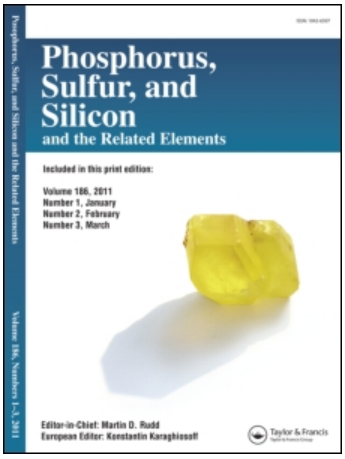

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Mari Sithambaram Karthikeyan[a]; Manjathuru Mahalinga[a]; Prakash Karegoundar[a]; Boja Poojary[a]; Bantwal Shivarama Holla[a]
[a] Department of Chemistry, Mangalore University, Mangalagangothri, India

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Mari Sithambaram Karthikeyan, Manjathuru Mahalinga, Prakash Karegoundar, Boja Poojary, and Bantwal Shivarama Holla
Department of Chemistry, Mangalore University, Mangalagangothri, India

A series of 7-substituted arylidene-1,3-thiazolo[2,3-c]-1,2,4-triazine-4,6-diones were synthesized in an one-pot multicomponent reaction of 6-arylmethyl-3-mercapto-1,2,4-triazin-5-ones with substituted benzaldehydes and monochloroacetic acid in the presence of acetic anhydride, acetic acid, and sodium acetate. The structures of the new compounds were supported by IR, ^1H NMR, MS, and analytical data. All the new compounds were tested for their antibacterial and antifungal activity. Arylidene thiazolo triazinediones with 1,3-benzodioxolo substituent at seventh and p*-chlorophenyl or 2,4-dichlorophenyl substituents at third displayed good antibacterial and antifungal activity. And also compound bearing 2,3,5-trimethoxyphenyl substituent at seventh and 2,4-dichlorophenyl substituent at third displayed good activity.*

Keywords Antibacterial; antifungal activity; arylidene thiazolo triazinediones; one pot; 1,2,4-triazin-5-ones

INTRODUCTION

Triazinones and their condensation products find important applications in the fields of medicine and agriculture.[1] Thioaza-uracils and their thioalkyl derivatives have been reported to possess antidiuretic and neurodepressant activities.[2] Some of the *S*-alkylated mercaptotriazinones such as 4-amino-3-methylthio-6-*tert*-butyl-1,2,4-triazin-5(4*H*)-one (Sencor or Metribuzin, $C_8H_{14}N_4OS$) are used as commercial herbicides.[3–5]

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Address correspondence to (present address) Mari Sithambaram Karthikeyan, Syngene International Limited, Plot No. 2 & 3 C Unit-II, Bommansandra Industrial Area, Bangalore- 560099, India. E-mail: karthims02@rediffmail.com

Thiazolidin-4-one derivatives constitute an important class of heterocyclic compounds. (–)2-(5-Carboxypentyl)thiazolidin-4-one, (i.e., actithiazic acid, $C_9H_{15}NO_3$), which is isolated from *Streptomyces* strains, contains a thiazolidin-4-one ring system and displays highly specific activity against *Mycobacterium tuberculosis*.[6]

Considerable interest has been shown towards the chemistry of the thiazolidin-4-one ring system, which is a key structure in various synthetic pharmaceuticals displaying various biological activities.[7] Thiazolidin-4-one derivatives are known to exhibit diverse bioactivities such as anticonvulsant,[8] antimicrobial,[9] antitubercular,[10] antidiabetic,[11] cycloxygenase inhibitory,[12] Ca^{2+} channel blocker,[13] anticancer,[14] and anti-HIV.[15]

Prompted by these observations, we planned to synthesize a new series of 7-substituted arylidene-1,3-thiazolo[2,3-*c*]-1,2,4-triazine-4,6-diones and to screen them for their in vitro antimicrobial properties. The results of these studies are presented in this article.

