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3,5-DISUBSTITUTED TETRAHYDRO-2*H*-1,3,5-THIADIAZINE-THIONE ESTER DERIVATIVES AND THEIR ANTIMICROBIAL EVALUATION

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Abstract – A diverse set of 3,5-disubstitited tetrahydro-2*H*-1,3,5-thiadiazine-thiones (THTT) and their ester derivatives were synthesized in good to excellent yields. The ester derivatives were screened for their antibacterial and antifungal potential and compared with their acidic counterparts. Structure-activity relationship revealed that the THTTs with N-3 carboxylic functionality have very promising potential against all tested pathogens in general. However, ester analogues showed suppressed or no activities against the same pathogens thus indicate that these compounds can be used as a template study for the development of improved antimicrobial agents/prodrugs.

INTRODUCTION

Tetrahydro-2*H*-1,3,5-thiadiazine-thione (THTT) derivatives have already been received extraordinary considerations because of their exhibiting pharmacological properties, including antibacterial,[1] antifungal (**1**),[1-3] anticancer (**2**),[4] antiprotozoa (**3**)[5] (Figure 1), leishmanicidal[6] and antitubercular activities.[7] Motifs having THTT nucleus are recognized as bio-labile prodrugs,[8] the pharmacologically inactive drug derivatives. Many derivatives from this class of compounds have been used as protected drug molecules for curing human diseases; for instance, 3,5-bis(phenylmethyl)-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione (**4**) (Figure 1) is used for arteriosclerosis treatment[9] and as an antiepileptic prodrug.[10] In general, prodrugs

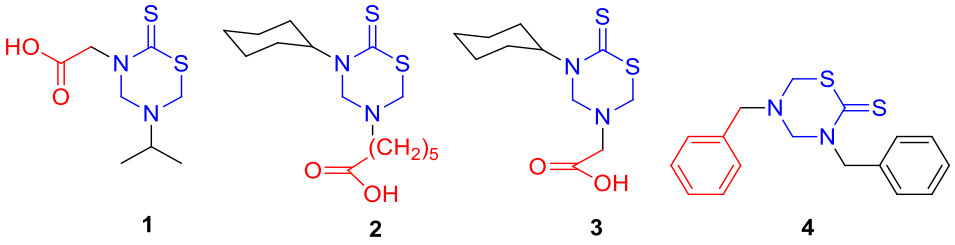


Figure 1. Important drug molecules containing THTT skeleton

are designed to maximize the availability of the active drug to its target site, through manipulation of the physicochemical and pharmacokinetic profile of the drugs.[11,12]

The most frequent rationale behind the design of a prodrug is to introduce lipophilicity and protect hydrogen bonding groups (such as alcohols and carboxylic acids) of an active compound; commonly by ester functionality, thus facilitating immobility against any pharmacological target and also enhancing the chemical stability against a pH range during the absorption phase.[11,12] In addition, the ester prodrugs are known to have a competitive advantage of enhanced passive intestinal absorption.[13,14] There are several ester prodrugs found in market like benazepril (**5**), fosinopril (**6**) (Figure 2) and pivampicillin.[13,15] These prodrugs are metabolized to the active drug molecule *via* ubiquitous esterases with varying rates depending upon chemical nature and type of biological genus.[11]

To the above illustration, we became interested to introduce ester functionality at N-3 position of THTT nucleus of type (**3**) (Figure 1); aiming to generate more pharmacologically interesting compounds in continuation to our recent report.[6b]

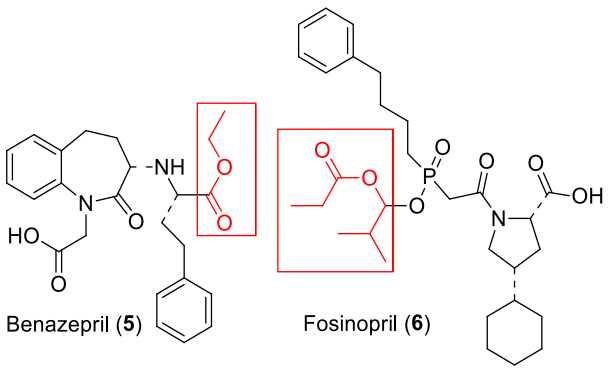
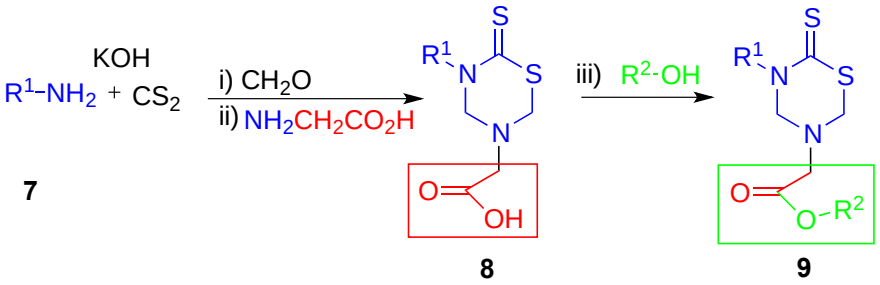


