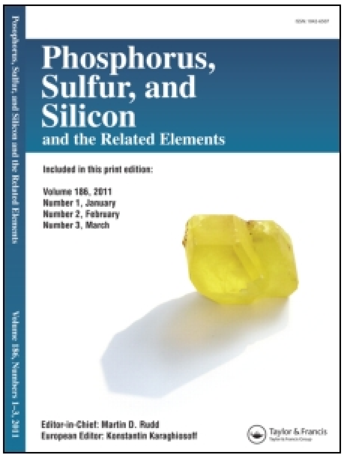

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Synthesis of Some Thiadiazolotriazinone Derivatives as Possible Antimicrobial Agents

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Synthesis of Some Thiadiazolotriazinone Derivatives as Possible Antimicrobial Agents

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A series of 7-substituted -3-t-butyl 4(H)-1,3,4-thiadiazolo(2,3-c)-1,2,4-triazine-4-one **(3)** *have been synthesized by condensing 4-amino-6-t-butyl-3-mercapto-1,2,4-triazin-5(4H)-one* **(1)** *with substituted Arylfuroic acids* **(2)** *using* $POCl_3$ *as a cyclizing agent. The newly synthesized compounds are characterized by elemental analysis, IR,* ^1H *NMR, and mass spectral studies. The compounds were screened for their antibacterial and antifungal activities. Some of the compounds showed promising antibacterial and antifungal activity.*

Keywords Antimicrobial; Aryl furoic acids; phosphorus oxychloride; thiadiazolotriazinones

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INTRODUCTION

Triazinones and their condensation products find important applications in the field of medicine and agriculture.[1] Many such compounds exhibit antidiuretic and neuro-depressent activities.[2] Mannich bases derived from substituted triazinones find a significant place in the field of pharmaceutical chemistry[3] Some of the S-alkylated mercapto triazinones exhibit herbicidal activities.[4] Among such derivatives Sencor[5] (4-amino-3-methylthio-6-t-butyl-1,2,4-triazin-5-(4*H*)-one) is being used as a commercial herbicide for the control of weeds in potato crops. Heterocyclic compounds containing substituted arylfuran moiety are found to exhibit diverse biological activities such as antimalarial, and antiviral, antifungal, antibacterial, and anticancer activities.[6–12] A number of thiadiazole derivatives possess antibacterial properties comparable with those of sulfonamide drugs.[13] Subsequently, thiadiazole derivatives have found applications as anti-tumor agents and pesticides.[14–17]

literature survey revealed that only a few thiadiazolotriazinones containing arylfuran moiety are reported. Prompted by the varied biological activities of 1,2,4 triazinones and 1,3,4 thiadiazoles and in continuation of our work on the synthesis of N- bridged heterocycles derived from 1,3,4 triazinones, a series of 7-substituted -3-t-butyl 4(*H*)-1,3,4-thiadiazolo(2,3-c)-1,2,4-triazine-4-one **(3)** have been synthesized and screened for their antibacterial and antifungal activities.

Materials and Methods

For the present work different aryl furoic acids have been synthesized according to the procedure described in the literature.[18] (Here we made use of of meerweins reaction[19] where a copper ion would be the catalyst.) Thus the Aryl furoic acids **(1)** obtained by this method were cyclized with commercially obtained Sencor compound, i.e., 4-amino-6-t-butyl-3-methylthio-1,2,4-triazine-5(*H*)–ones **(2)** in the presence of $POCl_3$ at 80°C. The products thiadiazolotriazinones were obtained in good yields and were confirmed by elemental analysis and spectral studies.

The characterization data of thiadiazolotriazinones **(3)** are given in Table I.

Results and discussion

The formation of 7-[5-(2,4,5-trichlorophenyl)-2-furyl]-3-t-butyl-4(*H*)-1,3,4-thiadiazolo (2,3-c)-1,2,4-triazin-4-ones **3a** and the formation of 7-(2-trifluoromethyl-5-aryl-2-furyl)-3-t-butyl-4*H*-1,3,4-thiadiazolo (2,3-c)-1,2,4-triazin- 4-ones **3c** were confirmed by elemental analysis,

TABLE I Characterization Data of the Preparation of 7-substituted-3-t-butyl- 4(*H*)-1,3,4-thiadiazolo (2, 3-c)-1,2,4-triazine-4–ones (3)

