European Journal of Medicinal Chemistry 84 (2014) 194–199

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of novel thiadiazolotriazin-4-ones and study of their mosquito-larvicidal and antibacterial properties

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ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form
17 June 2014
Accepted 29 June 2014
Available online 30 June 2014

Keywords:
Thiadiazolotriazin-4-one
Mosquito-larvicidal studies
Antibacterial studies
1,3,4-Thiadiazole moiety
Triazin-4-one moiety

ABSTRACT

A series of novel 3-*tert*-butyl-7-(aryloxymethyl)-4*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**) were synthesized by refluxing 3,3-dimethyl-2-oxobutanoic acid (trimethyl pyruvic acid) (**1**) and thiocarbohydrazide (**2**) in ethanol as solvent for 12 h, to yield 3-mercapto-4-amino-6-*tert*-butyl-1,2,4-triazine-5(4*H*)-one (**3**) (Scheme 1), then the compound (**3**) was condensed with different substituted aryloxyacetic acids (**4**) in $POCl_3$ at 90 °C for 8 h (Scheme 2). The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, elemental analyses and mass spectroscopic studies. Few of the synthesized compounds exhibited moderate mosquito-larvicidal and antibacterial activities. Among the novel derivatives, the compound (**5f**) showed relatively high larvicidal activity against a malaria vector. Compounds (**5i**) and (**5m**) exhibited a broad spectrum antibacterial activity against Gram positive and Gram negative species and hence they may be considered as drug candidates for bacterial pathogens.

1. Introduction

The recent decades have experienced the developments in the field of science; particularly a huge upsurge in the drug synthesis due to the advances in technology and the demands. With certainty and clarity one can pronounce that the novel inventions and the availability of voluminous literature have boosted the progress of research. Marching in the same direction it was observed that triazinone derivatives drew considerable interest because of enormous importance in the field of medicine and agriculture [1–6]. Studies have shown that they have potential herbicidal [7–13], insecticidal [14], antimicrobial [15–18], antifungal [19,20], anticancer [21–25], antibacterial [26], anti-HIV [27], antioxidant [27], antifish parasites [28,29], anticonvulsant [30,31], antitumour [32] and antimetastatic properties [33] and so on. In addition to the activities of triazinones, thiadiazoles are useful as antitumour [34–36], antimicrobial [37], cytotoxic [38] antituberculosis [39], antidepressant [40] and pesticidal agents [41,42]. Enthused by the enormous pharmacological importance of triazinones and 1,3,4-thiadiazoles, it was decided to synthesize some novel thiadiazolotriazinones (**5a**–**5n**) and screen them for mosquito-larvicidal and antibacterial activities.

2. Pharmacology

The novel 3-*tert*-butyl-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**) were synthesized and evaluated for mosquito-larvicidal and antibacterial activity studies.

2.1. Mosquito-larvicidal activity

All the synthesized compounds were tested for their larvicidal activity in mosquito using Malaria Vector *Anopheles stephensi* as per the standard WHO guidelines [43]. In 500 mL beakers containing 250 mL of water and 25 numbers of late III or early IV instar mosquito larvae for various concentrations of the extracts. A negative control was kept with each set of experiment and mortality was recorded after 24 h of exposure. Malathion, the commercial insecticide (Hindustan Insecticides Ltd, New Delhi, India) was used

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http://dx.doi.org/10.1016/j.ejmech.2014.06.072
0223-5234/

as the reference standard. Experiments were performed in triplicates for each sample. Median lethal concentration (LC_{50}) with 95% confidence limit (CI) was calculated using Abbott's formula and Log probit analysis [44] and results are expressed as μg/mL. Relative potency was determined for comparison with the reference standard using the formula.

2.2. Antibacterial activity

All the synthesized compounds were evaluated for their antibacterial property by Disc diffusion method [45]. The microorganisms were collected from Institute of Microbial Technology (IMTECH), Chandigarh, India. Two Gram positive bacteria namely *Staphylococcus aureus* MTCC-7443 and *Bacillus subtilis* MTCC-441; two Gram negative bacteria namely *Escherichia coli* MTCC-725, *Aeromonas hydrophila* MTCC-1739 were used for the screening.

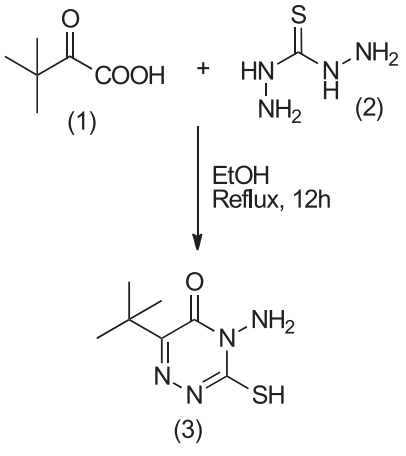
The bacterial strains were inoculated on Nutrient Agar (NA) and incubated for 24 h at 37 °C. Sterile empty discs (6 mm diameter) were purchased from Himedia Laboratories Pvt. Ltd. Mumbai. The test compounds were dissolved in 5 mL of DMSO taken as the solvent; from the stock solution 100 μL of respective compound in the selected concentration (500 μg/disc) was loaded on the disc individually and aseptically, dried and were used for screening the antibacterial assay.

