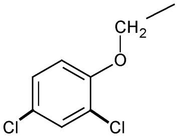
IR spectra of the cyclized products **6a–d** and **7a–d** showed the absence of absorption bands above 3100, thereby indicating the absence of a free NH_2 group, which is involved in the cyclization process. Also their ^1H NMR spectrum showed the absence of two singlets corresponding to NH_2 and SH protons, thereby further confirming the cyclization. The IR spectrum of (**6a**) exhibited absorption bands at 1705 cm^{-1} and 1598 cm^{-1}, which are due to C=O and C=N functional groups, respectively. The ^1H NMR spectrum of (**6a**) showed two singlets at δ 2.46 and δ 4.30, which are due to the SCH_3 and CH_2 protons, respectively. The four protons of the 4-methylthiophenyl ring resonated as a pair of doublets centred at δ 7.21 and δ 7.42. The five protons of the phenyl ring appeared as a multiplet at δ 7.55–7.96. The mass spectrum of (**6a**) showed a molecular ion peak (M^+) at m/z 366 in accordance with its molecular formula $C_{18}H_{14}N_4OS_2$. The experimental data of all the newly synthesized compounds are presented in Table I. The spectral details of some of the compounds have also been discussed herein.

An antibacterial and antifungal study carried out for the newly synthesized compounds **6a–d** and **7a–d** reveals that the all the compounds possess moderate to good antibacterial and antifungal activity. However, compounds **7c** and **7d** showed the maximum activity against all the tested organisms. The presence of a potential fused triazinothiadiazole ring system along with multiple halogen atoms may be the reason for their increased activity. In addition to this the presence of an aryloxyalkyl moiety in a molecule is also known to enhance its biological property.[22] The antibacterial and antifungal activity data of the newly synthesized compounds are given in Tables II and III.

Antibacterial Activity

We investigated the newly synthesized 7-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-*4H*-1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazin-4-ones (**6a–d**) and (**7a–d**) for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Psuedomonas aeruginosa* (ATCC-27853), and *Klebsiella pneumoniae* (recultured) bacterial stains by the disc diffusion method.[23–25] The discs, measuring 6.25 mm in diameter, were punched from Whatman No. 1 filter paper. Batches of 100 discs were dispensed in each screw-capped bottle and sterilized by dry heat at 140°C for an hour. The test compounds were prepared in different concentrations using DMF. Exactly 1 mL containing 100 times the amount of chemical

TABLE I Characterization Data of Thiadiazolotriazinones (6a–d and 7a–d)

Compound no.	Ar/Ar′	Molecular formula	M.P. (°C)	Yield (%)	Elemental Analysis Found (Calcd.) C	H	N
6a		$C_{18}H_{14}N_4OS_2$	196–198	72	59.06 (59.00)	3.81 (3.85)	15.20 (15.29)
6b		$C_{19}H_{16}N_4O_2S_2$	188–190	76	57.61 (57.56)	4.09 (4.07)	14.20 (14.13)
6c		$C_{18}H_{13}ClN_4OS_2$	202–204	79	53.92 (53.93)	3.29 (3.27)	13.91 (13.97)
6d		$C_{18}H_{12}Cl_2N_4OS_2$	170–172	80	49.56 (49.66)	2.76 (2.78)	12.88 (12.87)
7a		$C_{19}H_{16}N_4O_2S_2$	164–166	73	58.61 (58.56)	4.02 (4.07)	14.18 (14.13)
7b		$C_{20}H_{18}N_4O_2S_2$	166–168	73	58.57 (58.52)	4.43 (4.42)	13.69 (13.65)
7c		$C_{19}H_{15}ClN_4O_2S_2$	172–174	78	52.98 (52.96)	3.57 (3.51)	13.06 (13.00)
7d		$C_{19}H_{14}Cl_2N_4O_2S_2$	154–156	82	49.08 (49.04)	3.02 (3.03)	12.07 (12.04)

in each disc was added to each bottle, which contained 100 discs. The discs of each concentration were placed separately in nutrient agar medium inoculated with fresh bacterial strains. The plates were incubated at 37°C for 24 h. Ciprofloxacin was used as the standard drug. Ciprofloxacin has an inhibition length of 19–29 mm for *S. aureus*, 25–33 mm for *P. aeruginosa*, 20–25 mm for *K. pneumoniae*, and 18–26 mm for *E. coli* at 10 μg/mL concentration. Solvent and growth controls

TABLE II Antibacterial Activity of the Newly Synthesized Compounds (6a–d) and (7a–d)

	Diameter of the inhibition zone (in mm) (MIC)*			
Compound no.	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
6a	12 (25)	10 (12.5)	10 (12.5)	10 (25)
6b	12 (25)	10 (12.5)	10 (12.5)	10 (25)
6c	15 (12.5)	20 (12.5)	15 (6)	13 (12.5)
6d	15 (12.5)	20 (6)	18 (6)	16 (12.5)
7a	10 (25)	13 (12.5)	10 (12.5)	12 (25)
7b	12 (25)	12 (12.5)	10 (12.5)	10 (25)
7c	18 (12.5)	22 (6)	20 (6)	17 (12.5)
7d	16 (12.5)	24 (6)	19 (6)	17 (12.5)
Standard (Ciprofloxacin)	**19–29** **(12.5)**	**25–33** **(6)**	**20–25** **(6)**	**18–26** **(12.5)**

12 mm or less: resistant or no inhibition.