					Elemental Analysis (%) Found (Calculated)		
Compound	R	Mol. Formula	Yied (%)	M.P (°C)	C	H	N
3a	2,4,5-tri -Cl	$C_{18}H_{13}N_4SO_2Cl_3$	95	237–240	50.80 (50.83)	3.86 (3.83)	23.70 (23.71)
3b	2-NO_2-4 -Cl	$C_{18}H_{14}N_5SO_4Cl$	95	220–224	61.52 (61.52)	4.17 (4.19)	17.93 (17.93)
3c	2-CF_3	$C_{19}H_{15}N_4SO_2F_3$	88	176–180	54.26 (54.28)	3.57 (3.57)	13.30 (13.30)
3d	3-NO_2	$C_{18}H_{15}N_5SO_4$	86	235–236	51.37 (51.36)	2.55 (2.58)	15.96 (15.97)
3e	3,4-Cl_2	$C_{18}H_{14}N_4SO_2Cl_2$	92	205–206	54.18 (54.18)	3.75 (3.74)	14.84 (14.86)
3f	2,5-Cl	$C_{18}H_{14}N_4SO_2Cl_2$	85	192–194	54.18 (54.18)	3.75 (3.74)	14.84 (14.86)
3g	4-MeO-2- Nitro	$C_{19}H_{17}SO_5N_5$	84	210–214	53.39 (52.19)	4.01 (3.98)	16.38 (16.22)
3h	2-Cl-5-CF_3	$C_{19}H_{14}N_4SO_2ClF_3$	91	219–223	50.17 (50.03)	3.14 (2.99)	16.38 (16.28)
3i	4-Me	$C_{19}H_{18}\ SO_2N_4$	87	08–210	62.28 (62.01)	4.95 (4.76)	15.29 (15.07)
3j	4-Meo	$C_{19}H_{18}\ SO_3N_4$	90	218–223	59.67 (59.68)	4.70 (4.71)	14.64 (14.65)
3k	2-NO_2-4- -Me	$C_{19}H_{18}\ SO_4N_5$	85	202–205	55.47 (55.47)	4.36 (4.37)	17.03 17.03
3l	3-CF_3	$C_{19}H_{15}SO_2N_4F_3$	90	183–185	54.26 (54.28)	3.57 (3.57)	13.30 (13.30)

IR, ^{1}H NMR, and mass spectral studies. IR spectrum of compound **3a** showed no absorption band due to $-NH_2$ group; this confirmed the involment of N-NH2 group in the cyclization. The carbonyl stretching frequency was observed at 1699 cm^{-1}. The absorption band due to C-N stretching was observed at 1652 and 1573 cm^{-1}. The absorption band at 785 cm^{-1} and 628 cm^{-1} are due to the presence of chlorine atoms on the benzene ring of the aryl furan moiety. The aromatic C-H proton was observed at 3069 cm^{-1} and t-butyl C-H proton was observed at 2925 cm^{-1}. The characteristic absorption band of the SH group of the parent triazinone **(2)** was absent in the IR spectrum of the cyclized product. Similarly, in compound **3c** the carbonyl stretching frequency appeared at 1697 cm^{-1}. The aromatic C-H, C=N-, and C-F appeared at 3066, 1591, 1542, and at 810 cm^{-1}, respectively. The ^{1}H NMR spectra of compound **3a** showed a singlet at δ 1.44 integrating for nine protons, which were due to t-butyl group. The furan C_3-H signal appeared as a closely spaced doublet at δ 7.58 (J = 4Hz), while the signal due to C_4-H of the furan ring appeared at δ 8.09(J = 4Hz). The aromatic protons of the trichloro phenyl group were observed as two signets at δ 8.09 and 8.20. The ^{1}H NMR spectra of compound **3c** showed a singlet at δ 1.43 integrating for nine protons, which were assigned to t-butyl group. The furan C_3-H signal appeared as a closely spaced doublet at δ 7.25 (J = 3.7Hz), while the signal due to C_4-H of the furan ring appeared at δ 7.80(J = 3.7Hz). The aromatic C_3 and C_6protons appeared as doublet at δ 7.98, C_4 proton appeared as a triplet at δ 7.86, and C_5 proton appeared as triplet at 7.75. The formation of **3a** was also confirmed by recording its mass spectrum. The molecular ion peak m/z appeared at 455, which is in accordance with its molecular formula. Similarly, in **3c** the molecular ion peak m/z appeared at 420, thus confirming the formation of the previously discussed compound.