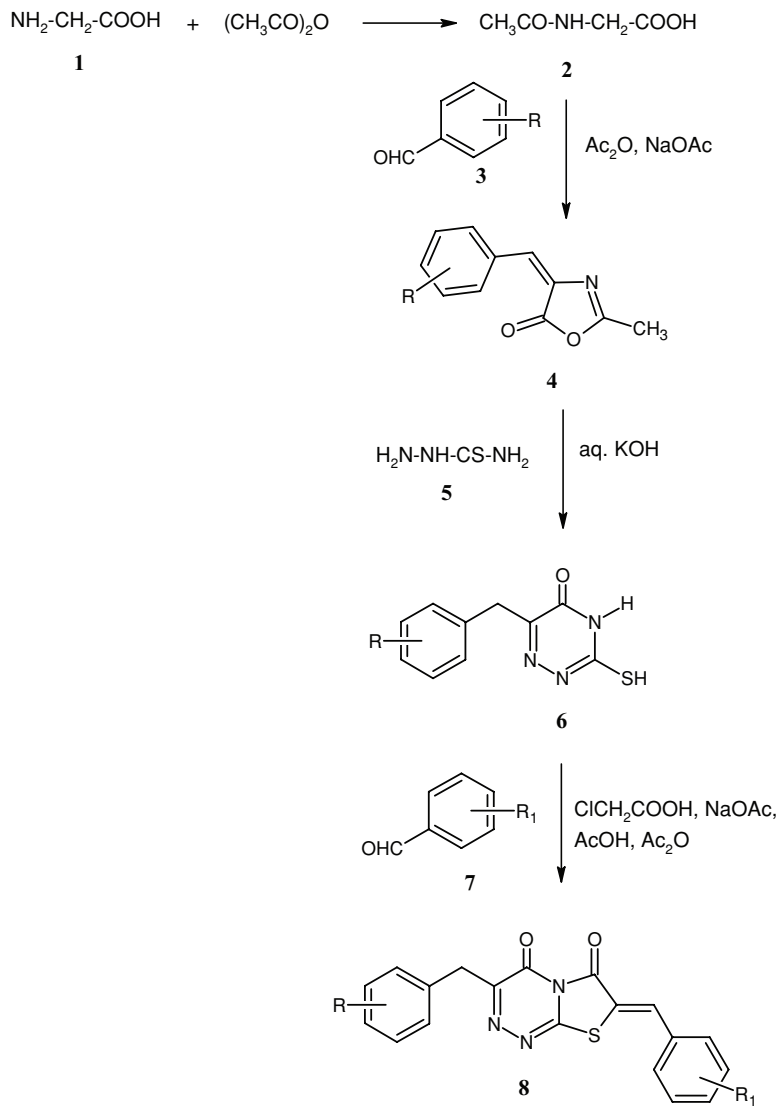
RESULTS AND DISCUSSION

6-Arylmethyl-3-mercapto-1,2,4-triazin-5-ones (**6**) were synthesized according to the reported method.[16] 7-Arylidene-1,3-thiazolo[2,3-*c*]-1,2,4-triazine-4,6-diones (**8**) were obtained by a one-pot reaction of 6-arylmethyl-3-mercapto-1,2,4-triazin-5-ones (**6**) with monochloroacetic acid and substituted benzaldehydes (**7**) in the presence of anhydrous sodium acetate, acetic anhydride, and acetic acid. The reaction sequence is outlined in Scheme 1.

Compound **8b** was taken as representative. The IR spectrum shows an absorption band at 3090 cm^{-1} indicating the presence of Ar-H. Other prominent absorption bands are observed at 1730 cm^{-1}(C = O), 1545 cm^{-1} (C = N), and 821 cm^{-1} (C-Cl).

The 400 MHz ^{1}H NMR spectrum of compound **8b** shows a singlet at $\delta = 3.87$ integrating for two protons, which is attributed to the methylene protons of the *p*-chlorobenzyl group. The protons of the OCH_3 group resonate as a singlet at $\delta = 4.01$. The signal due to the exocyclic vinylic proton appears as a singlet at $\delta = 8.21$. The signal of the aromatic protons of the *p*-anisyl ring are observed as two doublets at $\delta = 7.17$ and 7.46 ($J = 8.7$ Hz). The corresponding signals for the aromatic protons of the *p*-chlorophenyl ring are found as two doublets at $\delta = 7.62$ and 7.76 ($J = 9$ Hz).

Further evidence for the formation of compound **8b** was obtained from its mass spectrum. The mass spectrum of compound **8b** showed a



R = 4-Cl, 2,4-Cl_2; R_1 = 4-OCH_3, 4-Cl, 2,4-Cl_2, 3,4-$(OCH_3)_2$, 3,4-(OCH_2O), 2,4,5$(OCH_3)_3$

SCHEME 1

molecular ion peak at m/z 416 along with (M+2) and (M+4) peaks at m/z 418 and m/z 420 respectively, which is consistent with its molecular formula $C_{20}H_{14}ClN_3O_3S$. Yields and analytical data of arylidene thiazolo triazinediones **8a–l** are given in Table I.