Sterile discs were saturated with 100 μL of the test solution, dried under laminar air flow and placed on the Nutrient Agar (NA) plate for bacteria, which was inoculated with a lawn of the test microorganisms. Plates were incubated at 37 °C, for 18–24 h for bacteria. The compounds that produced distinct circular zones of inhibition around the discs and the diameters of clear zones were determined and used as an indication of antibacterial activity. Streptomycin, an antibiotic drug at a dose of 10 μg/disc was used as the reference standard.

3. Results and discussion

3.1. Chemistry

4-Amino-6-*tert*-butyl-3-mercapto-1,2,4-triazine-5(4*H*)-one (**3**) was synthesized according to the well established procedure of Dornow and co-workers [46]. The equimolar mixture of 3,3-dimethyl-2-oxobutanoic acid(trimethyl pyruvic acid) (**1**) and thiocarbohydrazide (**2**) were refluxed in ethanol for 12 h (Scheme 1). The resultant 4-amino-6-*tert*-butyl-3-mercapto-1,2,4-triazine-5(4*H*)-one (**3**) was condensed with different aryloxyacetic acids (**4**) in $POCl_3$ at 80 °C for 8 h to yield the resultant series of novel 3-*tert*-butyl-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**) (Scheme 2). The structures of the novel compounds (**5a**–**5n**) were confirmed by spectral and elemental analyses. The data are incorporated in experimental section.



Scheme 1. The synthesis of 4-amino-6-*tert*-butyl-3-mercapto-1,2,4-triazine-5(4*H*)-one (**3**).

3.2. Pharmacological screening

3.2.1. Mosquito-larvicidal activity

A. stephensi is a malarial vector, breeds commonly stagnant water known to be the root cause of urban and domestic malaria. Insects have the tendency of developing resistance to insecticidal/larvicidal agents due to continual exposure. Therefore, there is always demand for new agents possessing the larvicidal property to control the disease vectors. In the present study, 3-*tert*-butyl-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**) were subjected to larvicidal study and all exhibited larvicidal activity but with different potentiality, the median lethal dose or LC_{50} values ranging from 21.7 to 102.8 μg/mL (Fig. 1) (Table 1). Among the tested compounds, (**5f**) was found to be highly effective followed by (**5g**) and (**5c**) with LC_{50} values 21.7, 25.7 and 39.8 μg/mL respectively. Compounds with moderate larvicidal activity include (**5b**) and (**5n**) with LC_{50} values 48.7 and 49.3 μg/mL. Among all the synthesized compounds (**5a**–**5n**), the compound (**5f**) exhibited highest larvicidal activity against *A. stephensi* mosquito. The presence of hydroxyl moiety may be the plausible reason for the toxicity of (**5f**) against mosquito larvae. Similar to hydroxyl moiety, the moiety with electron releasing groups have been reported to possess potential larvicidal activity [47], which in fact supports the rationale for the larvicidal activity of compound (**5f**). Relative potency indicates that novel compounds (**5a**–**5n**) tested in the present study are not as effective as that of Malathion. However (**5f**) may be considered as mosquito-larvicidal agent, the further studies on its half-life, persistence in the nature and non health hazards to non-target organisms may give insight into the usefulness of (**5f**) as a larvicidal agent for mosquito.

3.2.2. Antibacterial activity

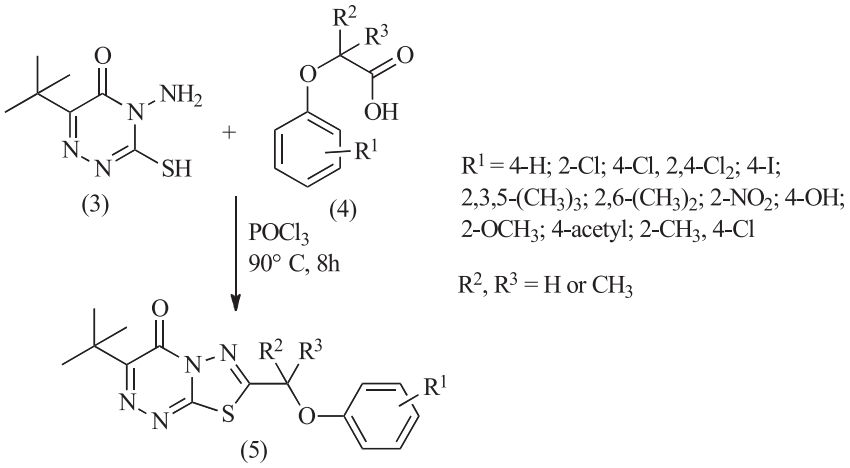
All the tested compounds (**5a**–**5n**) possess antibacterial activity in *in vitro* test system but with different sensitivity (Fig. 2) (Table 2). Compounds which gave the values >12 mm are considered as effective and following interpretations are drawn.

Among the tested compounds (**5g**) and (**5k**) are effective against only Gram positive bacteria and (**5h**) only against Gram negative bacterial species; (**5i**) and (**5m**) against Gram positive and Gram negative species. Among the tested compounds, highly effective include (zone inhibition > 18 mm), (**5g**) and (**5k**) (against *S. aureus*) (**5m**) (against *B. subtilis* and *P. aeruginosa*), (**5i**) against (*P. aeruginosa* and *K. pneumoniae*). Among the tested compounds, (**5i**) (18.4–22.4) and (**5m**) (18.7–25.7) exhibited a broad spectrum antibacterial activity against Gram positive and Gram negative species.