Figure 2. Important ester prodrug with improved absorption

The effect of the substituents at N-3 and N-5 positions of THTT framework on antimicrobial activity[16] and toxicity has been already recognized.[5,17,18] Furthermore, the lipophilic groups at N-3 and hydrophilic ones

at N-5 position are also developed.[19] However, to the best of our knowledge introduction of ester functionality at N-3 position of THTT nucleus and its related pharmacology is not explored yet except to our recent studies on antileismaniasis.[6b]

Herein, we report a diverse series of tetrahydro-2*H*-1,3,5-thiadiazine-6-thione ester derivatives (THTT) of type (**9**) by utilizing THTT acidic precursors (**8**) (Scheme 1) along with their comparative antifungal and antibacterial potential.



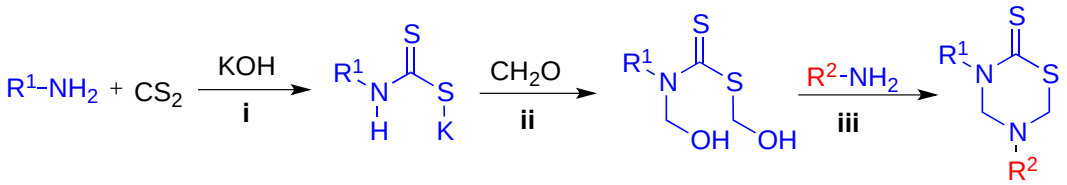
Scheme 1. Tetrahydro-2*H*-1,3,5-thiadiazine-6-thione (THTT) ester derivatives of type **9**

RESULTS AND DISCUSSION

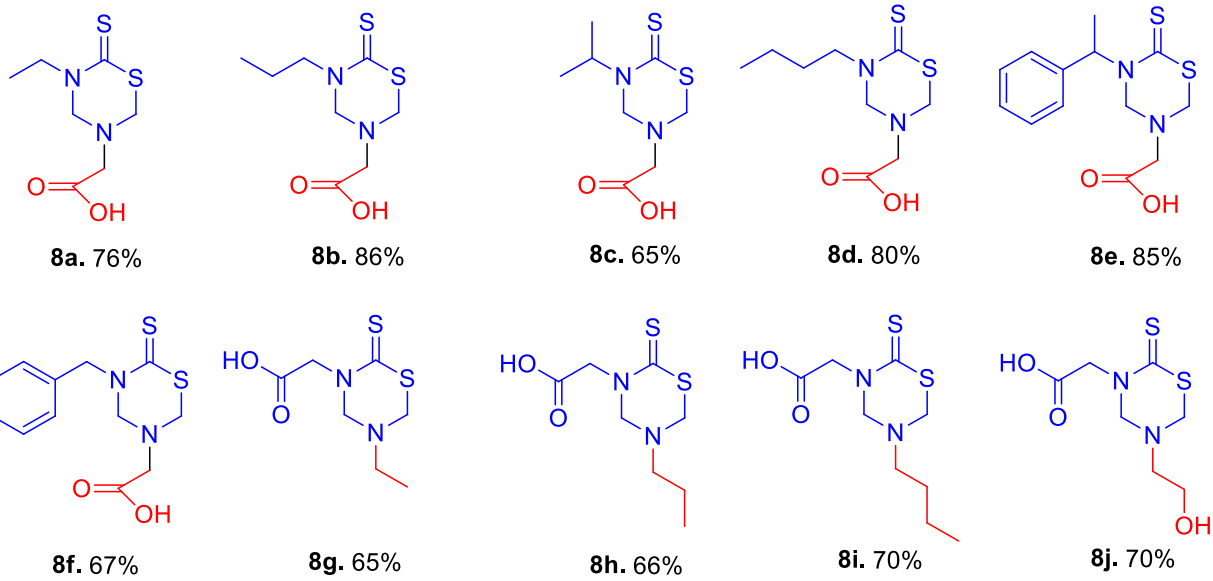
Chemistry. As a starting point of our study, we synthesized two different sets (10 examples) of tetrahydro-2*H*-1,3,5-thiadiazine-6-thiones (**8a–f**) and tetrahydro-2*H*-1,3,5-thiadiazine-2-thiones (**8g–j**) in overall excellent yields (Table 1) at 10 mmol scale by following literature protocol.[3,6b,20] Initially, reaction of appropriate alkylamine/aralkylamine/glycine (**7a–j**) with carbon disulfide and potassium hydroxide provided corresponding dithiocarbamate salts (**10a–j**).[19] The dithiocarbamate salts (**10a–j**) were then treated with formaldehyde solution to give intermediates of type (**11**). Afterwards, intermediates (**11a–j**) were allowed to react with glycine or primary amines to deliver THTT derivatives **8a–j** in good to excellent yields (65–86%, Table 1). Diversity was successfully achieved by introducing glycine or various substituted primary amines at N-3 and N-5 positions of THTT core.

