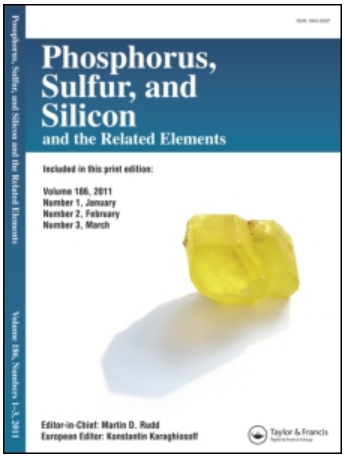

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Synthesis and Antimicrobial Evaluation of Some New Thiadiazinotriazinones Carrying 4-Methylthiobenzyl Moiety

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Synthesis and Antimicrobial Evaluation of Some New Thiadiazinotriazinones Carrying 4-Methylthiobenzyl Moiety

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*A series of 8*H*-7-(substituted aryl)-3-(4-methylthiobenzyl)-1,3,4-thiadiazino[2,3-*c*]-1,2,4-triazin-4-ones (**4**) were synthesized in good yield by condensing 4-amino-6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(4*H*)-one **1** with various substituted phenacyl bromides (**2**) in presence of anhydrous sodium acetate, followed by the cyclization of the intermediate triazinone derivatives (**3**) in presence of concentrated sulphuric acid. The structures of the newly synthesized compounds were formulated on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral studies. The newly synthesized compounds were tested for their in vitro antibacterial and antifungal activity against a variety of microorganisms.*

Keywords 1,2,4-triazin-5(4*H*)-one; 1,3,4-thiadiazine; 4-methylthiobenzyl; antimicrobial

INTRODUCTION

1,2,4-Triazines and their derivatives are important biological agents and a significant amount of research activity has been directed towards 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] The 1,3,4-thiadiazine nucleus is known to be pharmacologically[10] important and

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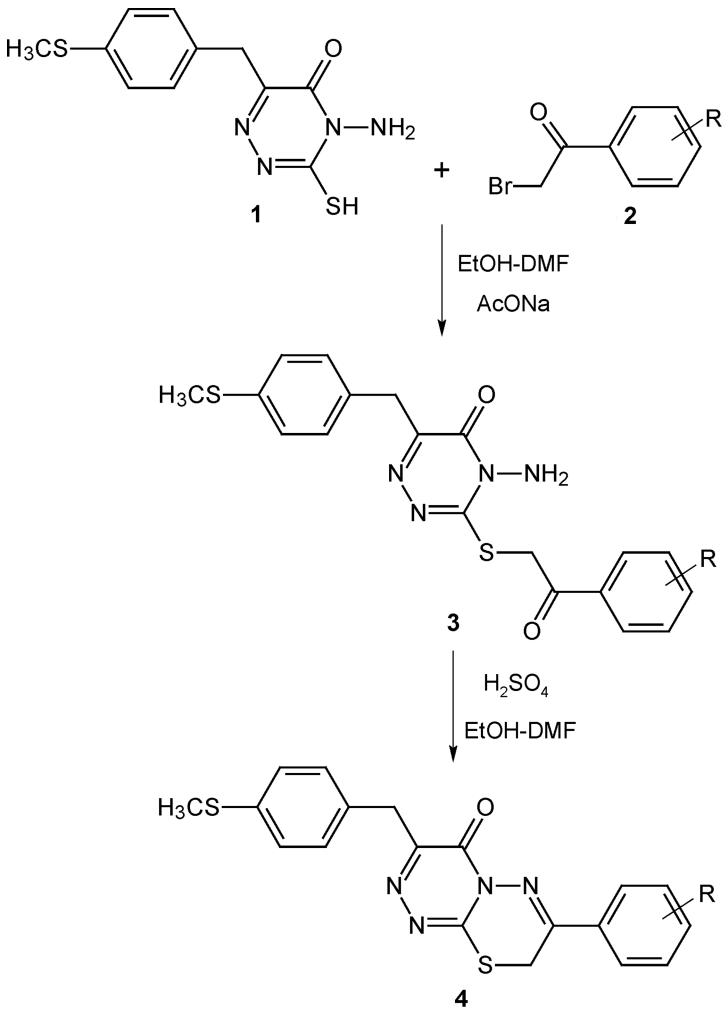
possess antiplatelet and antithrombotic properties.[11]The presence of 4-methylthiophenyl moiety is found to increase the biological activity of the molecules. We have recently reported a few heterocyclic analogues carrying 4-methylthiophenyl moiety as potent antimicrobial agents.[12,13] Prompted by the chemotherapeutic importance of 1,2,4-triazine derivatives and in a view to synthesize bioactive molecules, we have herein synthesized some new 8*H*-7-(substituted aryl)-3-(4-methylthiobenzyl)-1,3,4-thiadiazino[2,3-*c*]-1,2,4-triazin-4-ones and studied their antibacterial and antifungal properties.