TABLE I Yields and Analytical Data of Arylidene Thiazolo Triazinediones 8a–l

						Analysis (%) Found (Calcd)		
8	R	R_1	Mol. Formula	Mp °C	Yield %	C	H	N
a	4-Cl	4-Cl	$C_{19}H_{11}Cl_2N_3O_2S$	230–32	90	54.48 (54.82)	2.45 (2.64)	9.89 (10.10)
b	4-Cl	4-OCH_3	$C_{20}H_{14}ClN_3O_3S$	219–21	85	58.09 (58.32)	3.33 (3.40)	10.03 (10.21)
c	4-Cl	2,4-Cl_2	$C_{19}H_{10}Cl_3N_3O_2S$	246–48	75	50.34 (50.61)	2.15 (2.22)	9.14 (9.32)
d	4-Cl	3,4-OCH_2O	$C_{20}H_{12}ClN_3O_4S$	210–12	80	56.25 (56.40)	2.68 (2.82)	9.54 (9.87)
e	4-Cl	3,4-$(OCH_3)_2$	$C_{21}H_{16}ClN_3O_4S$	214–16	68	56.81 (57.08)	3.50 (3.62)	9.32 (9.51)
f	4-Cl	2,4,5-$(OCH_3)_3$	$C_{22}H_{18}ClN_3O_5S$	170–72	83	55.64 (55.99)	3.75 (3.82)	8.66 (8.91)
g	2,4-Cl_2	4-Cl	$C_{19}H_{10}Cl_3N_3O_2S$	234–37	84	50.42 (50.61)	2.10 (2.22)	9.18 (9.32)
h	2,4-Cl_2	4-OCH_3	$C_{20}H_{13}Cl_2N_3O_3S$	222–24	88	53.48 (53.81)	2.83 (2.91)	9.21 (9.42)
i	2,4-Cl_2	2,4-Cl_2	$C_{19}H_9Cl_4N_3O_2S$	210–13	77	46.85 (47.01)	1.75 (1.86)	8.47 (8.66)
j	2,4-Cl_2	3,4-OCH_2O	$C_{20}H_{11}Cl_2N_3O_4S$	204–06	78	51.92 (52.17)	2.28 (2.39)	9.02 (9.13)
k	2,4-Cl_2	3,4-$(OCH_3)_2$	$C_{21}H_{15}Cl_2N_3O_4S$	202–04	68	52.68 (52.94)	3.02 (3.15)	8.64 (8.82)
l	2,4-Cl_2	2,3,5-$(OCH_3)_3$	$C_{22}H_{17}Cl_2N_3O_5S$	196–99	82	51.92 (52.17)	3.12 (3.36)	8.11 (8.30)

TABLE II Antibacterial Activity of Arylidene Thiazolo Triazinediones 8a–l

8	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*
a	18 (6.25)	23 (6.25)	29 (6.25)	21 (6.25)	20 (6.25)
b	–	10 (25)	11 (12.5)	13 (12.5)	10 (25)
c	20 (6.25)	–	–	9 (25)	14 (12.5)
d	23 (6.25)	25 (6.25)	30 (6.25)	19 (6.25)	18 (6.25)
e	14 (12.5)	–	10 (25)	15 (12.5)	–
f	–	–	25(6.25)	18 (6.25)	19 (6.25)
g	20 (6.25)	15 (12.5)	27 (6.25)	–	17 (6.25)
h	12 (12.5)	22 (6.25)	–	–	8 (25)
i	21 (6.25)	20 (6.25)	29 (6.25)	20 (6.25)	19 (6.250
j	23 (6.25)	24 (6.25)	27 (6.25)	18 (6.25)	18 (6.25)
k	–	14 (12.5)	12 (12.5)	8 (25)	–
l	23 (6.25)	25(6.25)	28 (6.25)	21 (6.25)	20 (6.25)
Standard	23 (6.25)	25 (6.25)	30 (6.25)	22 (6.25)	20 (6.25)

– Indicates bacteria resistant to the compounds at >100 μg /mL, MIC values are given in brackets. MIC (μg/mL) = minimum inhibitory concentration, i.e., lowest concentration to completely inhibit bacterial growth. Zone of inhibition in mm. Ciprofloxacin was used as standard.

BIOLOGICAL ACTIVITY

Antibacterial Activity

The new compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes*, and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method and microdilution methods.[1718] The results of the antibacterial studies are given in the Table II.