Antibacterial activity

The newly synthesized compounds **(3a–3l)** were screened for their antibacterial activities against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25922) *Pseudomonas aeruginosa* (ATTC-27853) and *Klebsiella pneumoniae* bacterial stains by the disc diffusion method.[20,21] 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 bottle and sterilized by dry heat at 140°C for an hour. The test compounds were prepared with different concentrations such as $< 10\mu$g/mL and > 10 μg/mL in *N*, *N*dimethyl formamide. One milliliter containing 100 times the amount of chemical in each disc was added to each bottle, which contained 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium separately seeded with fresh bacteria. The incubation was carried out at 37°C for 24 h. Nitrofurazone was used as standard drug at a concentration of 10 μg/mL. Solvent and growth controls and zones of inhibition were noted. The results of such studies are given in Table II.

The antibacterial results indicated that among the tested compounds, **3b**, **3c**, **3f**, **3g**, **3h**, **3j**, and **3l** have shown excellent antibacterial activity against *E. coli*. Compounds **3b**, **3g**, **3h**, **3j**, and **3l** have shown excellent activity against *S. aureus*. Compounds **3b**, **3g**, **3h**, **3j**, **3k**, and **3l** have shown good activity against *P. aeruginosa*. Compounds **3b**, **3c**, **3g**, **3h**, **3j**, and **3k** have shown excellent antibacterial activity against *K. pneumoniae*, and were comparable with the standard drug active against *K. Pneumonia*. The antibacterial activity of rest of the

TABLE II Antibacterial Activity Data of Compounds 3a–3l (MIC Values in μg/mL)

Compound	Escherichia coli	Staphylococcus aureus	Pseudomonas aeruginosa	Klebsiella Pneumoniae
3a	12.5	—	—	12.5
3b	6.25	6.25	6.25	6.25
3c	6.25	—	—	6.25
3d	—	—	—	—
3e	12.5	12.5	12.5	—
3f	6.25	—	12.5	12.5
3g	6.25	6.25	6.25	6.25
3h	6.25	6.25	6.25	6.25
3i	—	—	—	—
3j	6.25	6.5	6.25	6.25
3k	12.5	12.5	6.25	—
3l	6.25	6.25	6.25	6.25
Nitrofurazone	6.25	12.5	12.5	12.5

compounds were comparable with the standard drug. It is noteworthy to mention that **3d** and **3i** were not active against any of the four bacterial stains used. It was concluded that compounds **3b**, **3g**, **3h**, **3j**, and **3l** were found to be promising antibacterial agents.

Antifungal Activity

All newly synthesized compounds **(3a–3l)** were screened for their antifungal activity against *Candida Albicans* (NICM No. 300*), Aspergillus Fumigatus* (NICM No. 902), *Aspergillus Flavus* (NICM No. 524), *and Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method.[22,23] Sabourands agar media was 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. Normal saline was used to make suspension of the spore of fungal strain for lawning. A loopful of particular fungal stain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty ml of agar media was poured into each Petri dish. Excess suspension was decanted, and the plates were dried by being placed in an incubator at 37°C for 1 h. Using an agar punch, wells were made on the seeded agar plates, and, $< 10\mu$g/mL and >10 μg/mL of the test compounds in *N*, *N* dimethyl formamide were added into each well labeled. A control also was prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound

TABLE III Antifungal Activity Data of Compounds 3a–3l, (MIC Valves in μg/mL)

Compound	*Candida albicans*	*Aspergillus fumigatus*	*Trichophyton mentagrophytes*	Aspergillus flavus
3a	—	12.5	12.5	12.5
3b	6.25	6.25	6.25	6.5
3c	6.25	6.25	6.25	6.5
3d	—	—	—	—
3e	12.5	12.5	6.25	12.5
3f	—	—	—	—
3g	6.25	6.25	6.25	6.25
3h	12.5	12.5	6.25	6.25
3i	—	6.25	—	—
3j	6.25	6.25	6.25	6.5
3k	6.25	—	—	6.5
3l	6.25	—	12.5	12.5
Fluconazole (standard)	6.25	12.5	12.5	12.5

was compared with Fluconazole as standard. The minimum inhibitory concentration (MIC) for Fluconazole in DMF was in the range 0.01–16 μg/mL against the tested species. Results of screening studies are given in Table III. Screening studies revealed that compounds **3b**, **3c**, **3g**, **3j**, **3k**, and **3l** have shown excellent antifungal activity against *C. albicans*. Compounds **3b**, **3c**, **3g**, **3i**, and **3j** have shown excellent activity against *A. fumigatus*. Compounds **3b**, **3c**, **3e**, **3g**, and **3j** have shown very good activity against *T. mentagrophytes*. Similarly compounds **3b**, **3c**, **3g**, **3h**, and **3k** have shown very good activity against *A. flavus*. The antifungal activities of the rest of the compounds were comparable with the standard drug. It was concluded that compounds **3b**, **3c**, **3g**, and **3j** were found to be promising antifungal agents.