RESULTS AND DISCUSSION

4-Amino-6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(4*H*)-one (**1**) was synthesized and reported by us, recently.[14] In the present article, 4-amino-6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(4*H*)-one (**1**) is condensed with various substituted phenacyl bromides (**2**) in presence of anhydrous sodium acetate to afford a series of 4-amino-6-(4-methylthiobenzyl)-3-*s*-(substituted aryl)-1,2,4-triazin-5(4*H*)-ones (**3**). The compounds (**3**) are further cyclized in presence of concentrated sulphuric acid to give a series of 8*H*-7-(substituted aryl)-3-(4-methylthiobenzyl)-1,3,4-thiadiazino[2,3-*c*]-1,2,4-triazin-4-ones (**4**) (Scheme 1). Some of the newly synthesized compounds are well characterized by their elemental analysis, IR, ^{1}H NMR and mass spectral studies. The results of such studies are discussed herein.

The IR spectra of the uncyclized products (**3a–h**) showed the characteristic free NH_2 absorption peaks. Their ^{1}H NMR spectrum also showed the presence of a broad singlet corresponding to the free NH_2 group. The IR spectrum of (**3a**) showed the characteristic NH_2 group stretchings at 3324 cm^{-1} and 3186 cm^{-1}. Two absorption bands at 1670 cm^{-1} and 1582 cm^{-1} are due to C=O and C=N functional groups, respectively. The ^{1}H NMR spectrum of (**3a**) showed a sharp singlet at δ 2.44 which is due to the SCH_3 protons. Two singlets at δ 4.06 and δ 4.76 integrating for two protons each are due to the CH_2 and SCH_2 protons, respectively. A characteristic broad singlet at δ 4.87 integrating for two protons is due to the NH_2 protons. The aromatic protons resonated as a multiplet at δ 7.18–8.08 which integrated for nine protons. The mass spectrum of (**3a**) showed a molecular ion peak (M^+) at m/z 398 in accordance with its molecular formula $C_{19}H_{18}N_4O_2S_2$.

The IR spectra of the cyclized products (**4a–h**) showed the absence of absorption bands corresponding to the free NH_2 group which is involved in the cyclization process. Their ^{1}H NMR spectra also showed the absence of a broad singlet corresponding to the free NH_2 group, thereby

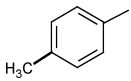
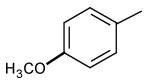
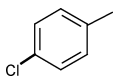
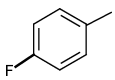
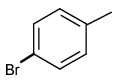
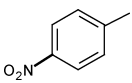
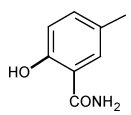