13–17 mm, intermediate or moderate inhibition.

18 mm or more, sensitive or maximum inhibition.

*The values within the parentheses indicate MIC, which is defined as the lowest concentration of an antibacterial that considerably inhibits growth of the organism as visually detected.

were kept separately and the diameter of the inhibition zone, and MIC were noted. The results of such studies are given in Table II.

Antifungal Activity

The newly synthesized 7-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-*4H*-1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazin-4-ones (**6a–d**) and (**7a–d**) were screened for their antifungal activity against *Aspergillus flavus* (NCIM No. 524), *Aspergillus fumigatus* (NCIM No. 902), *Candida albicans* (NCIM No. 3100), and *Penicillium marneffei* (recultured) in DMSO by the serial plate dilution method.[23–25] Sabourands agar media (prepared by dissolving peptone [1 g], D-glucose [4 g] and agar [2 g] in distilled water [100 mL] and adjusting the pH to 5.7) was used as the medium for fungal growth. Normal saline was used to make the spore

TABLE III Antifungal Activity of Newly Synthesized Compounds (6a–d) and (7a–d)

	Diameter of the inhibition zone (in mm) (MIC)*			
Compound no.	*A. fumigatus*	*A. flavus*	*C. albicans*	*P. marneffei*
6a	10	10	10	10
	(12.5)	(25)	(12.5)	(12.5)
6b	12	10	10	10
	(12.5)	(25)	(12.5)	(12.5)
6c	20	15	17	15
	(6)	(12.5)	(6)	(6)
6d	20	16	17	18
	(6)	(12.5)	(6)	(6)
7a	10	10	10	10
	(12.5)	(25)	(12.5)	(12.5)
7b	10	10	10	10
	(12.5)	(25)	(12.5)	(12.5)
7c	22	16	18	20
	(6)	(12.5)	(6)	(6)
7d	20	18	19	19
	(6)	(12.5)	(6)	(6)
Standard	**22–30**	**18–26**	**20–25**	**20–25**
(Ciclopiroxolamine)	**(6)**	**(12.5)**	**(6)**	**(6)**

12 mm or less, resistant or no inhibition.

13–17 mm, intermediate or moderate inhibition.

18 mm or more, sensitive or maximum inhibition.

*The values within the parentheses indicate MIC, which is defined as the lowest concentration of an antifungal that considerably inhibits growth of the organism as visually detected.

suspension of the fungal strains (i.e., a loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of the corresponding species). Then 20 mL of the previously discussed prepared agar media was poured into each of the petridishes. Excess media was decanted, and the plates were dried by placing them in an incubator at 37°C for 1 h. Wells were made on these seeded agar plates using an agar punch. A 10 μg/mL solution of the test compounds in DMSO was then added into each of these labeled wells. A control was also prepared in the same way using DMSO. The petridishes were then incubated at 37°C for 3–4 days. Ciclopiroxolamine was used as the standard drug. Ciclopiroxolamine has an inhibition length of 22–30 mm for *A. fumigatus*, 18–26 mm for *A. flavus*, 20–25 mm each for *C. albicans* and *P. marneffei* at 10 μg/mL concentration. The antifungal activity was determined by measuring the diameter of the inhibition zone and the MIC. The results of such studies are given in Table III.

EXPERIMENTAL

Melting points were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AMX-400 (400 MHz) spectrophotometer using TMS as an internal standard. Mass spectra were recorded on a Jeol JMS-D 300 spectrometer operating at 70 eV. The purity of the compounds was checked by TLC on a silica gel plate using n-hexane and ethyl acetate (4:1, v/v).

Procedure for the Preparation of 4-(4-Methylthiobenzylidene)-2-methyl-oxazol-5-one (3)

A mixture of acetyl glycine **1** (11.70 g, 100 mmol), 4-methylthiobenzaldehyde **2** (15.20 g, 100 mmol), anhydrous sodium acetate (4.1 g, 50 mmol), and 95% acetic anhydride (25 mL) were heated on a water bath for 3 h. The reaction mixture was then allowed to cool and left overnight in a refrigerator. The solid mass thus obtained was filtered, washed with cold water, and dried. It was then recrystallized from ethanol. Yield 85%, m.p. 150–152°C.

Procedure for the Preparation of 4-Amino-6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(*4H*)-one (5)

To a solution of thiocarbohydrazide **4** (11.65 g, 50 mmol) in warm water, a solution of **3** (5.3 g, 50 mmol) in ethanol was added dropwise with stirring. The resulting reaction mixture was then refluxed for 3 h and allowed to cool to r.t. The solid thus obtained was filtered, washed thoroughly with water, and dried. It was then recrystallized from ethanol to obtain the pure triazinone. Yield: 81%, m.p. 206–208°C.

