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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

α,α-Dibromoacetophenones mediated synthesis of some new 7*H*-7-alkoxy-3-alkyl/phenyl-6-aryl-*s*-triazolo[3,4-b][1,3,4] thiadiazines and their antimicrobial evaluation

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Abstract A series of new 7*H*-7-alkoxy-3-alkyl/phenyl-6-aryl-*s*-triazolo[3,4-b][1,3,4]thiadiazines **(3**, **4)** were synthesized by the reaction of various α,α-dibromoacetophenones **1** with 3-alkyl/phenyl-4-amino-5-mercapto-*s*-triazoles (**2**) in different alcoholic solvents in good yields. All the newly synthesized compounds (**3**, **4**) were screened for their in vitro antibacterial and antifungal activity. Biological activities of these compounds were compared with those of the commercially available antibiotic, ciprofloxacin and antifungal agent, amphotericin-B. The title compounds showed good activity against the Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* and the yeasts, *Saccharomyces cerevisiae* and *Candida albicans*.

Keywords α,α-Dibromoacetophenones · 7*H*-7-alkoxy-3-alkyl/phenyl-6-aryl-s-triazolo[3,4-b][1,3,4]thiadiazines · 3-alkyl/phenyl-4-amino-5-mercapto-s-triazoles · Antibacterial activity · Antifungal activity

Introduction

Triazole fused six-membered ring systems have engrossed substantial interest because of their utility in organic chemistry, medicine, agriculture, and industry (Holla *et al.*, 2002; Dandia *et al.*, 2006; Jin *et al.*, 2007a, b; Ammar *et al.*, 2002). Among them, 1,2,4-triazoles fused with thiadiazines, incorporating the N–C–S linkage in their skeleton, constitute a chemical entity of particular interest. The broad biological and pharmacological activities of triazolo thiadiazines have been extensively studied and especially, *s*-triazolo[3,4-b][1,3,4]thiadiazines have been shown to possess a wide spectrum of bioactivities such as antibacterial, antifungal (Almajan *et al.*, 2010; Karabasanagouda *et al.*, 2007; Mohan and Kataria, 1995; Ilhan *et al.*, 1996; Holla *et al.*, 2006a, b; Chadha *et al.*, 1992; Filmwala *et al.*, 2002), anti-inflammatory (Zitouni *et al.*, 2007), anthelmintic (Nadkarni *et al.*, 2001), antitubercular (Laddi *et al.*, 2001), plant-growth regulating (Ding *et al.*, 2010; Jin *et al.*, 2007a, b; Zhou *et al.*, 2007), antiviral (Farghaly *et al.*, 2006; Holla *et al.*, 2001), and anticancer properties (Holla *et al.*, 2006a, b). In view of the importance of triazolo[3,4-b][1,3,4]thiadiazines, our effort in the present study is to identify some new derivatives of this class that may be valuable in designing new, potent, selective, and less toxic antimicrobial agents.

The synthetic utility of α-halocarbonyl compounds in heterocyclic chemistry is well known for more than a century and they have been widely used as versatile intermediates for the synthesis of a variety of heterocyclic systems (Wilhelm and Schmidt, 1969; Garcia *et al.*, 1973; Little and Webber, 1994). However, as the α-haloketones suffer with serious handling problems due to highly lachrymatory properties associated with them, there is an increasing tendency to search a substitute for them. Recently, α,α-dihaloketones are gaining particular interest in view of their convenient preparation, high reactivity, selectivity, and easy handling (Prakash *et al.*, 2007a, b, c; Boeykens and Kimpe, 1994). In addition, α,α-dihaloketones and α-haloketones show analogous behavior in most

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of their reactions and α,α-dihaloketones seem to act as masked α-haloketones (Ahluwalia *et al.*, 1992, 1987; Prakash *et al.*, 2007a, b, c; Prakash and Sharma, 2007). In view of the above facts and in continuation with our ongoing program on exploring the synthetic utility of α, α-dihaloketones, it was thought worthwhile to synthesize fused heterocycles containing 1,2,4-triazole and 1,3,4-thiadiazine moieties using α,α-dibromoacetophenones. In this study, we are extending the application of α,α-dibromoacetophenones in the synthesis of some new 7*H*-7-alkoxy-3-alkyl/phenyl-6-aryl-*s*-triazolo [3,4-b][1,3,4]thiadiazines (**3**, **4**) of potential biological interest.

Chemistry

The present study was started with α,α-dibromoacetophenone **1a** (Ar = Ph) as a model substrate. The reaction of **1a** was carried out with 4-amino-3-ethyl-5-mercapto-*s*-triazole **2a** (R = C_2H_5) in ethanol under reflux conditions. Usual work-up of the reaction afforded the expected product, 7*H*-7-ethoxy-3-ethyl-6-phenyl-*s*-triazolo [3,4-b][1,3,4] thiadiazine **(3a)** in 78% yield. The product **3a** was characterized by the combined application of IR, NMR (^{1}H, ^{13}C) measurements, and elemental analysis. IR spectrum of the product showed the absence of any peak in the C=O or –NH_2 region. ^{1}H NMR spectrum of **3a** showed a characteristic singlet at δ 5.75 for C*H* of the thiadiazine ring. The spectrum also produced a triplet at δ 1.47 and two multiplets at δ 3.62 and 4.03 corresponding to the –OCH_2CH_3 group. The ^{13}C NMR of the product presented all the carbons corresponding to the triazolothiadiazine nucleus and the substituents; δ 11.09 (–$\mathbf{CH_2}CH_3$), 14.46 (–$OCH_2\mathbf{CH_3}$), 18.35 (–$CH_2\mathbf{CH_3}$), 63.93 (–OCH_2–), 73.13 (C_7), 127.09–142.46 (C_6 and aromatic carbons), 150.25 (C_3 of triazole), 155.05 (C_5 of triazole). Structure of the product was further confirmed by mass spectral data and elemental analysis. To assess the generality of the reaction, various α,α-dibromoacetophenones **(1b–1f)** were treated with the triazole **2a** under similar conditions. The reaction, indeed, afforded the corresponding 7-ethoxy triazolothiadiazines **3b–3f** in all the cases in good yields (65–81%) (Scheme 1).

From the results, it is apparent that the reaction followed a similar trend by changing the α,α-dibromo substrate **1**. In order to extend scope of the study, it was considered worthwhile to study the effect of changing the (i) solvent and (ii) triazole on the reaction.

Accordingly, several experiments were carried out on the substrate α,α-dibromoacetophenone **1a** using the solvents MeOH, *n*-PrOH, *i*-PrOH, *n*-BuOH, *i*-BuOH, and CH_3CN. The reaction did not follow a similar trend in all the cases. However, one of the two products obtained with solvents MeOH and *n*-PrOH are similar to those reported in literature (Bala *et al.*, 1978). Using MeOH and *n*-PrOH as a solvent in this reaction, a mixture of two products, namely 7*H*-7-alkoxy-6-phenyl-3-ethyl-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**4a** or **4e)** and 7*H*-3-ethyl-6-phenyl-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**5a)** (Bala *et al.*, 1978) was obtained. With *i*-PrOH, the 7-isopropoxy compound, 7*H*-6-aryl-3-ethyl-7-isopropoxy-*s*-triazolo[3,4-b][1,3,4]thiadiazines (**3i)** was obtained as the sole product. With solvents *n*-BuOH and *i*-BuOH, only the 7-unsubstituted product, i.e., the compound **5a** was obtained. Also, by using CH_3CN in place of alcohol, 7-unsubstituted thiadiazine **5a** was the only product.