All these results are based on preliminary tests; more studies are to be conducted to get conclusive results.

EXPERIMENTAL SECTION

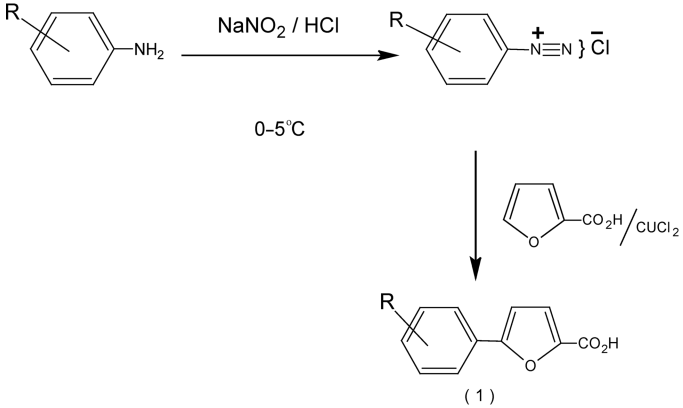
Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Merk (Darmstadt, Germany) of manufacturer at first mention. silica gel 60 F_{254} coated alumina plates. IR spectra were recorded on a SHIMADZU-FTIR infrared spectrometer in KBr (ν_{max} in cm^{-1}). ^{1}HNMR spectra were recorded in DMSO-d_6 on a amx (400 MHz) Spectrometer using TMS as internal standard. FABMS spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using argon/Xenon (6 kv, 10 mA) as the FAB gas. Elemental analysis was recorded on Vario EL, elementar.

Preparation of Substituted aryl furoic acid (1)

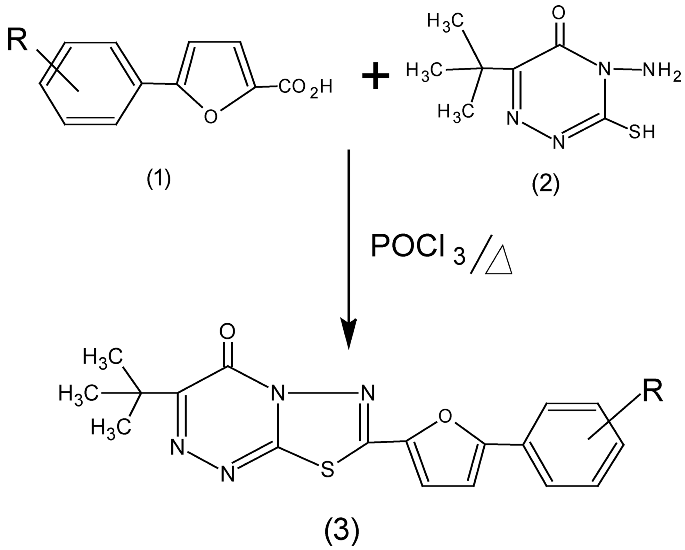
A mixture containing substituted aniline (100 mmol), hydrochloric acid (15%, 60 mL), and water (90 mL), was heated until clear solution was obtained; it was cooled to O°, diazotized with aqueous sodium nitrite (30%, 24 mL), and filtered. To the filtered solution, water (50 mL) and furoic acid (9.6 g, 100 mmol) were added. Aqueous solution of cupric chloride (2.5 g in 10 mL of water) was added dropwise and stirred for 4 h at r.t. and kept aside for 16 h. The resulting solid was filtered off, suspended in water, and purified by steam distillation in the presence of sodium carbonate to give the corresponding aryl furoic acid. These compounds were then recrystallized from a mixture of dimethyl formamide and ethanol.

Preparation of 7-[5-(Substituted-aryl)-2-furyl]-3-t-butyl-4(*H*)-1,3,4-thiadiazolo (2,3-c)-1,2,4-triazine-4-ones (3)

A mixture of triazinone (1 mmol), substituted aryl furoic acid (1.2 mmol), and phosphorus oxy chloride (5 mL) were refluxed on a water bath for about 6 h (Scheme 2). Excess phosphorus oxychloride was removed under reduced pressure, and the reaction mixture was cooled and poured onto crushed ice. The solid product was filtered washed with sodium bicarbonate solution (2%), and followed by distilled water. It was dried and recrystalized from a mixture of ethanol and diaxane. The characterization data of thiadiazolotriazinones (3) prepared according to the method is given in Table I.