The antibacterial screening data reveal that all tested compounds show moderate to good bacterial inhibition. Compounds **8a**, **8d**, **8g**, **8i**, **8j**, and **8l** were active against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* at 6.25 μg/mL concentrations. Compounds **8a**, **8d**, **8i**, **8j**, and **8l** showed very good activity against all the bacterial strains tested.

Antifungal Activity

The newly synthesized compounds were screened for their antifungal activity against *Aspergillus Flavus* (NCIM No. 524), *Aspergillus fumigatus* (NCIM No. 902), *Candida albicans* (NCIM No. 300), *Penicillium*

TABLE III Antifungal Activity of Arylidene Thiazolo Triazinediones 8a–l

8	*Aspergillus fumigatus*	*Aspergillus flavus*	*Trichophyton mentagrophytes*	*Penicillium marneffei*	*Candida albicans*
A	28 (6.25)	–	15 (12.5)	–	17 (6.25)
B	–	8 (25)	–	14 (12.5)	13 (12.5)
C	–	–	11 (12.5)	8 (25)	–
D	29 (6.25)	27 (6.25)	28 (6.25)	21 (6.25)	18 (6.25)
E	–	14 (12.5)	9 (25)	–	7 (25)
F	26 (6.25)	–	–	10 (25)	–
G	12 (12.5)	29 (6.25)	12 (12.5)	–	10 (25)
H	27 (6.25)	–	25 (6.25)	9 (25)	17 (6.25)
I	–	12 (12.5)	–	12 (12.5)	–
J	26 (6.25)	30 (6.25)	27 (6.25)	20 (6.25)	20 (6.25)
K	–	8 (25)	8 (25)	–	10 (25)
L	27 (6.25)	28 (6.25)	26 (6.25)	22 (6.25)	19 (6.25)
Standard	29 (6.25)	30 (6.25)	28 (6.25)	23 (6.25)	20 (6.25)

– Indicates fungus resistant to the compounds at >100 μg /mL, MIC values are given in brackets. MIC (μg/mL) = minimum inhibitory concentration, i.e., lowest concentration to completely inhibit fungal growth. Zone of inhibition in mm. Griesofluvin was used as standard.

marneffei, and *Trichophyton mentagrophytes* (recultured) in DMSO by the serial plate dilution method.[19,20] The results of the antifungal studies are given in Table III.

The antifungal screening data show moderate to good activity. Compounds **8a**, **8d**, **8j**, and **8l** were active against *A. fumigatus* and *C. albicans*. Compounds **8d**, **8j**, and **8l** were revealed as very active against all the fungal strains tested.

CONCLUSION

The antibacterial screening data reveal that among the 12 compounds screened, five compounds show good bacterial inhibition almost equivalent to that of the standard. The antifungal screening results show that only three of the compounds display good activity. Arylidene thiazolo triazinediones with 1,3-benzodioxolo substituent at seventh with *p*-chlorophenyl and 2,4-dichlorophenyl substituents at third displayed good antibacterial and antifungal activity. In addition, compounds bearing 2,3,5-trimethoxyphenyl substituent at seventh and 2,4-dichlorophenyl substituent at third displayed good activity.

EXPERIMENTAL

Chemistry

Melting points were determined in a Thomas-Hoover melting apparatus by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded with a Shimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded with a Bruker 400 MHz NMR spectrometer using TMS as internal standard. The mass spectra were recorded with a MASPEC low resolution mass spectrometer operating at 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates using petroleum ether and ethyl acetate (6:4).

Preparation of 6-Arylmethyl-3-mercapto-triazin-5(4*H*)-ones (6)

6-Arylmethyl-3-mercapto-triazin-5-(4H)-ones (**6**) were synthesized according to the reported method.[16]

Preparation of 7-Substituted Arylidene-1,3-thiazolo[2,3-*c*]-1,2,4-triazine-4,6-diones (8): General Procedure

A mixture of the appropriate mercaptotriazinone **6**, (0.01 mol), monochloroacetic acid (0.015 mol), anhydrous sodium acetate (0.002 mol), glacial acetic acid (20 mL), acetic anhydride (15 mL), and the substituted benzaldehyde **7**, (0.01 mol) was heated to reflux for 5 h. The reaction mixture was cooled and poured into crushed ice with vigorous stirring. The solid obtained was filtered, washed with excess of water, dried, and recrystallized from a mixture of ethanol and dimethylformamide (2:1).