All these reactions in different solvents were also repeated with the substrate α,α-dibromo-4-chloroacetophenone **(1b)**. Similar results were obtained in all the cases as with the substrate **1a**.

Furthermore, the reaction of α,α-dibromoacetophenone **1a** was studied with various 3-substituted triazoles **2** (**b**; R = H, **c**; R = *n*-Pr and **d**; R = C_6H_5). Similar results were obtained with triazoles **2b** and **2c** as with the triazole **2a** in various alcoholic solvents (MeOH, EtOH, *n*-PrOH, and *i*-PrOH), a single product (7-alkoxy) was obtained in EtOH and *i*-PrOH, while a mixture of two products (7-alkoxy and 7-unsubstituted) was obtained in MeOH and *n*-PrOH. However, with the triazole **2d**, the reaction proceeded differently and a mixture of two products was obtained in all the alcoholic solvents used.

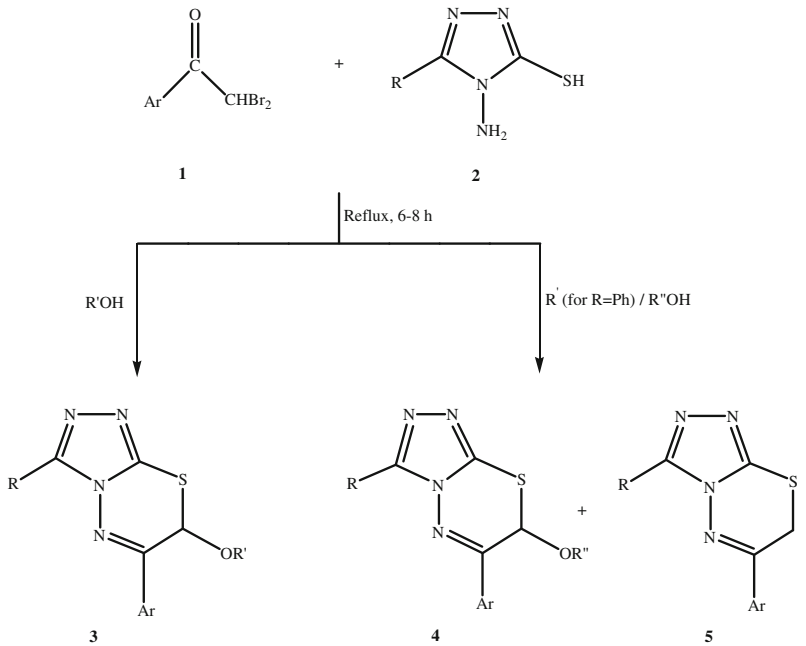
Mechanistic aspects of this synthetic approach have not been investigated in the present study. Detailed investigations regarding the mechanism of the reaction and separation of the intermediates are underway.

Biological results and discussion

In vitro antibacterial activity

The newly synthesized 7*H*-7-alkoxy-3-alkyl/phenyl-6-aryl-7-*s*-triazolo[3,4-b][1,3,4]thiadiazines **(3a–3n, 4a–4j)** were screened for their antibacterial activity against four strains of bacteria namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa* by agar well diffusion technique. All the tested chemical compounds possessed variable antibacterial activity against both the Gram-positive (*S. aureus* and *B. subtilis*) bacteria. Positive controls produced significantly sized inhibition zones against the tested bacteria; however, negative control produced no observable inhibitory effect against any of the test organism as shown in Tables 1 and 2. The activity of the title compounds was compared to well-known commercial antibiotic, ciprofloxacin. On the basis of maximum inhibitory activity shown against Gram-positive bacteria, compound **4i** was found to be most effective against

Scheme 1 Reaction of α,α-dibromoacetophenones **1** with triazoles 2 in different alcohols



Compound	Ar	R	R'	Compound	Ar	R	R"
3a	C_6H_5	C_2H_5	Et	**4a**	C_6H_5	C_2H_5	Me
3b	4-MeC_6H_4	C_2H_5	Et	**4b**	4-ClC_6H_4	C_2H_5	Me
3c	4-ClC_6H_4	C_2H_5	Et	**4c**	C_6H_5	H	Me
3d	4-BrC_6H_4	C_2H_5	Et	**4d**	C_6H_5	n-C_3H_7	Me
3e	4-FC_6H_4	C_2H_5	Et	**4e**	C_6H_5	C_2H_5	n-Pr
3f	4-$NO_2C_6H_4$	C_2H_5	Et	**4f**	4-ClC_6H_4	C_2H_5	n-Pr
3g	C_6H_5	H	Et	**4g**	C_6H_5	H	n-Pr
3h	C_6H_5	n-C_3H_7	Et	**4h**	C_6H_5	n-C_3H_7	n-Pr
3i	C_6H_5	C_2H_5	i-Pr	**4i**	C_6H_5	C_6H_5	Me
3j	4-ClC_6H_4	C_2H_5	i-Pr	**4j**	C_6H_5	C_6H_5	n-Pr
3k	C_6H_5	H	i-Pr	**5a**	C_6H_5	C_2H_5	Me/n-Pr
3l	C_6H_5	n-C_3H_7	i-Pr	**5b**	4-ClC_6H_4	C_2H_5	Me/n-Pr
3m	C_6H_5	C_6H_5	Et	**5c**	C_6H_5	H	Me/n-Pr
3n	C_6H_5	C_6H_5	iso-Pr	**5d**	C_6H_5	n-C_3H_7	Me/n-Pr
				5e	C_6H_5	C_6H_5	Et/Me/ n-Pr/i-Pr

S. aureus with zone of inhibition of 25.6 mm, while the compound **4a** was found to be most effective against *B. subtilis* showing the maximum zone of inhibition of 21.6 mm, when compared with commercial antibiotic, ciprofloxacin, which showed maximum zone of inhibition of 26.6 and 24.0 mm against *S. aureus* and *B. subtilis,* respectively (Table 1). In the whole series, the MIC of various tested chemical compounds ranged between 16 and 256 μg/ml against Gram-positive bacteria (Table 2). Compounds **3m** and **4i** exhibited the lowest MIC of 16 μg/ml against *S. aureus* and the compound **4a** was found to be best against *B. subtilis* with the lowest MIC of 32 μg/ml. None of the tested compounds possessed any activity against Gram-negative bacteria.

In vitro antifungal activity

All the title compounds, **3(a–n)** and **4(a–j)** were screened for their in vitro antifungal activity against two fungi, namely *Saccharomyces cerevisiae* and *Candida albicans.* The activity of the compounds were compared to the standard antifungal agent, amphotericin-B. The compounds

Table 1 In vitro antimicrobial activity of tested compounds through agar well diffusion method

Compound nos.	Diameter of growth of inhibition zone (mm)[a]			
	S. aureus	*B. subtilis*	*S. cerevisiae*	*C. albicans*
3a	13.6	18.3	–	–
3b	12.3	17	10.6	–
3c	–	–	18.3	15.6
3d	12.3	13.6	–	–
3e	–	–	13.6	13
3f	–	–	15.6	13.3
3g	16.3	20.3	13.6	13.6
3h	13.6	17.6	–	–
3i	13.6	12.3	–	–
3j	11.3	12.3	11.3	–
3k	14.6	13.6	13.3	–
3l	12.3	14.6	–	–
3m	24.3	17.6	17.6	15.6
3n	18.3	15.6	13.3	–
4a	22.3	21.6	15.3	16.3
4b	19.3	16.3	12.6	13.0
4c	19.6	18.6	13.6	13.3
4d	16.0	18.3	12.6	–
4e	14.0	13.6	–	–
4f	12.6	16.3	10.3	–
4g	15.6	18.3	–	–
4h	13.6	16.3	15.3	–
4i	25.6	18.0	18.6	–
4j	20.6	17.3	17.3	–
Ciprofloxacin	26.6	24.0	Nt	Nt
Amphotericin-B	Nt	Nt	13.6	14.3