R = H, 4-CH_3, 4-OCH_3, 4-Cl, 4-F, 4-Br, 4-NO_2, 4-OH-3-$CONH_2$

SCHEME 1

confirming the cyclization. The IR spectrum of (**4a**) showed characteristic absorption bands at 1693 cm^{-1} and 1594 cm^{-1}, which are due to the C=O and C=N functional groups, respectively. The ^1H NMR spectrum of (**4a**) showed a characteristic singlet at δ 2.44 integrating for three protons which is due to the SCH_3 protons. Two singlets at δ 3.89 and δ 4.17 integrating for two protons each are due to the CH_2 and SCH_2 protons, respectively. The nine protons of the aromatic ring appeared as

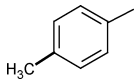
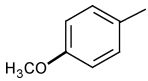
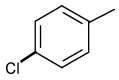
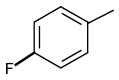
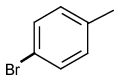
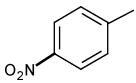
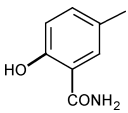
TABLE I Characterization Data of Compounds (3a–h)

Compd no.	R	Molecular formula	M.P. (°C)	Yield (%)	Elemental analysis found (calc.) C	H	N
3a		$C_{19}H_{18}N_4O_2S_2$	174–76	82	57.29 (57.27)	4.58 (4.55)	14.00 (14.06)
3b		$C_{20}H_{20}N_4O_2S_2$	178–80	80	58.21 (58.23)	4.92 (4.89)	13.52 (13.58)
3c		$C_{20}H_{20}N_4O_3S_2$	182–84	82	56.02 (56.06)	4.72 (4.70)	13.06 (13.07)
3d		$C_{19}H_{17}ClN_4O_2S_2$	168–70	86	52.76 (52.71)	3.92 (3.96)	12.98 (12.94)
3e		$C_{19}H_{17}FN_4O_2S_2$	174–76	86	59.92 (54.91)	4.09 (4.11)	13.48 (13.45)
3f		$C_{19}H_{17}BrN_4O_2S_2$	166–68	88	47.87 (47.80)	3.52 (3.59)	11.76 (11.74)
3g		$C_{19}H_{17}N_5O_4S_2$	146–48	84	51.48 (51.46)	3.81 (3.86)	15.76 (15.79)
3h		$C_{20}H_{19}N_5O_4S_2$	222–24	82	52.48 (52.50)	4.22 (4.19)	15.37 (15.31)

a multiplet at δ 7.22–8.12. The mass spectrum of (**4a**) showed a molecular ion peak (M^+) at m/z 380 in accordance with its molecular formula $C_{19}H_{16}N_4OS_2$. The experimental data of all the newly synthesized compounds (**3a–h**) and (**4a–h**) are presented in Table I and Table II. The spectral characterization data of some of the selected compounds have also been discussed in this paper.

An antibacterial and antifungal study carried out for the newly synthesized compounds (**4a–h**) reveals that the compound (**4d**) showed moderate activity against the tested microorganisms. However, the compound (**4e**) and (**4f**) showed the maximum activity against all the tested organisms. The presence of a potential triazino-thiadiazine ring system along with halogen atoms may be the reason for their increased activity. The antibacterial and antifungal activity data of the newly synthesized compounds are given in Tables III and Table IV.

TABLE II Characterization Data of Compounds (4a–h)

Compd no.	R	Molecular formula	M.P. (°C)	Yield (%)	Elemental analysis found (calc.) C	H	N
4a		$C_{19}H_{16}N_4OS_2$	170–72	78	59.90 (59.98)	4.25 (4.24)	14.68 (14.72)
4b		$C_{20}H_{18}N_4OS_2$	164–66	76	60.81 (60.89)	4.65 (4.60)	14.28 (14.20)
4c		$C_{20}H_{18}N_4O_2S_2$	176–78	76	58.57 (58.52)	4.47 (4.42)	13.66 (13.65)
4d		$C_{19}H_{15}ClN_4OS_2$	132–34	82	55.06 (55.00)	3.69 (3.64)	13.48 (13.50)
4e		$C_{19}H_{15}FN_4OS_2$	168–70	80	57.32 (57.27)	3.71 (3.79)	14.10 (14.06)
4f		$C_{19}H_{15}BrN_4OS_2$	152–54	84	49.67 (49.68)	3.22 (3.29)	12.15 (12.20)
4g		$C_{19}H_{15}N_5O_3S_2$	136–38	82	53.68 (53.63)	3.51 (3.55)	16.42 (16.46)
4h		$C_{20}H_{17}N_5O_3S_2$	204–06	78	54.68 (54.66)	3.92 (3.90)	15.97 (15.93)