The resulting compounds were then subjected to transform into diverse N-3 ester derivatives of type (**9**). For the conversion of THTT precursors (**8**) into N-3 ester moieties (**9**), optimization of the reaction conditions were carried out initially by adapting Avila-Zárraga et al. protocol,[21] employing slurry of potassium hydroxide in dimethyl sulfoxide and iodomethane (entries 1–6, Table 2) and Ramalinga et al. protocol[22] under reflux conditions with a desired alcohol in the presence of iodine (entries 7–9, Table 2). Ultimately, these protocols with variable additives, solvent, temperature and time duration were found to

Table 1. Synthesis of substituted tetrahydro-2*H*-1,3,5-thiadiazine scaffolds **8a-j**[a,b]



R^1= alkyl, aralkyl, carboxymethyl
R^2= alkyl, carboxymethyl



8a. 76% **8b.** 86% **8c.** 65% **8d.** 80% **8e.** 85%

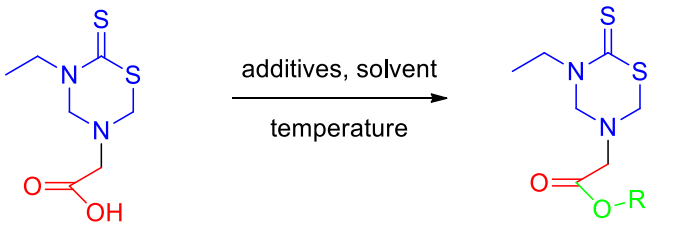
8f. 67% **8g.** 65% **8h.** 66% **8i.** 70% **8j.** 70%

[a] Reaction conditions: **i)** 1º amines **7a-j** (10 mmol), CS_2 (6 equiv), KOH (20%, 1 equiv), EtOH (10 mL), rt, 3 h; **ii)** CH_2O (35% in water, 2.2 equiv), rt, 1 h; **iii)** 1º amines (1 equiv), phosphate buffer (pH 7.8, 20 mL), rt, 1 h. [b] Isolated yields.

be ineffective for this transformation. It was also observed that THTT moiety was sensitive to elevated temperatures and longer heating time which resulted in decomposition of the compound.

Gratifyingly, the subsequent screening of reaction conditions revealed that esterification of THTT could be achieved by the use of thionyl chloride in ethanol as solvent.[23] It was observed that 1.5 equiv of thionyl chloride in ethanol for 2 h under ice cooling (0–4 ºC), was optimal to afford corresponding ester (**9**) in good isolated yield (entry 10, Table 2, 60% desired product by flash chromatography). After the optimized conditions in hands, we further proceeded to expand modularity in ester functionality. It is noteworthy that this protocol found applicable to obtain various cyclic and acyclic ester-functionalities (**9a-h**, Table 3) in good to excellent yields (60–85%) by using the corresponding alcohols. All new compounds were characterized by spectral techniques including ^{1}H NMR, ^{13}C NMR, DEPT-90/135, IR, MS, HRMS and are described in experimental section.