– no activity, *Nt* not tested

[a] Values, including diameter of the well (8 mm), are means of three replicates

3c (18.3 and 15.6 mm), **3m** (17.6 and 15.6 mm) and **4a** (15.3 and 16.3 mm), showed antifungal activity even better than the commercial drug amphotericin-B (13.6 and 14.3 mm) against both the fungal strains, *S. cerevisiae* and *C. albicans,* respectively. Against *S. cerevisiae*, the activity of the title compounds **3f** (15.6) and **4h**–**4j** (15.3, 18.6, and 17.3, respectively) is better than; while the activity of **3e, 3g,** and **4c** is comparable with amphotericin-B (Table 1). In case of yeast, MIC ranged between 32 and 256 μg/ml (Table 2). The compounds **3c**, **3m**, and **4a** showed lowest MIC of 64 μg/ml against *C. albicans,* while compounds **3c**, **3m**, and **4i** showed the lowest MIC of 32 μg/ml against *S. cerevisiae.* Thus, these compounds seems to be very interesting from medicinal point of view as many of them exhibited MIC values even lower than the standard antifungal drug, amphotericin-B which showed MIC value 100 μg/ml for both fungal strains.

Table 2 MIC (in μg/ml) of tested compounds by using macrodilution method

Compound nos.	*S. aureus*	*B. subtilis*	*S. cerevisiae*	*C. albicans*
3a	256	64	–	–
3b	>256	128	>256	–
3c	–	–	32	64
3d	>256	256	–	–
3e	–	–	128	128
3f	–	–	64	128
3g	128	64	128	–
3h	256	128	–	–
3i	256	>256	–	–
3j	>256	>256	>256	–
3k	256	256	128	–
3l	>256	256	–	–
3m	16	128	32	64
3n	128	256	128	–
4a	32	32	64	64
4b	64	128	128	128
4c	64	64	128	128
4d	128	64	256	–
4e	256	256	–	–
4f	>256	128	>256	–
4g	128	64	–	–
4h	256	128	64	–
4i	16	64	32	–
4j	64	128	64	–
Ciprofloxacin	5	5	Nt	Nt
Amphotericin-B	Nt	Nt	100	100

Conclusions

In this study, application of α,α-dibromoacetophenones in an efficient and facile synthesis of new 7*H*-7-alkoxy-3-alkyl/phenyl-6-aryl-*s*-triazolo[3,4-b][1,3,4]thiadiazines is extended. The title compounds showed good activity against the Gram-positive bacteria, *S. aureus* and *B. subtilis* and the yeasts, *S. cerevisiae* and *C. albicans*. Among all the tested chemical compounds, compounds **3c**, **3m**, and **4a** showed antifungal activity even better than the commercial drug amphotericin-B against both the tested fungal pathogens and these compounds can further be used as antifungal agents in pharmaceutical industry, after testing their toxicity to human beings.

Experimental

Melting points were taken in open capillaries and were uncorrected. Elemental analyses (C, H, N) and mass

spectra were carried out at University Science Instrumentation Centre, University of Delhi, Delhi, India. ^{1}H NMR spectra were recorded on a Bruker 300 MHz instrument using TMS as an internal standard. Infrared (IR) spectra were recorded on a Perkin-Elmer 1800 FT-IR spectrophotometer. The purity of the synthesized compounds was tested by thin-layer chromatography (TLC). The starting materials, 4-amino-3-alkyl-5-mercapto-*s*-triazoles **(2a–2c)** were prepared (Bala *et al.*, 1978; Dhaka *et al.*, 1974) starting from carbondisulphide and hydrazine hydrate. 4-amino-3-phenyl-5-mercapto-s-triazole **2d** was prepared according to literature procedure (Chande *et al.*, 1990). The α,α-dibromoacetophenones needed for the present study were prepared by stirring different acetophenones with bromine (2.5 eq.) in $CHCl_3$ (Boeykens and Kimpe, 1994) and were confirmed by comparison with literature mp. All chemicals used were taken from the commercial suppliers and are used as such without further purification.

Preparation of 7*H*-7-alkoxy-6-aryl-3-alkyl/phenyl-*s*-triazolo[3,4-b][1,3,4]thiadiazines (3a–3n, 4a–4j)

General procedure

A solution of appropriate α,α-dibromoacetophenone (**1a–1f**, 2 mmol) was refluxed with triazole **2** (2 mmol) in various alcohols (MeOH, EtOH, *n*-PrOH, iso-PrOH) for 6–8 h. The reaction mixture was cooled and neutralized with ammonium hydroxide.

(a) With alkyl triazoles **2a–2c** (i) in EtOH and iso-PrOH as solvent, a solid was separated out. The solid was filtered, washed with water, and recrystallized from EtOH/H_2O to obtain the pure products **3a–3l**; (ii) In MeOH, a gummy mass, containing a mixture of two products, was obtained which was purified by column chromatography on silica gel using pet ether-ethyl acetate (20–25%) as eluent to obtain the pure products, **4a–4d** and **5a–5c**; (iii) In *n*-PrOH, a white solid, containing a mixture of two products, was obtained. The mixture was separated by column chromatography on silica gel using pet ether-ethyl acetate (20–30%) as eluent to obtain the products, **4e–4h** and **5a–5c**

(b) With 3-phenyl triazole **2d**, a mixture of two products was obtained in all the alcoholic solvents used (MeOH, EtOH, *n*-PrOH, iso-PrOH). The mixture was separated by column chromatography on silica gel using pet ether-ethyl acetate (30–40%) as eluent to obtain the pure compounds (**3m**, **3n, 4i**, **4j**) and **5d**.

Characterization data of the new compounds (3a–3n, 4a–4j)

7H-3-ethyl-7-ethoxy-6-phenyl-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**3a**)

Light brown; mp 110–112°C; yield 68%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.26 (t, 3H, $-CH_2\mathbf{CH_3}$), 1.47 (t, 3H, $-OCH_2\mathbf{CH_3}$), 3.0 (m, 2H, $-\mathbf{CH_2}CH_3$), 3.62 (m, 1H, $-O\mathbf{CH_2}CH_3$), 4.03 (m, 1H, $-O\mathbf{CH_2}CH_3$), 5.75 (s, 1H, –CH), 7.59–7.89 (m, 5H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.09 ($-\mathbf{CH_2}CH_3$), 14.46 ($-OCH_2\mathbf{CH_3}$), 18.35 ($-CH_2\mathbf{CH_3}$), 63.93 ($-OCH_2-$), 73.13 (C_7), 127.09–142.46 (C_6 and aromatic carbons), 150.25 (C_3 of triazole), 155.05 (C_5 of triazole); Anal. Calculated for $C_{14}H_{16}N_4OS$: C, 58.31; H, 5.59; N, 19.43. Found: C, 58.27; H, 5.64; N, 19.32; ESI-MS, *m/z*: 289.32 $[M + 1]^+$.