8a: IR (KBr) ν/cm^{-1}: 3089 (Ar-H), 1735 (C = O), 1535 (C = N); ^{1}H NMR (400 MHz, $CDCl_3$) : $\delta = 4.01$ (s, 2H, CH_2), 7.45 (d, $J = 8.2$Hz, 2H, Ar-H), 7.60 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.66 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.79 (d, $J = 8.2$ Hz, 2H, Ar-H), 8.25 (s, 1H, CH); MS (m/z,%): 412 (M^+, 20).

8c: IR (KBr) ν/cm^{-1}: 3092 (Ar-H), 1740 (C = O), 1527 (C = N), 812 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) : $\delta = 4.05$ (s, 2H, CH_2), 7.32- 7.39 (m, 4H, Ar-H), 7.65–7.73 (m, 4H, Ar-H), 8.20 (s, 1H, CH); MS (m/z,%): 451 (M+1, 25).

8d: IR (KBr) ν/cm^{-1}: 3098 (Ar-H), 1753 (C = O), 1537 (C = N), 792 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) : $\delta = 4.00$ (s, 2H, CH_2), 6.18 (s, 2H, OCH_2O), 7.15–7.62 (m, 6H, Ar-H), 8.16 (s, 1H, CH); MS (m/z,%): 426 (M+1, 35).

8f: IR (KBr) ν/cm^{-1}: 3082 (Ar-H), 1745 (C = O), 1528 (C = N), 787 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 3.89 (s, 2H, CH_2), 3.91–4.05 (3s, 3H, OCH_3), 7.15 (s, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.61 (d, J = 8.5 Hz, 2H, Ar-H), 7.87 (d, J = 8.5 Hz, 2H, Ar-H), 8.36 (s, 1H, CH); MS (m/z,%): 471 (M+1, 27).

8g: IR (KBr) ν/cm^{-1}: 3090 (Ar-H), 1731 (C = O), 1536 (C = N), 732 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 4.14 (s, 2H, CH_2), 7.58 (d, J = 8.4 Hz, 2H, Ar-H), 7.79 (d, J = 8.4 Hz 2H, Ar-H), 7.65–7.67 (m, 2H, Ar-H) 7.41–7.46 (m, 2H, Ar-H), 8.20 (s,1H, CH); MS (m/z,%): 486 (M+1, 31).

8h: IR (KBr) ν/cm^{-1}: 3085 (Ar-H), 1740 (C = O), 1545 (C = N), 792 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 3.87 (s, 2H, CH_2), 4.13 (s, 3H, OCH_3), 7.58 (d, J = 8.5 Hz, 2H, Ar-H), 7.74 (d, J = 8.5Hz, 2H, Ar-H), 7.70 (m, J = 1.4Hz, 1H, Ar-H) 7.40–7.46 (m, 2H, Ar-H), 8.18 (s, 1H, CH).

8i: IR (KBr) ν/cm^{-1}: 3090 (Ar-H), 1745 (C = O), 1529 (C = N), 810 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 3.95 (s, 2H, CH_2), 7.32–7.39 (m, 4H, Ar-H), 7.61–7.67 (m, 2H, Ar-H), 8.37 (s, 1H, CH); MS (m/z,%): 485 (M+1, 15).

8k: IR (KBr) ν/cm^{-1}: 3099 (Ar-H), 1715 (C = O), 1527 (C = N), 721 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 3.93 (s, 2H, CH_2), 4.12 (s, 3H, OCH_3), 4.14 (s, 3H, OCH_3), 7.06 (s, 1H, Ar-H), 7.42–7.70 (m, 5H, Ar-H), 8.15 (s,1H, CH); MS (m/z,%): 477 (M+1, 20).

Microbiological Assay

Antibacterial Assay

Discs measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 °C for 1 h. The test compounds were prepared with different concentrations using dimethylformamide. 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 in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Ciprofloxacin was used as a standard drug. Solvent and growth controls were kept and zones of inhibition and minimum inhibitory concentrations (MIC) were noted.

Antifungal Assay

Sabourands agar medium was 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. Normal saline was used to make a suspension of spores of the fungal strain for lawning. A loopful of a particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 mL of agar medium was poured into each petric dish. Excess of suspension was decanted, and the plates were dried by placing in an incubator at 37°C for 1 h. Each well was labeled. A control was also prepared in triplicate and kept at 37°C for 3–4 days. Zone of inhibition and minimum inhibitory concentration (MIC) were noted. The activity of each compound was compared with Griesofluvin as standard drug.

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