SCHEME 1 Synthesis of Aryl furoic acids (1).



SCHEME 2 Synthesis of 7-substituted-3-t-butyl-4(*H*)-1,3,4-thiadiazolo(2,3-c)-1,2,4-triazine-4-one.

3b IR (KBr) ν/cm^{-1}: 1697(**C**=O str), 1649, 1572(C=N str), 780 and 622 (C-Cl str), 3055(Ar-H str), 2893 (t-butyl C-H str), 1518 (NO$_2$ assy str) 1345 (NO$_2$Sym str) ^{1}HNMR (DMSO-d$_6$)δ: 1.43 (s, 9H of t-butyl), 7.8 (d, 1H of furan ring), 7.4 (d, 1H of furan ring), 8.8 (d, 1H of phenyl ring), 8.7 (d, 1H of phenyl ring), 8.96 (d, 1H of phenyl ring), MS(m/z,%): 431 (M$^{+\cdot}$), 403 (M$^+$, 18%), 433 (M+2), 222 (25%), 194 (22%).

3c IR (KBr) ν/cm^{-1}: 1699 (**C**=O str), 1652 and 1591 (C=N str), 810 (C-F str), 2925(t-butyl C-H str), 3069 (Ar-H str). ^{1}HNMR (DMSO-d$_6$)δ: 1.43(s, 9H, t-butyl), 7.25(d, 1H, furan ring C$_3$, j = 3.7Hz), 7.80 (d, 1H, furanC$_4$, J = 3.7Hz), 7.98 (d, C$_3$and C$_4$proton of phenyl ring), 7.82–7.86 (t, 1H, C$_4$ of phenylring) 7.7–7.86 (t, 1H, C$_5$of phenyl ring). MS (m/z, %): 420 (M$^{+\cdot}$), 392 (M$^+$, 40%), 211(20%), 183 (13), 222 (25%).

3e IR (KBr) ν/cm^{-1}: 1699 (**C**=O str), 1658 (C=N str), 785 (C-Cl str), 2890 (t-butyl C-H str), 3028 (Ar-H str). ^{1}HNMR (DMSO-d$_6$)δ: 1.44 (s, 9H, t-butyl), 7.24 (d, 1H of furan ring J = 3.6Hz), 6.91 (d, 1H of furan ring J = 3.6Hz), 7.33–7.36 (m, 2H 3,4-dichloro phenyl), 7.43 (s, 1H of phenyl ring).

3f IR (KBr) ν/cm^{-1}: 1699 (**C**=O str), 1662 (C=N str), 785 (C-Cl str), 2918 (t-butyl C-H str), 3033 (Ar-H str). ^{1}HNMR (DMSO-d$_6$)δ: 1.40(s, 9H, t-butyl), 7.7(d, 1H of furan ring J = 3Hz), 7.31 (d, 1H of furan ring J = 3Hz), 7.63 (d, 1H of phenyl ring J = 3.8Mz), 7.38 (d, 1H of phenyl ring J = 3.8Mz), 7.48 (s, 1H of phenyl ring), MS (m/z,%): 420 ($M^{+\cdot}$), 422(M+2), 424(M+4), 392 (M^+, 47%), 211(24%), 183 (15%).

3g IR (KBr) ν/cm$^-$: 1695 (**C**=O str), 1648 (C=N str), 753 (C-Cl str), 2836 (t-butyl C-H str), 3045 (Ar-H str). 1513 (NO_2 assy str) 1340 (NO_2Sym str), ^{1}HNMR (DMSO-d_6)δ: 1.43 (s, 9H, t-butyl), 7.17 (d, 1H of furan ring J = 3Hz), 6.71(d, 1H of furan ring J = 3Hz), 8.3 (d, 1H phenyl ring, J = 8.5 Hz), 7.9 (d, 1H Phenyl ring, J = 8.5Hz) 7.6 (d, 1H, J = 7Hz, phenyl ring, meta coupling), 3.9 (s, 3H, OCH_3) MS (m/z, %): 427($M^{+\cdot}$), 399 (M^+, 52%), 218(30%), 190 (17%).

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