7H-3-ethyl-7-ethoxy-6-(4-methylphenyl)-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**3b**)

Brown; mp 138–140°C; yield 72%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.25 (t, 3H, $-CH_2\mathbf{CH_3}$), 1.50 (t, 3H, $-OCH_2\mathbf{CH_3}$), 2.68 (s, 3H, $-CH_3$), 3.16 (m, 2H, $-\mathbf{CH_2}CH_3$), 3.67 (m, 1H, $-O\mathbf{CH_2}CH_3$), 4.05 (m, 1H, $-O\mathbf{CH_2}CH_3$), 5.94 (s, 1H, –CH), 7.34 (d, 2H, $J = 8.4$, Ar-H), 7.77 (d, 2H, $J = 7.8$, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.31 ($-\mathbf{CH_2}CH_3$), 14.46 ($-OCH_2\mathbf{CH_3}$), 18.40 ($-CH_2\mathbf{CH_3}$), 21.54 (Ar-CH_3), 63.94 ($-OCH_2-$), 73.13 (C_7), 127.09-142.49 (C_6 and aromatic carbons), 150.05 (C_3 of triazole), 155.18 (C_5 of triazole); Anal. Calculated for $C_{15}H_{18}N_4OS$: C, 59.58; H, 6.00; N, 18.53. Found: C, 59.45; H, 6.07; N, 18.39; ESI-MS, *m/z*: 303.28 $[M + 1]^+$.

7*H*-3-ethyl-7-ethoxy-6-(4-chlorophenyl)-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**3c**)

Brown; mp 141–142°C; yield 75%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (t, 3H, $-CH_2\mathbf{CH_3}$), 1.463 (t, 3H, $-OCH_2\mathbf{CH_3}$), 3.05 (m, 2H, $-\mathbf{CH_2}CH_3$), 3.64 (m, 1H, $-O\mathbf{CH_2}CH_3$), 4.05 (m, 1H, $-O\mathbf{CH_2}CH_3$), 5.73 (s, 1H, –CH), 7.50 (d, 2H, $J = 8.4$, Ar-H), 7.80 (d, 2H, $J = 8.4$, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.10 ($-\mathbf{CH_2}CH_3$), 14.45 ($-OCH_2\mathbf{CH_3}$), 18.38 ($-CH_2\mathbf{CH_3}$), 64.00 ($-OCH_2-$), 72.94 (C_7), 128.50–138.15 (C_6 and aromatic carbons), 148.99 (C_3 of triazole), 155.23 (C_5 of triazole); Anal. Calculated for $C_{14}H_{15}ClN_4OS$: C, 52.09; H, 4.68; N, 17.36. Found: C, 51.85; H, 4.76; N, 17.27; ESI-MS, *m/z*: 323.47 $[M + 1]^+$, 325. 21 $[M + 3]^+$.

7*H*-3-ethyl-7-ethoxy-6-(4-bromophenyl)-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3d**)

Brown; mp 135–138°C; yield 78%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.26 (t, 3H, –CH_2**CH_3**), 1.46 (t, 3H, –OCH_2**CH_3**), 3.05 (m, 2H, –**CH_2**CH_3), 3.68 (m, 1H, –O**CH_2**CH_3), 4.05 (m, 1H, –O**CH_2**CH_3), 5.70 (s, 1H, –CH), 7.66 (d, 2H, $J = 8.7$, Ar-H), 7.72 (d, 2H, $J = 8.7$, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.10 (–**CH_2**CH_3), 14.45 (–OCH_2**CH_3**), 18.38 (–CH_2**CH_3**), 64.00 (–OCH_2–), 72.88 (C_7), 126.59–137.01 (C_6 and aromatic carbons), 149.12 (C_3 of triazole), 155.23 (C_5 of triazole); Anal. Calculated for $C_{14}H_{15}BrN_4OS$: C, 45.78; H, 4.12; N, 15.26. Found: C, 45.95; H, 3.98; N, 15.32; ESI-MS, *m/z*: 368.06 $[M + 1]^+$, 370.14 $[M + 3]^+$.

7*H*-3-ethyl-7-ethoxy-6-(4-fluorophenyl)-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3e**)

Light brown; mp 152–154°C; yield 70%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.25 (t, 3H, –CH_2**CH_3**), 1.46 (t, 3H, –OCH_2**CH_3**), 3.07 (m, 2H, –**CH_2**CH_3), 3.62 (m, 1H, –O**CH_2**CH_3), 4.03 (m, 1H, –O**CH_2**CH_3), 5.72 (s, 1H, –CH), 7.20 (d, 2H, $J = 8.4$, Ar–H), 7.87 (d, 2H, $J = 8.4$, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.11 (–**CH_2**CH_3), 14.45 (–OCH_2**CH_3**), 18.38 (–CH_2**CH_3**), 63.97 (–OCH_2–), 73.07 (C_7), 116.25–136.96 (C_6 and aromatic carbons), 149.04 (C_3 of triazole), 155.19 (C_5 of triazole) 166.55 (–C_5H_4**C-F**); Anal. Calculated for $C_{14}H_{15}FN_4OS$: C, 54.89; H, 4.94; N, 18.29. Found: C, 55.02; H, 4.83; N, 18.17; ESI-MS, *m/z*: 307.18 $[M + 1]^+$.

7*H*-3-ethyl-7-ethoxy-6-(4-nitrophenyl)-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3f**)

Yellow–brown; mp 210-212°C; yield 81%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.28 (t, 3H, –CH_2**CH_3**), 1.48 (t, 3H, –OCH_2**CH_3**), 3.15 (m, 2H, –**CH_2**CH_3), 3.76 (m, 1H, –O**CH_2**CH_3), 4.10 (m, 1H, –O**CH_2**CH_3), 6.28 (s, 1H, –CH), 8.18(d, 2H, $J = 8.7$, Ar-H), 8.40 (d, 2H, $J = 8.7$, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.07 (–**CH_2**CH_3), 14.44 (–OCH_2**CH_3**), 18.38 (–CH_2**CH_3**), 64.12 (–OCH_2–), 72.81 (C_7), 124.29–147.92 (C_6 and aromatic carbons), 149.53 (C_3 of triazole), 155.37 (C_5 of triazole); Anal. Calculated for $C_{14}H_{15}N_5O_3S$: C, 50.44; H, 4.54; N, 21.01. Found: C, 50.26; H, 4.62; N, 21.18; ESI-MS, *m/z*: 334.24 $[M + 1]^+$.

7*H*-7-ethoxy-6-phenyl-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3g**)

Dark brown; mp 120–122°C; yield 75%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (t, 3H, –OCH_2**CH_3**), 3.65 (m, 1H, –O**CH_2**CH_3), 4.06 (m, 1H, –O**CH_2**CH_3), 5.79 (s, 1H, –CH), 7.32-7.72 (m, 5H, Ar-H), 8.70 (s, 1H, triazole); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 14.42 (–OCH_2**CH_3**), 64.12 (–OCH_2–), 74.56 (C_7), 126.48–142.76 (C_6 and aromatic carbons), 150.65 (C_3 of triazole), 155.35 (C_5 of triazole); Anal. Calculated for $C_{12}H_{12}N_4OS$: C, 55.37; H, 4.65; N, 21.52. Found: C, 55.28; H, 4.68; N, 21.43; ESI-MS, *m/z*: 261.17 $[M + 1]^+$.