Antibacterial Activity

We investigated the newly synthesized 8*H*-7-(substituted aryl)-3-(4-methylthiobenzyl)-1,3,4-thiadiazino[2,3-*c*]-1,2,4-triazin-4-ones (**4a–h**) 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.[15–17] 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 bottles and sterilized by dry heat at 140°C for 1 h. The test compounds were prepared in different concentrations using DMF. Exactly 1 mL, containing 100 times the amount of chemical in each disc, was added to each bottle, which contains 100 discs. The discs of each concentration were placed

TABLE III Antibacterial Activity of the Newly Synthesized Compounds (4a–h)

Compound no.	Diameter of the inhibition zone (mm) (minimum inhibitory concentration)*			
	S. aureus	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
4a	10 (25)	10 (12.5)	10 (12.5)	12 (25)
4b	12 (25)	10 (12.5)	10 (12.5)	12 (25)
4c	12 (25)	12 (12.5)	10 (12.5)	12 (25)
4d	16 (12.5)	18 (6)	15 (6)	12 (12.5)
4e	19 (12.5)	22 (6)	18 (6)	17 (12.5)
4f	18 (12.5)	22 (6)	18 (6)	18 (12.5)
4g	10 (25)	12 (12.5)	10 (12.5)	10 (25)
4h	10 (25)	12 (12.5)	10 (12.5)	12 (25)
Standard (Ciprofloxacin)	19–29 (12.5)	25–33 (6)	20–25 (6)	18–26 (12.5)

Standard drug—Ciprofloxacin; 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 minimum inhibitory concentration (MIC), which is defined as the lowest concentration of an antibacterial that considerably inhibits growth of the organism as visually detected.

in nutrient agar medium inoculated with fresh bacterial strains separately. 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 were kept separately, and the diameter of the inhibition zone and minimum inhibitory concentrations (MIC) were noted. The results of such studies are given in the Table III.

Antifungal Activity

The newly synthesized 8*H*-7-(substituted aryl)-3-(4-methylthiobenzyl)-1,3,4-thiadiazino[2,3-*c*]-1,2,4-triazin-4-ones (**4a–h**) 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.[15–17] Sabourauds agar media [prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7] was used as the medium for fungal growth. Normal saline was used to make the spore 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).

TABLE IV Antifungal Activity of the Newly Synthesized Compounds (4a–h)

Compound no.	Diameter of the inhibition zone (mm) (minimum inhibitory concentration)*			
	A. fumigatus	*A. flavus*	*C. albicans*	*P.marneffei*
4a	10 (12.5)	12 (25)	10 (12.5)	10 (12.5)
4b	12 (12.5)	12 (25)	10 (12.5)	10 (12.5)
4c	10 (12.5)	10 (25)	10 (12.5)	12 (12.5)
4d	15 (6)	16 (12.5)	12 (6)	15 (6)
4e	20 (6)	18 (12.5)	16 (6)	19 (6)
4f	22 (6)	18 (12.5)	18 (6)	19 (6)
4g	10 (12.5)	12 (25)	10 (12.5)	12 (12.5)
4h	10 (12.5)	10 (25)	10 (12.5)	12 (12.5)
Standard (Ciclopiroxolamine)	22–30 (6)	18–26 (12.5)	20–25 (6)	20–25 (6)

Standard drug—Ciclopiroxolamine; 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 minimum inhibitory concentration (MIC), which is defined as the lowest concentration of an antifungal that considerably inhibits growth of the organism as visually detected.