The reference standard, Streptomycin, a well known antibiotic drug was highly sensitive against Gram positive and Gram negative bacterial species. However, tested compounds cannot directly be compared with the reference standard for their efficacy since it depends on therapeutic index, which varies for different bioactive compounds depending upon the ratio between lethal dose and therapeutic dose.

3.2.3. Toxicity studies

Among the novel derivatives, (**5f**), (**5i**) and (**5m**) which showed comparatively high larvicidal and antibacterial activities were analyzed for oral acute toxicity considering their future potential utilities and safer mode of exposures. Median lethal dose (MLD)



Scheme 2. Syntheses of 3-*tert*-butyl-7-(aryloxymethyl)-4*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**).

was determined for each of these derivatives using Swiss albino mice (*Mus musculus*) as per the OECD guidelines [47]. Handling and animal experimentation were done according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals), India [48] with the ethical clearance certificate IAEC/KMC/69/2012. The oral MLD for (**5f**), (**5i**) and (**5m**) were found to be 1.36, 2.26 and 1.82 g/kg b.wt. respectively at 72 h duration. It is evident that the oral LD_{50} values of these compounds were within the range between 500 and 5000 mg/kg b.wt. Hence, as per the Hodge and Sterner Scale toxicity [49], these compounds can be classified as Slightly Toxic as far as mammalian oral route of exposure is concerned. The LD_{50} values obtained for (**5i**) and (**5m**) will be useful for determination of therapeutic index in view of their relatively good antibacterial activity.

4. Conclusion

The present study shows that among the thiadiazolotriazin-4-one derivatives (**5a**–**5n**), one with hydroxyl moiety at 4th position of phenyl ring (**5f**) possesses larvicidal activity against a malaria vector. This compound may further be screened against other insect larvae, which may open up the possibility of using the compound as a pesticide. Thiadiazolotriazin-4-one derivatives with chloro and acetyl groups at 4th position of the phenyl ring (**5i** and **5m**) exhibiting broad spectrum antibacterial activity against both Gram positive and Gram negative species may contribute to the development of potential antibiotics against bacterial pathogens.

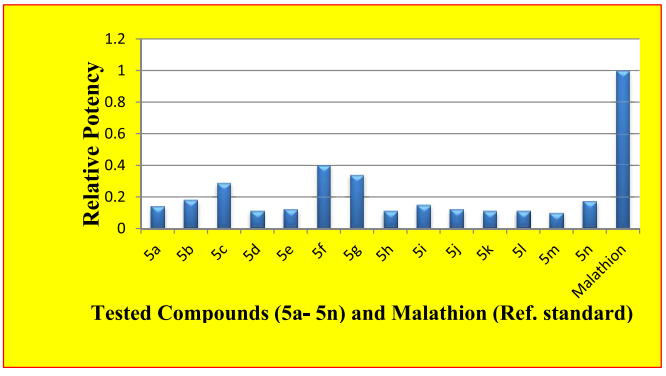


Fig. 1. Mosquito-larvicidal activity of [1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**) against ***Anopheles stephensi*** mosquito and **Malathion** (Ref. standard).

The three compounds with larvicidal and antibacterial activities exhibiting oral toxicity (LD_{50} values) which come under the category 'slightly toxic' stimulate further studies for the potential use of these lead compounds.

5. Experimental section

Melting points of the compounds (**5a**–**5n**) were determined in open capillary tubes and are uncorrected. The purity of synthesized compounds was checked by TLC observing single spot on Merck silica gel 60 F_{254} coated alumina plates. The structures of thiadiazolotriazin-4-ones (**5a**–**5n**) were confirmed by spectral studies. The IR spectra (cm^{-1}) were recorded on a **Shimadzu-FTIR 577** Infrared spectrophotometer in KBr pellets. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a **Brucker AMX-400 (400 MHz)** spectrometer using $CdCl_3$-*d* as solvent and TMS as the internal standard. The Mass spectra were recorded on **Perkin**–**Elmer 018444-Y**, **Triple Quadrupole LC/MS** Spectrometer. The novel synthesized compounds showed the molecular ion peak (*m*/*z*) equivalent to their molecular weight. The elemental analysis was carried out on an **Elementar Vario EL III** analyzer.

5.1. General procedure for the synthesis of 4-amino-6-tert-buty-3-mercapto-1,2,4-triazine-5(4H)-one (3) [50]

The synthesis of 4-amino-6-*tert*-butyl-3-mercapto-1,2,4-triazine-5(4*H*)-one (**3**) was done according to the procedure well described in the literature. An equimolar mixture of 3,3-dimethyl-2-oxobutanoic acid (trimethyl pyruvic acid) [CAS no: 815-17-8] (**1**) and thiocarbohydrazide (**2**) were refluxed in ethanol as solvent for 12 h. The progress of the reaction and the purity of the compound were checked by TLC. The reaction mixture was poured into crushed ice, the precipitate obtained was dried; yield and melting points were noted and recrystallized from ethanol (Scheme 1).