7*H*-3-propyl-7-ethoxy-6-phenyl-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3h**)

Light brown; mp 148–150°C; yield 72%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.06 (t, 3H, –CH_2CH_2**CH_3**), 1.25 (t, 3H, –OCH_2**CH_3**), 1.91 (m, 2H, –CH_2**CH_2**CH_3), 3.02 (m, 2H, –**CH_2**CH_2CH_3), 3.62 (m, 1H, –O**CH_2**CH_3), 4.03 (m, 1H, –O**CH_2**CH_3), 5.75 (s, 1H, –CH), 7.52–7.88 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 18.52 (–CH_2CH_2**CH_3**), 19.34 (–OCH_2**CH_3**), 25.06 (–CH_2**CH_2**CH_3), 31.15 (–**CH_2**CH_2CH_3), 68.57 (–OCH_2–), 77.75 (C_7), 132.12–142.33 (C_6 and aromatic carbons), 155.37 (C_3 of triazole), 158.54 (C_5 of triazole); Anal. Calculated for $C_{15}H_{18}N_4OS$: C, 59.58; H, 6.0; N, 18.53. Found: C, 59.52; H, 6.04; N, 18.62; ESI-MS, *m/z*: 303.15 $[M + 1]^+$.

7*H*-3-ethyl-6-phenyl-7-iso-propoxy-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3i**)

Pale yellow; mp 183–185°C; yield 68%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.16 (d, 3H, –OCH(**CH_3**)$_2$), 1.33 (d, 3H, –OCH(**CH_3**)$_2$), 1.47 (t, 3H, –CH_2**CH_3**), 3.03 (m, 2H, –**CH_2**CH_3), 4.28 (m, 1H, –O**CH**–), 5.85 (s, 1H, –CH), 7.53–7.90 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.10 (–**CH_2**CH_3), 18.42 (–CH_2**CH_3**), 20.60 (–OCH(**CH_3**)$_2$), 23.04 (–OCH(**CH_3**)$_2$), 69.98 (–OCH–), 70.99 (C_7), 127.12-137.35 (C_6 and aromatic carbons), 150.47 (C_3 of triazole), 155.19 (C_5 of triazole); Anal. Calculated for $C_{15}H_{18}N_4OS$: C, 59.58; H, 6.00; N, 18.53. Found: C, 59.73; H, 6.07; N, 18.38; ESI-MS, *m/z*: 303.09 $[M + 1]^+$.

7*H*-3-ethyl-6-(4-chlorophenyl)-7-iso-propoxy-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**3j**)

Brown; mp 146–148°C; yield 74%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.15 (d, 3H, –OCH(**CH_3**)$_2$), 1.31 (d, 3H, –OCH(**CH_3**)$_2$), 1.46 (t, 3H, –CH_2**CH_3**), 3.04 (m, 2H, –**CH_2**CH_3), 4.27 (m, 1H, –O**CH**–), 5.81 (s, 1H, –CH), 7.49 (d, 2H, $J = 8.4$, Ar–H), 7.81 (d, 2H, $J = 8.4$, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.12 (–**CH_2**CH_3), 18.38 (–CH_2**CH_3**), 20.63 (–OCH(**CH_3**)$_2$), 23.07 (–OCH(**CH_3**)$_2$), 70.02 (–OCH–), 71.05 (C_7), 127.07–137.31 (C_6 and aromatic carbons), 150.42 (C_3 of triazole), 155.18 (C_5 of triazole); Anal. Calculated for $C_{15}H_{17}ClN_4OS$: C, 53.49; H,

5.09; N, 16.63. Found: C, 53.58; H, 4.99; N, 16.48; ESI-MS, *m/z*: 337.52 $[M + 1]^+$, 339.27 $[M + 3]^+$.

7*H*-6-phenyl-7-iso-propoxy-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3k**)

Dark brown; mp 153–155°C; yield 76%; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.18 (d, 3H, –OCH(**C**$\mathbf{H_3}$)$_2$), 1.35 (d, 3H, –OCH(**C**$\mathbf{H_3}$)$_2$), 4.28 (m, 1H, –O**C**$\mathbf{H}$–), 6.46 (s, 1H, –CH), 7.66–8.01 (m, 5H, Ar-H), 9.31 (s, 1H, triazole); ^{13}C NMR (DMSO-d_6, 300 MHz): δ 20.94 (–OCH(**C**$\mathbf{H_3}$)$_2$), 23.44 (–OCH(**C**$\mathbf{H_3}$)$_2$), 70.24 (–OCH–), 71.86 (C_7), 127.34–137.85 (C_6 and aromatic carbons), 150.77 (C_3 of triazole), 155.34 (C_5 of triazole); Anal. Calculated for $C_{13}H_{14}N_4OS$: C, 56.91; H, 5.14; N, 20.42. Found: C, 56.83; H, 5.17; N, 20.38; ESI-MS, *m/z*: 275.17 $[M + 1]^+$.

7*H*-3-propyl-6-phenyl-7-iso-propoxy-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3l**)

Light brown; mp 172–174°C; yield 70%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.17 (t, 3H, –CH_2CH_2**C**$\mathbf{H_3}$), 1.37 (d, 3H, –OCH(**C**$\mathbf{H_3}$)$_2$), 1.45 (d, 3H, –OCH(**C**$\mathbf{H_3}$)$_2$), 2.10 (m, 2H, –CH_2**C**$\mathbf{H_2}$$CH_3$), 3.07 (m, 2H, –**C**$\mathbf{H_2}$$CH_2CH_3$), 4.27 (m, 1H, –O**C**$\mathbf{H}$–), 5.80 (s, 1H, –CH), 7.41–7.83 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 18.44 (–CH_2CH_2**C**$\mathbf{H_3}$), 20.57 (–OCH(**C**$\mathbf{H_3}$)$_2$), 22.98 (–OCH(**C**$\mathbf{H_3}$)$_2$), 24.92 (–CH_2**C**$\mathbf{H_2}$$CH_3$), 31.07 (–**C**$\mathbf{H_2}$$CH_2CH_3$), 70.14 (–OCH–), 71.35 (C_7), 127.26–137.65 (C_6 and aromatic carbons), 150.45 (C_3 of triazole), 155.24 (C_5 of triazole); Anal. Calculated for $C_{16}H_{20}N_4OS$: C, 60.73; H, 6.37; N, 17.71. Found: C, 60.78; H, 6.35; N, 17.58; ESI-MS, *m/z*: 317.12 $[M + 1]^+$.

7*H*-3-phenyl-7-ethoxy-6-phenyl-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3m**)

Light brown; mp 174–176°C; yield 36%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.27 (t, 3H, –OCH_2**C**$\mathbf{H_3}$), 3.65 (m, 1H, –O**C**$\mathbf{H_2}$$CH_3$), 4.07 (m, 1H, –O**C**$\mathbf{H_2}$$CH_3$), 5.76 (s, 1H, –CH), 7.44–8.17 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 10.45 (–OCH_2**C**$\mathbf{H_3}$), 63.76 (–OCH_2–), 73.28 (C_7), 127.09–142.46 (C_6 and aromatic carbons), 150.48 (C_3 of triazole), 155.65 (C_5 of triazole); Anal. Calculated for $C_{18}H_{16}N_4OS$: C, 64.26; H, 4.79; N, 16.65. Found: C, 64.18; H, 4.75; N, 16.77; MS, *m/z*: 337.22 $[M + 1]^+$.