General Procedure for the Preparation of 7-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-4*H*-1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazin-4-ones (6a–d) and (7a–d)

A mixture of triazinone **5** (2.80 g, 10 mmol), substituted benzoic acids/aryloxyacetic acids (10 mmol), and phosphorus oxychloride

(10 mL) was heated on a water bath for 8 h. Excess of phosphorus oxychloride was removed under reduced pressure. The resulting reaction mass was cooled and poured into crushed ice with vigorous stirring. The solid product thus obtained was filtered and washed thoroughly with diluted sodium bicarbonate solution to remove any unreacted acid left. It was then washed well with cold water, dried, and recrystallized from ethanol. The characterization data of these compounds are given in the Table I.

6b: IR (KBr, cm^{-1}): 2919 (Aliph.CH), 1701 (C=O), 1589 (C=N), ^{1}H-NMR ($CDCl_3$) δ: 2.48 (s, 3H, SCH_3), 3.91 (s, 3H, OCH_3), 4.28 (s, 2H, CH_2) 7.02 (d, 2H, J = 8.52 Hz, 4-methoxyphenyl), 7.21 (d, 2H, J = 8.36Hz, 4-methylthiophenyl), 7.42 (d, 2H, J = 8.32 Hz, 4-methylthiophenyl), 7.89 (d, 2H, J = 8.56 Hz, 4-methoxyphenyl), MS (m/z, %): 397(M^++ 1, 80), 396(M^+, 40) 391(50), 369(10), 307(70), 289(30), 273(5), 167(5), 154(100), 137(70), 136(70), 120(10), 107(20), 105(5)

6c: IR (KBr, cm^{-1}): 2918 (Aliph.CH), 1703 (C=O), 1593 (C=N), 833 (C—Cl), ^{1}H-NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 4.27 (s, 2H, CH_2) 7.19 (d, 2H, J = 8.28 Hz, 4-methylthiophenyl), 7.39 (d, 2H, J = 8.28 Hz, 4-methylthiophenyl), 7.53 (d, 2H, J = 8.64 Hz, 4-chlorophenyl), 7.88 (d, 2H, J = 8.60 Hz, 4-chlorophenyl), MS (m/z, %): 401(M^++ 1, 20), 399(M^+, 20) 391(30), 308(10), 307(50), 289(25), 279(5), 242(5), 209(5), 178(5), 167(10), 154(100), 149(30), 137(65), 136(70), 120(10), 107(20), 90(10), 88(10).

7b: IR (KBr, cm^{-1}): 2917, 2859 (Aliph.CH), 1710 (C=O), 1589 (C=N), 1064 (C-O), ^{1}H-NMR ($CDCl_3$) δ: 2.30 (s, 3H, CH_3), 2.44 (s, 3H, SCH_3), 4.26 (s, 2H, CH_2), 5.33 (s, 2H, OCH_2), 6.86 (d, 2H, J = 8.60 Hz, 4-methylphenoxy), 7.12 (d, 2H, J = 8.36 Hz, 4-methylphenoxy), 7.18 (d, 2H, J = 8.28 Hz, 4-methylthiophenyl), 7.38 (d, 2H, J = 8.24 Hz, 4-methylthiophenyl), MS (m/z, %): 412(M^++ 2, 15), 410(M^+, 30), 391(5), 307(30), 289(20), 279(5), 242(5), 180(2), 165(5), 154(100), 137(55), 136(60), 120(10), 107(15), 88(10), 76(5).

7d: IR (KBr, cm^{-1}): 2921, 2852 (Aliph.CH), 1709 (C=O), 1588 (C=N), 1071 (C—O), 800 (C—Cl), ^{1}H-NMR ($CDCl_3$) δ: 2.43 (s, 3H, SCH_3), 4.25 (s, 2H, CH_2), 5.38 (s, 2H, OCH_2), 6.93 (d, 1H, J_o= 8.76 Hz, 2,4-dichlorophenoxy), 7.17 (d, 2H, J = 8.24 Hz, 4-methylthiophenyl), 7.23 (dd, 1H, $J_{o/m}$ = 8.76 Hz, 2.44 Hz, 2,4-dichlorophenoxy) 7.36 (d, 2H, J = 8.20 Hz, 4-methylthiophenyl), 7.44 (d, 1H, J_m = 2.44 Hz, 2,4-dichlorophenoxy), MS (m/z, %): 467(M^++ 2, 40), 465(M^+, 60), 391(2), 307(25), 304(20), 289(25), 234(10), 209(2), 165(10), 154(100), 137(55), 136(70), 120(10), 107(25), 91(10), 89(15), 77(10).

CONCLUSION

Some new 7-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-*4H*-1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazin-4-ones (**6a–d**) and (**7a–d**) were synthesized and screened for their antibacterial and antifungal activity. The antimicrobial study revealed that compounds **7c** and **7d** showed excellent antibacterial and antifungal activity against all the tested organisms. This enhanced activity could be attributed to the presence of potential 1,2,4-triazine functionality along with aryloxymethyl moiety carrying one or more chlorine atoms.

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