*5.2. General procedure for the syntheses of 3-tert-butyl-7-(aryloxymethyl)-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-ones (**5a**–**5n**)*

An equimolar mixtures of 4-amino-6-*tert*-butyl-3-mercapto-1,2,4-triazine-5(*H*)-one (0.01 mol) (**3**) and substituted aryloxyacetic acids (0.01 mol) (**4**) were condensed in presence of $POCl_3$ at 90 °C, for 8 h. The reactions were carried out in dry condition. The reaction mixtures were cooled and poured into crushed ice drop wise with

Table 1
Mosquito-larvicidal activity of [1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**) against ***Anopheles stephensi*** – a malaria vector and **Malathion** (Ref. standard).

Tested compounds	LC_{50} (μg/mL)	95% CI	LC_{90} (μg/mL)	95% CI	[b]Relative potency
5a	61.3	46.07–76.53	90.45	75.24–105.66	0.14
5b	48.7	35.81–60.13	73.2	56.63–85.92	0.18
5c	39.8	26.52–38.92	45.05	32.36–57.69	**0.29**
5d	102.8	60.54–92.81	115.2	97.14–133.27	0.11
5e	70.5	56.22–84.76	105.04	88.7–121.32	0.12
5f	21.7	14.72–28.69	32.11	20.72–43.45	**0.40**
5g	25.7	19.16–32.23	37.78	24.17–51.39	**0.34**
5h	79.2	62.23–96.04	120.98	103.36–138.58	0.11
5i	55.2	45.62–71.08	87.49	70.48–104.52	0.15
5j	72.8	55.44–89.58	113.57	96.08–131.04	0.12
5k	81.6	63.58–99.66	115.05	96.01–134.12	0.11
5l	77.5	61.46–93.56	124.77	106.13–143.39	0.11
5m	86.6	71.37–101.23	135.9	118.98–152.83	0.10
5n	49.3	38.28–60.32	71.45	57.66–85.24	0.17
[a]**Malathion**	8.7	6.66–10.79	12.01	8.39–15.69	1

Bold represents relatively more significant in terms of zone of inhibition.
CI – Confidence Interval.
[a] Malathion – Reference standard.
[b] Relative potency – LC_{50} standard/LC_{50} tested substance.

vigorous shaking, yielded solid product, filtered and recrystallized from ethanol to afford analytical samples (**5a**–**5n**) (Scheme 2).

*5.2.1. The analyses of 3-tert-butyl-7-[(aryloxy)methyl]-4H-[1,3,4] thiadiazolo[2,3-c]-1,2,4-triazin-4-one (**5a**–**5n**)*

*5.2.1.1. 3-tert-Butyl-7-[(4-chlorophenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5a**).* IR (KBr, cm^{-1}): 3075 (Ar–H), 2982 (–CH_3 of t-butyl), 1709 (–C=O), 1571 (–C=N stretch), 1524 and 1487 (C=C) and 945 (Ar–Cl). ^{1}H NMR ($CdCl_3$-*d*, δ ppm): 1.50 (9H, s, $3CH_3$), 5.36 (2H, s, $-CH_2$), 6.94 (2H, d, J = 8.8 Hz, 4-chlorophenoxy), 7.30 (2H, d, J = 8.8 Hz, chlorophenoxy). ^{13}C NMR (δ ppm): 27.67 ($3CH_3$ groups of *tert* butyl), 38.26 (C-atom of t-butyl), 65.28 ($-CH_2$), 116.23, 128.22, 129.97 and 146.90 (4-chlorophenoxy), 155.31, 158.93 and 161.78 (3C-atoms of thiadiazolotriazin-4-one) and 162.01 (–C=O of thiadiazolotriazin-4-one). LC–MS, [M^+], (*m*/*z*): 350.9/351.9. Anal. Cald for $C_{15}H_{15}ClN_4O_2S$: C, 55.35; H, 4.31; N, 15.97. Found: C, 55.33; H, 4.32; N, 15.98. m.p. 166–168 °C, yield: 72%.

*5.2.1.2. 3-tert-Butyl-7-[(2,4-chlorophenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5b**).* IR (KBr, cm^{-1}): 3098 (Ar–H), 2995 (–CH_3 of t-butyl), 1705 (–C=O), 1575 (–C=N stretch), 1521 and 1483 (C=C), 943 and 875 (Ar–Cl). ^{1}H NMR ($CdCl_3$-*d*, δ ppm): 1.50 (9H, s, $3CH_3$), 5.41 (2H, s, $-CH_2$), 6.97 (1H, d, J = 8.8 Hz, 2,4-dichlorophenoxy), 7.45 (1H, d, J = 2.4 Hz, 2,4-dichlorophenoxy) and 7.25 (dd, H, J = 2 Hz, 2,4-dichlorophenoxy). ^{13}C NMR (δ ppm): 27.67 (3 CH_3 groups of t-butyl), 38.26 (C-atom of t-butyl), 66.40 ($-CH_2$), 115.45, 124.75, 128.06, 128.81, 130.78 and 146.90 (2,4-dichlorophenoxy), 151.29, 158.96 and 161.28 (3C-atoms of thiadiazolotriazin-4-one) and 162.01 (–C=O of thiadiazolotriazin-4-one). LC–MS, [M^+], (*m*/*z*): 384.8/386.8/387.9/389.9. Anal. Cald for $C_{15}H_{14}Cl_2N_4O_2S$: C, 46.76; H, 3.66; N, 14.54. Found: C, 46.75; H, 3.67; N, 14.52. m.p. 180–182 °C, yield: 74%.