7*H*-3-phenyl-6-phenyl-7-iso-propoxy-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**3n**)

Brown; mp 161–164°C; yield 32%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.18 (d, 3H, –OCH(**C**$\mathbf{H_3}$)$_2$), 1.35 (d, 3H, –OCH(**C**$\mathbf{H_3}$)$_2$), 4.28 (m, 1H, –O**C**$\mathbf{H}$–), 5.76 (s, 1H, –CH), 7.51–8.17 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 21.46 (–OCH(**C**$\mathbf{H_3}$)$_2$), 24.12 (–OCH(**C**$\mathbf{H_3}$)$_2$), 70.18 (–OCH–), 74.39 (C_7), 126.12-137.85 (C_6 and aromatic carbons), 151.27 (C_3 of triazole), 155.79 (C_5 of triazole); Anal Cald for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99. Found: C, 65.21; H, 5.16; N, 15.88; ESI-MS, *m/z*: 351.35 $[M + 1]^+$.

7*H*-3-ethyl-7-methoxy-6-phenyl-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**4a**)

Dark brown; mp 122–124°C; yield 46%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.44 (t, 3H, –CH_2**C**$\mathbf{H_3}$), 3.03 (m, 2H, –**C**$\mathbf{H_2}$$CH_3$), 3.53 (s, 3H, –O**C**$\mathbf{H_3}$), 5.62 (s, 1H, –CH), 7.50–7.87 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.10 (–**C**$\mathbf{H_2}$$CH_3$), 18.38 (–$CH_2$**C**$\mathbf{H_3}$), 55.32 (–$OCH_3$), 74.58 ($C_7$), 128.50–138.18 ($C_6$ and aromatic carbons), 149.05 (C_3 of triazole), 155.20 (C_5 of triazole); Anal. Calculated for $C_{13}H_{14}N_4OS$: C, 56.91; H, 5.14; N, 20.42. Found: C, 56.78; H, 5.21; N, 20.31; ESI-MS, *m/z*: 275.14 $[M + 1]^+$.

7H-3-ethyl-7-methoxy-6-(4-chlorophenyl)-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**4b**)

Reddish brown; mp 130–132°C; yield 54%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.45 (t, 3H, –CH_2**C**$\mathbf{H_3}$), 3.04 (m, 2H, –**C**$\mathbf{H_2}$$CH_3$), 3.53 (s, 3H, –O**C**$\mathbf{H_3}$), 5.61 (s, 1H, –CH), 7.50 (d, 2H, $J = 8.4$, Ar-H), 7.79 (d, 2H, $J = 8.4$, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.15 (–**C**$\mathbf{H_2}$$CH_3$), 18.36 (–$CH_2$**C**$\mathbf{H_3}$), 55.29 (–$OCH_3$), 74.65 ($C_7$), 128.53-138.23 ($C_6$ and aromatic carbons), 149.07 (C_3 of triazole), 155.22 (C_5 of triazole); Anal. Calculated for $C_{13}H_{13}ClN_4OS$: C, 50.57; H, 4.24; N, 18.14. Found: C, 50.65; H, 4.35; N, 18.02; ESI-MS, *m/z*: 309.62 $[M + 1]^+$, 311.28 $[M + 3]^+$.

7*H*-7-methoxy-6-phenyl-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**4c**)

Brown; mp 102–104°C; yield 38%; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.46 (s, 3H, –O**C**$\mathbf{H_3}$), 6.46 (s, 1H, –CH), 7.66–7.98 (m, 5H, Ar-H), 9.31(s, 1H, triazole); ^{13}C NMR (DMSO-d_6, 300 MHz): δ 55.32 (–OCH_3), 74.58 (C_7), 128.50–138.18 (C_6 and aromatic carbons), 149.05 (C_3 of triazole), 155.20 (C_5 of triazole); Anal. Calculated for $C_{11}H_{10}N_4OS$: C, 53.64; H, 4.09; N, 22.75. Found: C, 53.76; H, 4.05; N, 22.62; ESI-MS, *m/z*: 247.21 $[M + 1]^+$.

7*H*-3-propyl-7-methoxy-6-phenyl-*s*-triazolo [3,4-b][1,3,4]thiadiazine (**4d**)

Brown; mp 134–136°C; yield 36%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.06 (t, 3H, –CH_2CH_2**C**$\mathbf{H_3}$), 1.92 (m, 2H, –CH_2**C**$\mathbf{H_2}$$CH_3$), 3.02 (m, 2H, –**C**$\mathbf{H_2}$$CH_2CH_3$), 3.54 (s, 3H, –O**C**$\mathbf{H_3}$), 5.66 (s, 1H, –CH), 7.52–7.92 (m, 5H, Ar-H); ^{13}C

NMR ($CDCl_3$, 300 MHz): δ 13.80 (–CH_2CH_2**CH_3**), 20.43 (–CH_2**CH_2**CH_3), 26.52 (–**CH_2**CH_2CH_3), 55.30 (–OCH_3), 74.91 (C_7), 127.12–133.29 (C_6 and aromatic carbons), 150.09 (C_3 of triazole), 156.28 (C_5 of triazole); Anal. Calculated for $C_{14}H_{16}N_4OS$: C, 58.31; H, 5.59; N, 19.43. Found: C, 58.38; H, 5.51; N, 19.31; ESI-MS, *m/z*: 289.14 $[M + 1]^+$.

7*H*-3-ethyl-6-phenyl-7-propoxy-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**4e**)

Light brown; mp 128–130°C; yield 34%; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.78 (t, 3H, –OCH_2CH_2**CH_3**), 1.35 (t, 3H, –CH_2**CH_3**), 1.50 (m, 2H, –OCH_2**CH_2**–), 2.95 (m, 2H, –**CH_2**CH_3), 3.40 (m, 1H, –O**CH_2**–), 3.83 (m, 1H, –O**CH_2**–), 5.71 (s, 1H, –CH), 7.46–7.87 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 10.41 (–OCH_2CH_2**CH_3**), 11.15 (–**CH_2**CH_3), 18.40 (–CH_2**CH_3**), 22.16 (–OCH_2**CH_2**–), 70.04 (–OCH_2–), 73.52 (C_7), 127.20–137.10 (C_6 and aromatic carbons), 150.10 (C_3 of triazole), 155.25 (C_5 of triazole); Anal. Calculated for $C_{15}H_{18}N_4OS$: C, 59.58; H, 6.00; N, 18.53. Found: C, 59.67; H, 5.89; N, 18.62; ESI-MS, *m/z*: 303.27 $[M + 1]^+$.

7*H*-3-ethyl-6-(4-chlorophenyl)-7-propoxy-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**4f**)

Pale yellow; mp 115–118°C; yield 38%; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.89 (t, 3H, –OCH_2CH_2**CH_3**), 1.45 (t, 3H, –CH_2**CH_3**), 1.60 (m, 2H, –OCH_2**CH_2**–), 3.05 (m, 2H, –**CH_2**CH_3), 3.46 (m, 1H, –O**CH_2**–), 3.92 (m, 1H, –O**CH_2**–), 5.70 (s, 1H, –CH), 7.50 (d, 2H, $J = 8.4$, Ar-H), 7.95(d, 2H, $J = 8.4$, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 10.44 (–OCH_2CH_2**CH_3**), 11.17 (–**CH_2**CH_3), 18.42 (–CH_2**CH_3**), 22.19 (–OCH_2**CH_2**–), 70.07 (–OCH_2–), 73.58 (C_7), 127.12–137.16 (C_6 and aromatic carbons), 150.07 (C_3 of triazole), 155.19 (C_5 of triazole); Anal. Calculated for $C_{15}H_{17}ClN_4OS$: C, 53.49; H, 5.09; N, 16.63. Found: C, 53.37; H, 5.16; N, 16.41; ESI-MS, *m/z*: 337.11 $[M + 1]^+$, 339.26 $[M + 3]^+$.