Then 20 mL of the above-prepared agar media was poured into each of the petri dishes. 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 petri dishes were then incubated at 37°C for 3 to 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 minimum inhibitory concentrations (MIC). The results of such studies are given in Table IV.

EXPERIMENTAL

The 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. The ^{1}H NMR spectra were recorded on a Bruker AMX-400 (400 MHz) spectrometer using TMS as an internal standard. The mass spectra were recorded on a Jeol JMS-D 300 spectrometer operating at 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plate using n-hexane and ethyl acetate (4:1, v/v).

General Procedure for the Preparation of 4-amino-6-(4-methylthiobenzyl)-3-*s*-(substituted aryl)-1,2,4-triazin-5(4*H*)-ones (3a–h)

A mixture of 4-amino-6-(4-methylthiobenzyl)-3-mercapto-1,2,4-triazin-5(4*H*)-one (**1**) (2.80 g, 10 mmol), substituted phenacyl bromides (**2**) (10 mmol) and anhydrous sodium acetate (10 mmol) in ethanol-DMF mixture were stirred at room temperature for 4 h. The precipitated solid was filtered, washed with water, dried, and recrystallized from ethanol-DMF mixture. The characterization data of these compounds are given in Table I.

3b: IR (KBr, cm^{-1}) : 3167(NH_2), 3068(Ar—H), 2918(C—H), 1670 (C=O), 1589 (C=N), ^{1}H-NMR ($CDCl_3$) δ: 2.44 (s, 3H, CH_3), 2.45 (s, 3H, SCH_3), 4.07 (s, 2H, CH_2), 4.78 (s, 2H, SCH_2), 4.89 (bs, 2H, NH_2), 7.19 (d, 2H, 4-methylthiophenyl), 7.25 (d, 2H, 4-methylphenyl), 7.31 (d, 2H, 4-methylthiophenyl), 7.95 (d, 2H, 4-methylphenyl), MS (m/z, %): 413(M^++1, 100), 412(M^+, 50), 391(20), 367(2), 307(2), 289(3), 279(3), 232(5), 209(2), 165(2), 154(20), 137(15), 136(10), 119(10), 107(5), 105(3), 95(5), 91(10).

3d: IR (KBr) ν/cm^{-1}: 3176(NH_2), 3081(Ar—H), 2915(C—H), 1680(C=O), 1587(C=N), 891(C—Cl),^{1}H-NMR ($CDCl_3$) δ: 2.45 (s, 3H, SCH_3), 4.07 (s, 2H, CH_2), 4.73 (s, 2H, SCH_2), 4.87 (bs, 2H, NH_2), 7.19 (d, 2H, 4-methylthiophenyl), 7.30 (d, 2H, 4-methylthiophenyl), 7.49 (d, 2H, 4-chlorophenyl), 8.00 (d, 2H, 4-chlorophenyl), MS (m/z, %): 435(M^+ + 2, 50), 433(M^+, 80), 391(90), 308(15), 307(60), 289(40), 279(10), 232(15), 167(15), 165(10), 154(100), 149(45), 137(70), 136(80), 120(15), 107(25), 105(10).

3f: IR (KBr) ν/cm^{-1}: 3227(NH_2), 3080(Ar—H), 2917(C—H), 1668(C=O), 1584(C=N), 733(C—Br),^{1}H-NMR ($CDCl_3$) δ: 2.45 (s, 3H, SCH_3), 4.07 (s, 2H, CH_2), 4.72 (s, 2H, SCH_2), 4.87 (bs, 2H, NH_2), 7.20 (d, 2H, 4-methylthiophenyl), 7.30 (d, 2H, 4-methylthiophenyl), 7.66 (d, 2H, 4-bromophenyl), 7.92 (d, 2H, 4-bromophenyl), MS (m/z,

%): 479($M^+ + 2$, 100), 477(M^+, 80), 453(2), 423(5), 404(10), 328(10), 306(10), 267(5), 222(10).