*5.2.1.3. 3-tert-Butyl-7-[(2,3,5-trimethylphenoxy)methyl]-4H-[1,3,4] thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5c**).* IR (KBr, cm^{-1}): 3021 (Ar–H), 2925 (–CH_3 of t-butyl), 2918 (–CH_3 of 2,3,5-trimethylphenoxy group), 1706 (–C=O), 1561 (–C=N stretch), 1518 and 1462 (C=C); ^{1}H NMR ($CdCl_3$-*d*, δ ppm): 1.51 (9H, s, $3CH_3$), 2.17 (3H, s, $-CH_3$, 2,3,5-trimethylphenoxy), 2.21 (3H, s, $-CH_3$ of

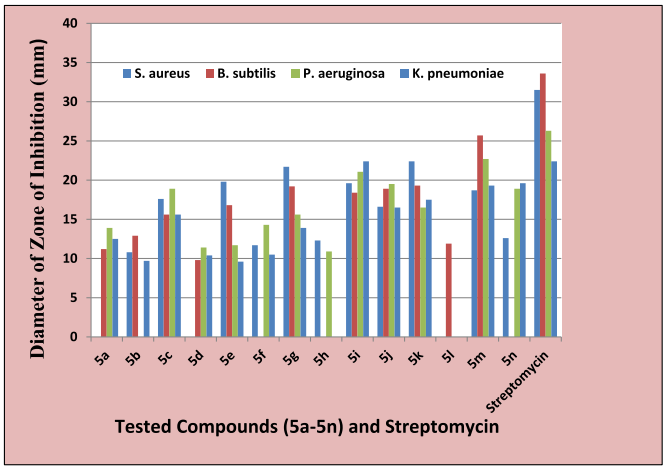


Fig. 2. Antibacterial activity (zone of inhibition) of [1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**) against Gram positive and Gram negative bacteria and **Streptomycin** (Ref. standard).

Table 2
Antibacterial activity (zone of inhibition) of [1,3,4]thiadiazolo[2,3-*c*][1,2,4]triazin-4-ones (**5a**–**5n**) against Gram positive and Gram negative bacteria and **Streptomycin** (Ref. standard).

Tested compounds (1 μg/mL) bacterial species	Diameter of zone of inhibition (mm ± SD)[a]			
	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*
5a	–	11.2	13.9 ± 0.59	12.5 ± 0.42
5b	10.8 ± 0.76	12.9 ± 0.54	–	9.7 ± 0.23
5c	17.6 ± 0.83	15.6 ± 0.67	18.9 ± 0.67	15.6 ± 0.49
5d	–	9.8 ± 0.59	11.4 ± 0.39	10.4 ± 0.37
5e	19.8 ± 0.74	16.8 ± 0.67	11.7 ± 0.45	9.6 ± 0.59
5f	11.7 ± 0.48	–	14.3 ± 0.47	10.5 ± 0.38
5g	**21.7 ± 0.97**	19.2 ± 0.61	15.6 ± 0.59	13.9 ± 0.58
5h	12.3 ± 0.54	–	10.9 ± 0.43	–
5i	19.6 ± 0.63	18.4 ± 0.62	**21.06 ± 0.48**	**22.4 ± 0.65**
5j	16.6 ± 0.74	18.9 ± 0.64	19.5 ± 0.59	16.5 ± 0.57
5k	**22.4 ± 0.69**	19.3 ± 0.42	16.5 ± 0.44	17.5 ± 0.51
5l	–	11.9 ± 0.53	–	–
5m	18.7 ± 0.74	**25.7 ± 0.65**	**22.7 ± 0.68**	19.3 ± 0.49
5n	12.6 ± 0.56	–	18.9 ± 0.75	19.6 ± 0.63
[b]**Streptomycin**	31.5 ± 0.84	33.6 ± 0.78	26.3 ± 1.08	22.4 ± 0.79

Bold represents relatively more significant in terms of zone of inhibition.
'0' Zone of inhibition <7 mm indicating no sensitivity.
[a] Mean values (n = 3).
[b] Reference standard (10 μg/disc) used as reference standard.

2,3,5-trimethylphenoxy), 2.29 (3H, s, $-CH_3$ of 2,3,5-trimethylphenoxy), 5.34 (2H, s, $-CH_2$), 6.54 (1H, s, 2,3,5-trimethylphenoxy), 6.73 (1H, s, 2,3,5-trimethylphenoxy). ^{13}C NMR (δ ppm): 11.51, 19.57 and 21.25 ($3CH_3$ groups on 2,3,5-trimethylphenoxy), 27.69 ($3CH_3$ groups of t-butyl), 38.23 (C-atom of t-butyl), 65.25 ($-CH_2$), 110.11, 122.34, 125.29, 136.09, 138.62 and 147.00 (2,3,5-trimethylphenoxy), 154.76, 159.11 and 161.85 (3C-atoms of thiadiazolotriazin-4-one) and 163.13 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 358.9. Anal. Cald for $C_{18}H_{22}N_4O_2S$: C, 60.31; H, 6.19; N, 15.63. Found: C, 60.30; H, 6.20; N, 15.62. m.p. 178–180 °C, yield: 72%.