7*H*-6-phenyl-7-propoxy-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**4g**)

Dark brown; mp 144–146°C; yield 32%; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.27 (t, 3H, –OCH_2CH_2**CH_3**), 2.05 (m, 2H, –OCH_2**CH_2**–), 3.68 (m, 1H, –O**CH_2**–), 4.05 (m, 1H, –O**CH_2**–), 5.84 (s, 1H, –CH), 7.43–7.84 (m, 5H, Ar-H), 8.74 (s, 1H, triazole); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 10.52 (–OCH_2CH_2**CH_3**), 22.92 (–OCH_2**CH_2**–), 69.87 (–OCH_2–), 74.08 (C_7), 127.15–138.62 (C_6 and aromatic carbons), 150.32 (C_3 of triazole), 153.01 (C_5 of triazole); Anal. Calculated for $C_{13}H_{14}N_4OS$: C, 56.91; H, 5.14; N, 20.42. Found: C, 59.82; H, 5.09; N, 20.54; ESI-MS, *m/z*: 275.22 $[M + 1]^+$.

7*H*-3-propyl-6-phenyl-7-propoxy-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**4h**)

Light brown; mp 196–198°C; yield 34%; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.89 (t, 3H, –CH_2CH_2**CH_3**), 1.05 (t, 3H, –OCH_2CH_2**CH_3**), 1.59 (m, 2H, –OCH_2**CH_2**CH_3), 1.90 (m, 2H, –CH_2**CH_2**CH_3), 3.03 (m, 2H, –**CH_2**CH_2CH_3), 3.50 (m, 1H, –O**CH_2**CH_2CH_3), 3.94 (m, 1H, –O**CH_2**CH_2CH_3), 5.73 (s, 1H, –CH), 7.52–7.88 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 10.90 (–OCH_2CH_2**CH_3**), 13.84 (–CH_2CH_2**CH_3**), 20.30 (–CH_2**CH_2**CH_3), 25.06 (–OCH_2**CH_2**CH_3), 31.15 (–**CH_2**CH_2CH_3), 69.63 (–OCH_2–), 72.90 (C_7), 129.15–139.91 (C_6 and aromatic carbons), 150.46 (C_3 of triazole), 154.06 (C_5 of triazole); Anal. Calculated for $C_{16}H_{20}N_4OS$: C, 60.73; H, 6.37; N, 17.71. Found: C, 60.62; H, 6.34; N, 17.82; ESI-MS, *m/z*: 317.18 $[M + 1]^+$.

7*H*-3-phenyl-7-methoxy-6-phenyl-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**4i**)

Dark brown; mp 132–135°C; yield 42%; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.57 (s, 3H, –OCH_3), 5.67 (s, 1H, –CH), 7.41–8.20 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 55.59 (–OCH_3), 73.98 (C_7), 126.39–137.72 (C_6 and aromatic carbons), 151.34 (C_3 of triazole), 155.78 (C_5 of triazole); Anal. Calculated for $C_{17}H_{14}N_4OS$: C, 63.33; H, 4.38; N, 17.38. Found: C, 63.26; H, 4.37; N, 17.49; ESI-MS, m/z: 323.22 $[M + 1]^+$.

H-3-phenyl-6-phenyl-7-propoxy-*s*-triazolo[3,4-b][1,3,4]thiadiazine (**4j**)

Brown; mp 188–190°C; yield 28%; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.92 (t, 3H, –OCH_2CH_2**CH_3**), 1.68 (m, 2H, –OCH_2**CH_2**–), 3.53 (m, 1H, –O**CH_2**–), 3.70 (m, 1H, –O**CH_2**–), 5.75(s, 1H, –CH), 7.52–8.16 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 10.45 (–OCH_2CH_2**CH_3**), 23.01 (–OCH_2**CH_2**–), 70.14 (–OCH_2–), 76.81 (C_7), 125.91–138.26 (C_6 and aromatic carbons), 152.97 (C_3 of triazole), 156.65 (C_5 of triazole); Anal. Calculated for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99. Found: C, 65.05; H, 5.19; N, 16.09; ESI-MS, *m/z*: 351.16 $[M + 1]^+$.

Biological assay

Test microorganisms

Total six microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (*S. aureus* MTCC 96 and *B. subtilis* MTCC 121); two Gram-negative bacteria (*E. coli* MTCC 1652 and *P. aeruginosa* MTCC 741), and two yeast

(*C. albicans* MTCC 227 and *S. cerevisiae* MTCC 170) were screened for evaluation of antibacterial and antifungal activity of the chemical compounds. All the microbial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were sub-cultured on Nutrient agar whereas yeast on Malt yeast agar.

Antimicrobial activity (bacteria and yeasts)

The antimicrobial activity of 24 chemical compounds was evaluated by the agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml. 20 ml of agar medium was poured into each Petri plate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8-mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 2.0 mg/ml of each compound reconstituted in the dimethylsulfoxide (DMSO). All the plates were incubated at 37°C for 24 h. Antimicrobial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control, whereas Ciprofloxacin were used as positive control for bacteria and amphotericin-B for yeast. This procedure was performed in three replicate plates for each organism (Ahmad and Beg, 2001; Andrews, 2001).

Determination of minimum inhibitory concentration (MIC) of chemical compounds

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation**.** MIC of the various compounds against bacterial and yeast strains was tested through a modified agar well diffusion method (Okeke *et al.*, 2001). In this method, a twofold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 256–0.5 µg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µl of standardized inoculum (10^6 cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 h and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that completely inhibited the growth of the microbe, showed by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin and amphotericin-B was used as positive control, while DMSO as negative control.

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References

Ahluwalia VK, Arora KK, Kaur G, Mehta B (1987) An unusual observation in the synthesis of thiazoles. Synth Commun 17:333–340

Ahluwalia VK, Mehta B, Rawat M (1992) An expeditious study of the nature of halogens at α- positions in carbonyl compounds. Synth Commun 22:2697–2701

Ahmad I, Beg AJ (2001) Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. J Ethnopharmacol 74:113–123

Almajan GL, Barbuceanu SF, Saramet I, Draghici C (2010) New 6-amino-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines and [1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-ones: synthesis, characterization and antibacterial activity evaluation. Eur J Med Chem 45:3191–3195

Ammar YA, Ghorab MM, El-Sharief AM, Mohamed Sh I (2002) Naproxen in heterocyclic chemistry: novel syntheses of triazoles, triazolothiadiazines, triazolothiadiazoles, and triazolothiadiazepine bearing an asymmetric carbon atom and radiostability of the biologically active compounds. Heteroatom Chem 13:199–206

Andrews JM (2001) Determination of minimum inhibitory concentrations. J Antimicrob Chemother 48:5–16

Bala S, Gupta RP, Sachdeva ML, Singh A, Pujari HK (1978) Heterocyclic systems containing bridgehead nitrogen atom: Part-33, Syntheses of s-triazolo[3,4-b][1,3,4]thiadiazine,*s*-triazolo[3,4-b][1,3,4]thiadiazino[6,7-b]quinoxaline and s-triazolo[3,4-b][1,3,4]thiadiazine. Indian J Chem 16B:481–483

Boeykens M, Kimpe ND (1994) Selective transformation of α, α-dibromomethyl ketones into α-monosulfenylated ketones. Tetrahedron 50:12349–12360