General Procedure for the Preparation of 8*H*-7-(Substituted aryl)-3-(4-methylthiobenzyl)-1,3,4-thiadiazino[2,3-*c*]-1,2,4-triazin-5(4*H*)-ones (4a–h)

A mixture of (**3**) (10 mmol) and concentrated sulphuric acid (4–5 drops) in ethanol-DMF mixture were heated to reflux for 4 h. The resulting reaction mass was cooled to room temperature and poured into crushed ice with vigorous stirring. The precipitated solid was filtered, washed with water, dried, and recrystallized from ethanol. The characterization data of these compounds are given in Table II.

4c: IR (KBr, cm^{-1}) : 3007(Ar—H), 2920(C-H), 1697 (C=O), 1598 (C=N), 1178(C—O), ^{1}H-NMR ($CDCl_3$)δ: 2.45 (s, 3H, SCH_3), 3.86 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2), 4.18 (s, 2H, SCH_2), 6.99 (d, 2H, 4-methoxyphenyl), 7.20 (d, 2H, 4-methylthiophenyl), 7.41 (d, 2H, 4-methylthiophenyl), 7.92 (d, 2H, 4-methoxyphenyl), MS (m/z, %): 411(M^++1, 100), 410(M^+, 20), 391(15), 307(5), 289(5), 248(5), 220(2), 180(2), 176(10), 154(40), 149(25), 137(30), 136(25), 107(10), 95(5).

4d: IR (KBr) ν/cm^{-1}: 3059(Ar-H), 2917(C—H), 1682(C=O), 1596(C=N), 842(C—Cl),^{1}H-NMR ($CDCl_3$) δ: 2.45 (s, 3H, SCH_3), 3.90 (s, 2H, CH_2), 4.17 (s, 2H, SCH_2), 7.19 (d, 2H, 4-methylthiophenyl), 7.38 (d, 2H, 4-methylthiophenyl), 7.45 (d, 2H, 4-chlorophenyl), 7.88 (d, 2H, 4-chlorophenyl), MS (m/z, %): 417(M^+ +2, 50), 415(M^+, 80), 391(90), 307(60), 289(40), 279(10), 232(15), 167(15), 154(100), 149(45), 137(70), 136(80), 120(15), 107(25), 105(10).

4f: IR (KBr) ν/cm^{-1}: 3048(Ar—H), 2921(C—H), 1693(C=O), 1587(C=N), 748(C—Br),^{1}H-NMR ($CDCl_3$)δ: 2.45 (s, 3H, SCH_3), 3.95 (s, 2H, CH_2), 4.15 (s, 2H, SCH_2), 7.22 (d, 2H, 4-methylthiophenyl), 7.39 (d, 2H, 4-methylthiophenyl), 7.64 (d, 2H, 4-bromophenyl), 7.82 (d, 2H, 4-bromophenyl), MS (m/z, %): 461(M^++2, 100), 459(M^+, 95), 429(10), 411(50), 391(60), 381(20), 367(10), 337(5), 323(10), 289(15), 280(30), 279(80), 265(10), 217(25), 205(15), 185(30), 183(30), 163(5), 149(70), 137(15), 136(25), 109(15).

CONCLUSION

Some new 8*H*-7-(substituted aryl)-3-(4-methylthiobenzyl)-1,3,4-thiadiazino[2,3-*c*]-1,2,4-triazin-4-ones (**4a–h**) were synthesized and screened for their antibacterial and antifungal activity. The antimicrobial study revealed that the compounds (**4e**) and (**4f**) showed excellent antibacterial and antifungal activity against all the tested organisms. This enhanced activity could be attributed to the presence

of potential 1,3,4-thiadiazino-1,2,4-triazine nucleus in combination with halogen containing phenyl rings.

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