*5.2.1.4. 3-tert-Butyl-7-[(2,6-dimethylphenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin -4-one (**5d**).* IR (KBr, cm^{-1}): 3035 (Ar–H), 2924 ($-CH_3$ of t-butyl), 1703 (−C═O), 1560 (−C═N stretch), 1516 and 1460 (C═C); ^1H NMR ($CdCl_3$-*d*, δ ppm): 1.51 (9H, s, $3CH_3$), 2.32 (6H, s, $2CH_3$ groups on 2,6-dimethylphenoxy), 5.12 (2H, s, $-CH_2$), 7.04 (3H, m, 2,6-dimethylphenoxy); ^{13}C NMR (δ ppm): 16.35 ($2CH_3$ groups on 2,6-dimethylphenoxy), 27.67 ($3CH_3$ groups of t-butyl), 38.23 (C-atom of t-butyl), 67.95 ($-CH_2$), 125.51, 129.41, 130.37 and 147.04 (2,6-dimethylphenoxy), 154.30, 159.14 and 161.84 (3C-atoms of thiadiazolotriazin-4-one) and 162.54 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 344.9/346.1. Anal. Cald for $C_{17}H_{20}N_4O_2S$: C, 59.28; H, 5.85; N, 16.27. Found: C, 59.26; H, 5.87; N, 16.28. m.p. 112–114 °C, yield: 76%.

*5.2.1.5. 3-tert-Butyl-7-[(2-nitrophenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5e**).* IR (KBr, cm^{-1}): 3007 (Ar–H), 2928 ($-CH_3$ of t-butyl), 1707 (−C═O), 1562 (−C═N stretch), 1542 and 1412 (sym and asym stretch of $-NO_2$), 1524 and 1468 (C═C). ^1H NMR ($CdCl_3$-*d*, δ ppm): 1.50 (9H, s, $3CH_3$), 5.51 (2H, s, $-CH_2$), 7.18–7.66 (3H, m, 2-nitrophenoxy), 7.97 (1H, dd, 2-nitrophenoxy); ^{13}C NMR (δ ppm): 27.67 ($3CH_3$ groups of t-butyl), 38.27 (C-atom of *tert* butyl), 66.57 ($-CH_2$), 115.60, 119.21, 123.31, 126.42, 134.73 and 146.93 (2-nitrophenoxy), 150.02, 159.05 and 160.79 (3 C-atoms of thiadiazolotriazin-4-one) and 161.97 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 362.3. Anal. Cald for $C_{15}H_{15}N_5O_4S$: C, 49.85; H, 4.18; N, 19.38. Found: C, 49.86; H, 4.19; N, 19.37. m.p. 142–144 °C, yield: 70%.

*5.2.1.6. 3-tert-Butyl-7-[(4-hydroxyphenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5f**).* IR (KBr, cm^{-1}): 3351 (-OH), 3098 (Ar–H), 2982 (CH_3 of -butyl), 1709 (−C═O),1571 (−C═N stretch), 1524 and 1487 (C═C); ^1H NMR ($CdCl_3$-*d*, δ ppm): 1.51 (9H, s, $3CH_3$), 5.41 (2H, s, $-CH_2$), 6.98 (d, 2H, J = 8.8 Hz, 4-hydroxyphenoxy), 7.26 (d, 2H, J = 8.8 Hz, 4-hydroxyphenoxy), 8.5 (1H, s, −OH). ^{13}C NMR (δ ppm): 27.61 ($3CH_3$ groups of t-butyl), 38.25 (C-atom of t-butyl), 66.34 ($-CH_2$), 124.45, 128.79, 130.19 and 136.90 (4-hydrophenoxy), 151.31, 158.85 and 161.21 (3C-atoms of thiadiazolotriazin-4-one) and 163.12 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 333.4. Anal. Cald for $C_{15}H_{16}N_4O_3S$: C, 54.20; H, 4.85; N, 16.86. Found: C, 54.19; H, 4.86; N, 16.85. m.p. 114–116 °C, yield: 68%.

*5.2.1.7. 3-tert-Butyl-7-[(2-methoxyphenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5g**).* IR (KBr, cm^{-1}): 3015 (Ar–H), 2938 (CH_3 of t-butyl), 1708 (−C═O), 1564 (−C═N stretch), 1526 and 1466 (C═C); ^1H NMR ($CdCl_3$-*d*, δ ppm): 1.51 (9H, s, $3CH_3$), 3.52 (3H, s, $-OCH_3$), 5.52 (2H, s, $-CH_2$), 7.16–7.62 (3H, m, 2-methoxyphenoxy), 7.98 (1H, dd, 2-methoxyphenoxy); ^{13}C NMR (δ ppm): 27.63 ($3CH_3$ groups of t-butyl), 31.23 (C atom of $-OCH_3$), 38.27 (C-atom of t-butyl), 66.55 ($-CH_2$), 115.62, 119.23, 123.35, 127.44, 135.71 and 145.92 (2-methoxyphenoxy), 151.02, 158.15 and 161.89 (3 C-atoms of thiadiazolotriazin-4-one) and 162.97 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 362.3. Anal. Cald for $C_{16}H_{18}N_4O_3S$: C, 55.48; H, 5.24; N, 16.17. Found: C, 55.46; H, 5.25; N, 16.16. m.p. 172–174 °C, yield: 74%.