Chadha VK, Joshi S, Bhargava A (1992) Syntheses of s-triazolo [3,4-b][1,3,4]benzothiadiazines,*s*-triazolo[3,4-b][1,3,4]thiadiazines, s-triazolo[3,4-b][1,3,4]thiadiazino[5,6-b]quinoxaline, imidazo[2,1-b]benzothiazole and benzimidazo[2,1-b]benzothiazole as potential antimicrobial agents. J Indian Chem Soc 69:884–887

Chande MS, Karnik BM, Inamdar AN, Ganguly N (1990) Design, synthesis and biological screening of new *s*-triazolothiadiazine derivatives. J Indian Chem Soc 67:220–222

Dandia A, Singh R, Khaturia S (2006) Microwave enhanced solid support synthesis of fluorine containing benzopyrano-triazolothiadiazepines as potent anti-fungal agents. Bioorg Med Chem 14:1303–1308

Dhaka KS, Mohan J, Chadha VK, Pujari HK (1974) Heterocyclic systems containing bridgehead nitrogen atom: Part16—Syntheses of *s*-triazolo[3,4-b][1,3,4]thiadiazines and related heterocycles. Indian J Chem 12:287–289

Ding Q, Zhang L, Zhang H (2010) Synthesis and biological activities of some novel triazolothiadiazines and schiff bases derived from 4-amino-3-(4-hydroxyphenyl)-1H–1, 2, 4-triazole-5(4H)-thione. Phosphorous Sulfur Silicon Relat Elem 185:567–572

Farghaly AR, De Clereq E, El-Kashef H (2006) Synthesis and antiviral activity of novel [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles, [1,2,4]triazolo[3,4-b][1,3,4]thiadiazines and[1,2,4]triazolo [3,4-b][1,3,4]thiadiazepines. Arkivoc 10:137–151

Filmwala ZA, Bhalekar SM, D'Souza JP, Fernandes PS (2002) Synthesis and biological activity of some nitrogen bridgehead heterocycles having pyridazinone/triazole moiety. Indian J Heterocycl Chem 11:225–228

Garcia EE, Benjamin LE, Fryer RI (1973) Acyl indoles, III (I): the synthesis of [1,4]diazepino[6,5-b]indoles. J Het Chem 10:51–53

Holla BS, Akberali PM, Shivanznda MK (2001) Studies on nitrophenylfuran derivatives part-Xii, synthesis, characterization, antibacterial and antiviral activities of some nitrophenylfurfurylidene-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines. II Farmaco 56: 919–927

Holla BS, Poojary NK, Rao SB, Shivananda MK (2002) New bis-aminomercaptotriazoles and bis- triazolothiadiazoles as possible anticancer agents. Eur J Med Chem 37:511–517

Holla BS, Rao SB, Sarojini BK, Akberali PM, Kumari SN (2006a) Synthesis and studies on some new fluorine containing triazolothiadiazines as possible antibacterial, antifungal and anticancer agents. Eur J Med Chem 41:657–663

Holla BS, Prasanna CS, Poojary B, Rao KS, Shridhara K (2006b) Synthesis, characterization and biological studies of some triazolothiadiazines and triazolothiadiazoles containing 3-chloropyridin-3-yl methyl moiety. Indian J Chem 45B:2071–2076

Ilhan E, Ergenc N, Ulusoy N, Otuk-Sanis G (1996) Synthesis and antimicrobial action of 4-arylideneamino-3-(alpha,alpha-diphenyl-alpha-hydroxmethyl)-1,4-dihydro-5H–1,2,4-triazino-5-thiones and 6-arene-3-(alpha,alpha-diphenyl-alpha-hydroxymethyl)-7H-s-triazolo(3,4-b) (1,2,4)thiadiazine. Pharmazie 51:123–124

Jin JY, Zhang LX, Chen XX, Zhang AJ, Zhang HL (2007a) Syntheses and biological activities of 6-aryl-3-(3-hydroxy-propyl)-7H–1,2,4-triazolo[3,4-b]thiadiazines. Molecules 12:297–303

Jin JY, Zhang ZX, Zhang AJ, Lei XX, Zhu JH (2007b) Synthesis and biological activity of some novel derivatives of 4-amino-3-(d-galactopentitol-1-yl)-5-mercapto-1,2,4-triazole. Molecules 12:1596–1605

Karabasanagouda T, Adhikari AV, Shetty NS (2007) Synthesis and antimicrobial activities of 1,2,4-triazolo[3,4-b]-1,3,4-thidiazoles and 1,2,4-triazolo[3, 4-b]-1,3,4-thidiazines carrying thioalkyl and sulphonyl phenoxy moieties. Eur J Med Chem 42:521–529

Laddi UV, Talawar MB, Desai SR, Bennur RS, Bennur SC (2001) Synthesis, antimicrobial and antituberculosis activity of N-bridgehead heterocycles. Indian J Chem B 40:828–833

Little TL, Webber SE (1994) A simple and practical synthesis of 2-aminoimidazoles. J Org Chem 59:7299–7305

Mohan J, Kataria S (1995) Heterocyclic systems containing bridgehead nitrogen atom: synthesis and bioactivity of 3-(1-adamantyl)-6-(*p*-chlorophenyl)-7H-s-triazolo[3,4-b][1,3,4]thiadiazine and 3,6-DI-(1-adamantyl)-*s*-triazolo[3,4-b][1,3,4]thiadiazole. Indian J Heterocycl Chem 5:143–144

Nadkarni BA, Kamat VR, Khadse BG (2001) Synthesis and anthelmintic activity of 3,6-disubstituted-7H-*s*-triazolo(3,4-b) (1,3,4)thiadiazines. Arznein-Forsch 51:569–573

Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO (2001) Evaluation of extracts of the roots of *Landolphia owerrience* for antibacterial activity. J Ethnopharmacol 78:119–127

Prakash O, Sharma N (2007) Reaction of α,α-dibromoketones with 4-amino-5-mercapto-3-methyl-*s*-triazole: synthesis of some 7H–7-alkoxy-6-aryl-3-methyl-*s*-triazolo[3,4-b][1,3,4]thiadiazines. Arkivoc 16:65–72

Prakash O, Sharma N, Pannu K (2007a) Synthesis of 2,2′-bi-2H-benzothiazines from α,α-Dibromoacetophenones and *o*-aminothiophenol. Synth Commun 37:1995–1999

Prakash R, Kumar A, Singh SP (2007b) Dehydroacetic acid and its derivatives in organic synthesis: synthesis of some new 2-substituted-4-(5-bromo-4-hydroxy-6-methyl-2H-pyran-2-one-3-yl)thiazoles. Indian J Chem 46B:1713–1715

Prakash R, Kumar A, Singh SP (2007c) α,α-Dibromoketones: a superior alternative to α-bromoketones in hantzsch thiazole synthesis. Synth Commun 37:2501–2505

Wilhelm M, Schmidt P (1969) A study of the synthesis and some reactions of 3-phenyl-1,4-benzothiazines. J Het Chem 6:635–638

Zhou S, Zhang L, Jin JY, Zhang AJ, Lei XX, Lin J, He J, Zhang H (2007) Synthesis and biological activities of some novel triazolothiadiazines and schiff bases derived from 1,2,4-triazole. Phosphorus Sulfur Silicon Relat Elem 182:419–432

Zitouni GT, Kaplancikl ZA, Özdemir A, Chevallet P, Kandilci HB, Gümüsel B (2007) Studies on 1,2,4-triazole derivatives as potential anti-inflammatory agents. Arch Pharm Chem Life Sci 11:586–590