*5.2.1.8. 3-tert-Butyl-7-(phenoxymethyl)-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5h**).* IR (KBr, cm^{-1}): 3065 (Ar–H), 2974 (CH_3 of t-butyl), 1701 (−C═O), 1593 (−C═N stretch), 1564 and 1516 (C═C); ^1H NMR ($CdCl_3$-*d*, δ ppm): 1.50 (9H, s, $3CH_3$), 5.38 (2H, s, $-CH_2$), 6.98–7.37 (5H, m, phenoxy); ^{13}C NMR (δ ppm): 27.68 ($3CH_3$ groups of t-butyl), 38.24 (C-atom of t-butyl), 64.99 ($-CH_2$), 114.88, 123.05, 130.03, 146.95 (6C atoms of phenoxy), 156.76, 159.08 and 161.90 (3C-atoms of thiadiazolotriazin-4-one) and 162.55 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 317.08. Anal. Cald for $C_{15}H_{16}N_4O_2S$: C, 56.94; H, 5.10; N, 17.71. Found: C, 56.93; H, 5.11; N, 17.70. m.p. 150–152 °C, yield: 76%.

*5.2.1.9. 3-tert-Butyl-7-[2-(4-chlorophenoxy)propan-2yl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5g**).* IR (KBr, cm^{-1}): 3011 (Ar–H), 2937 (CH_3 of t-butyl), 2922 (CH_3 groups), 1704 (−C═O), 1562 (−C═N stretch), 1521 and 1468 (C═C), 941 (Ar–Cl); ^1H NMR ($CdCl_3$-*d*, δ ppm): 1.50 (9H, s, $3CH_3$), 1.80 (6H, s, 2 CH_3 groups), 6.90 (2H, d, J = 8.8 Hz, 4-chlorophenoxy), 7.28 (2H, d, J = 8.8 Hz, 4-chlorophenoxy); ^{13}C NMR (δ ppm): 23. 15 (2C atoms of CH_3), 27.69 ($3CH_3$ groups of t-butyl), 38.27 (C-atom of t-butyl), 67.98 ($-CH_2$), 125.52, 129.43, 130.39 and 147.07 (4-chlorophenoxy), 154.31, 159.16 and 161.87 (3 C-atoms of thiadiazolotriazin-4-one) and 162.59 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 379.8/381.8. Anal. Cald for $C_{17}H_{19}ClN_4O_2S$: C, 53.89; H, 5.05; N, 14.79. Found: C, 53.88; H, 5.06; N, 14.78. m.p. 66–68 °C, yield: 70%.

*5.2.1.10. 3-tert-Butyl-7-[(2-chlorophenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5j**).* IR (KBr, cm^{-1}): 3015 (Ar–H), 2926 (CH_3 of t-butyl), 1705 (−C═O), 1564 (−C═N stretch), 1522 and 1464 (C═C) and 932 (Ar–Cl); ^1H NMR ($CdCl_3$-*d*, δ ppm): 1.50 (9H, s, $3CH_3$), 5.08 (2H, s, $-CH_2$), 7.58 (1H, d, 2-chlorophenoxy), 7.73 (1H, d, 2-chlorophenoxy), 7.31 and 7.54 (2H, 2t, 2-chlorophenoxy); ^{13}C NMR (δ ppm): 27.69 ($3CH_3$ groups of t-butyl), 38.27 (C-atom of t-butyl), 67.98 ($-CH_2$), 125.52, 129.43, 130.39, 132.25, 134.26 and 147.07 (2-chlorophenoxy), 154.31, 159.16 and 161.87 (3C-atoms of thiadiazolotriazin-4-one) and 162.59 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 351.82/353.9. Anal. Cald for $C_{15}H_{15}ClN_4O_2S$: C, 51.35; H, 4.31; N, 15.96. Found: C, 51.35; H, 4.32; N, 15.96. m.p. 116–118 °C, yield: 74%.

*5.2.1.11. 3-tert-Butyl-7-[(2, 4, 5-trichlorophenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5k**).* IR (KBr, cm^{-1}): 3025 (Ar–H), 2982 (CH_3 of t-butyl), 1710 (−C═O), 1571 (−C═N stretch), 1532 and 1491 (C═C). 945, 927 and 906 (Ar–Cl); ^1H NMR ($CDCl_3$-*d*, δ, ppm): 1.50 (9H, s, $3CH_3$), 5.42 (2H, s, $-CH_2$), 7.14 (1H, s, 2,4,5-trichlorophenoxy), 7.54 (1H, s, 2,4,5-trichlorophenoxy); ^{13}C NMR (δ ppm): 27.66 ($3CH_3$ groups of t-butyl), 38.28 (C-atom of t-butyl), 66.55 ($-CH_2$), 116.22, 122.95, 127.33, 131.66, 131.81 and 146.85 (2,4,5-trichlorophenoxy), 151.42, 158.88 and 160.62 (3C-atoms of thiadiazolotriazin-4-one) and 162.09 (−C═O of thiadiazolotriazin-4-one). LC–MS, [M^+], (m/z): 420.8/422.7/424.8/426.8; Anal. Cald for $C_{15}H_{13}Cl_3N_4O_2S$: C, 42.92; H, 3.12; N, 13.35. Found: C, 42.91; H, 3.14; N, 13.34. m.p. 120–122 °C, yield: 78%.

*5.2.1.12. 3-tert-Butyl-7-[(4-iodophenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5l**).* IR (KBr, cm^{-1}): 3011 (Ar–H), 2988 (CH_3 of t-butyl), 1707 (−C═O), 1574 (−C═N stretch), 1534 and 1497 (C═C); ^1H NMR ($CdCl_3$-*d*, δ ppm): 1.51 (9H, s, $3CH_3$), 5.42 (2H, s, $-CH_2$), 6.97 (2H, d, J = 8.8 Hz, 4-iodophenoxy), 7.24 (2H, d, J = 8.8 Hz, 4-iodophenoxy); ^{13}C NMR (δ ppm): 27.62 ($3CH_3$ groups of t-butyl), 38.27 (C-atom of t-butyl), 66.37 ($-CH_2$ group), 125.45, 128.85, 131.22 and 137.90 (4-iodophenoxy), 152.34, 159.87 and

162.44 (3C-atoms of thiadiazolotriazin-4-one) and 162.52 (−C═O of thiadiazolotriazin-4-one). LC−MS, [M^+], (*m*/*z*): 443.3. Anal. Cald for $C_{15}H_{15}IN_4O_2S$: C, 56.97; H, 5.06; N, 15.03. Found: C, 56.96; H, 5.07; N, 15.62. m.p. 156–158 °C, yield: 76%.

*5.2.1.13. 7-[(4-acetylphenoxy)methyl]3-tert-butyl-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5m**).* IR (KBr, cm^{-1}): 3021 (Ar−H), 2970 (CH_3 of t-butyl), 2978 (CH_3), 1708 (−C═O), 1690 (−C═O, of acetyl), 1572 (−C═N stretch), 1525 and 1402 (C═C); ^{1}H NMR ($CdCl_3$-*d*, *δ* ppm): 1.51 (9H, s, $3CH_3$), 2.29 (3H, s, CH_3), 5.40 (2H, s, $-CH_2$), 6.81 (2H, d, *J* = 8.8 Hz, 4-acetylphenoxy), 7.16 (2H, d, *J* = 8.8 Hz, 4-acetylphenoxy); ^{13}C NMR (*δ* ppm): 21.10 (CH_3 group on 4-acetyl), 27.69 ($3CH_3$ groups of t-butyl), 38.29 (C-atom of t-butyl), 66.27 ($-CH_2$), 112.65, 126.96, 128.69, 130.04, 131.34 and 138.34 (4-acetylphenoxy), 147.22, 152.74 and 157.81 (3C-atoms of thiadiazolotriazin-4-one) and 162.25 (−C═O of thiadiazolotriazin-4-one). LC−MS, [M^+], (*m*/*z*): 359.4; Anal. Cald for $C_{17}H_{18}N_4O_3S$: C, 56.97; H, 5.06; N, 15.63. Found: C, 56.96; H, 5.07; N, 15.62. m.p. 176–178 °C, yield: 72%.

*5.2.1.14. 3-tert-Butyl-7-[(4-chloro-2-methylphenoxy)methyl]-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-4-one (**5n**).* IR (KBr, cm^{-1}): 3028 (Ar−H), 297 (CH_3 of t-butyl), 1710 (−C═O), 1571 (−C═N stretch), 1523 and 1398 (C═C), 937 (Ar−Cl); ^{1}H NMR ($CdCl_3$-*d*, *δ* ppm): 1.50 (9H, s, $3CH_3$), 2.27 (3H, s, CH_3), 5.36 (2H, s, $-CH_2$), 6.79 (H, s, *J* = 8.8 Hz, 4-chloro-2-methylphenoxy), 7.14 (H, d, *J* = 2.4 Hz, 4-chloro-2-methylphenoxy), 7.16 (2H, dd, *J* = 2 Hz, 4-chloro-2-methylphenoxy), 7.19 (H, d, *J* = 2.4 Hz, 4-chloro-2-methylphenoxy); ^{13}C NMR (*δ* ppm): 16.10 (CH_3 group on 4-chloro-2-methylphenoxy), 27.68 ($3CH_3$ groups of t-butyl), 38.27 (C-atom of t-butyl), 65.23 ($-CH_2$), 112.45, 126.86, 127.69, 129.04 and 131.34 (4-chloro-2-methylphenoxy), 146.92, 153.54 and 158.91 (3C-atoms of thiadiazolotriazin-4-one) and 162.05 (−C═O of thiadiazolotriazin-4-one). LC−MS, [M^+], (*m*/*z*): 364.8/365.9. Anal. Cald for $C_{16}H_{17}ClN_4O_2S$: C, 56.67; H, 4.70; N, 15.36. Found: C, 56.66; H, 4.72; N, 15.35. m.p. 142–144 °C, yield: 74%.

Acknowledgements

The authors are grateful to Rt Rev. Dr Aloysius Paul D' Souza, the Bishop of Mangalore Diocese for the financial assistance. Dr Mahagundappa R. Maddani for his help in the manuscript. The Head, NMR Research Centre, SAIF-Cochin-22 for ^{1}H NMR, ^{13}C NMR spectroscopy and CHN analyses, USIC-Mangalore University for FT-IR analyses, Acquity Labs, Bengaluru and Syngenta, Goa for Mass spectroscopy.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.06.072.

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