Table 2. Optimization of reaction conditions for esterification of THTT **8a**[a]



8a **9**

R = methyl, ethyl

Entry	Additive	Base	Solvent	Temp (°C)	Time (h)	Yield (%)[b]
1.	MeI	KOH	DMSO	25	16	–
2.	MeI	KOH	DMSO	reflux	16	–
3.	MeI	KOH	DMSO	90[c]	1	–
4.	MeI	KOH	DMSO	150[c]	1	–
5.	MeI	NaOH	DMSO	25	16	–
6.	MeI	NaOH	DMSO	reflux	16	–
7.	I_2	–	EtOH	25	16	–
8.	I_2	–	EtOH	reflux	16	–
9.	I_2	–	EtOH	60[c]	1	–
10.	$SOCl_2$	–	EtOH	0-4	2	60

[a] Reaction conditions: THTT **8a** (1 mmol), additive (1.5 equiv), base (1.5 equiv), solvent (1.5 mL). [b] Isolated yields. [c] Microwave irradiation.

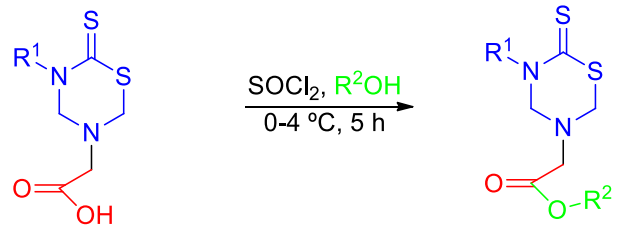
To inspect the prodrug phenomenon, preliminary studies were performed for enzymatic hydrolysis of ester derivative (**9h**) along with its acidic counterpart (**8e**) by using pig liver esterase (PLE). Experiments showed that the ester derivative was indeed hydrolyzable by esterase to the same metabolite as of corresponding carboxylic acid analogue under the similar set of reaction conditions (see Supporting Information).

In order to establish structure-activity relationship, we also investigated the in-vitro antimicrobial activity (antifungal, Table 4 and antibacterial, Table 5); whereas different biological activities are currently under examine in our laboratories.

Biological Evaluation.

Antifungal activity. Antifungal activity of synthesized THTT analogues was examined by employing the standard agar tube dilution protocol with the concentration of 200 μM in DMSO,[24-26] against five (05) human pathogens named as *Trichphyton rubrum* (clinical isolate), *Candida albicans* (ATCC 2091), *Aspergillus niger* (ATCC 32611), *Microsporum canis* (ATCC 11622), and *Fusarium lini* (ATCC 46066);

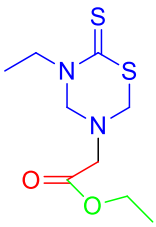
Table 3. Transformation of 3,5-disubstituted THTT analogues **8a-b/8d-e** into corresponding alkyl/aryl substituted esters derivatives **9a-h**[a-b]



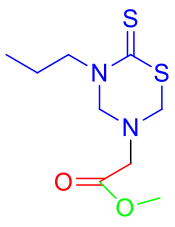
8a-b
8d-e

9a-h

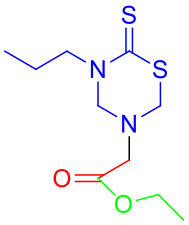
R^1 = ethyl, propyl, butyl, 1-phenylethyl
R^2 = methyl, ethyl, benzyl, phenylpropyl, 2-methylcyclohexyl



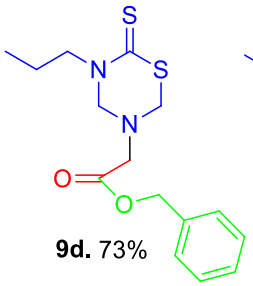
9a. 60%



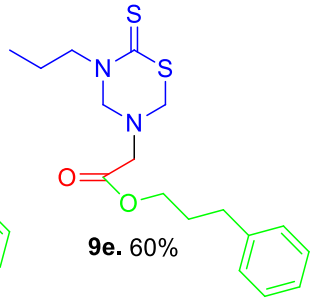
9b. 78%



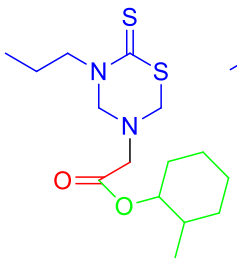
9c. 76%



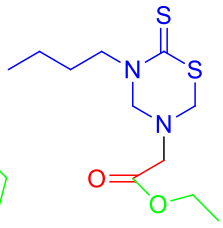
9d. 73%



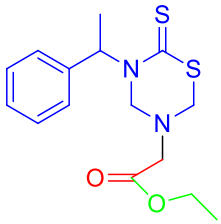
9e. 60%



9f. 62%



9g. 85%



9h. 75%

[a] Reaction conditions: THTT **8a-b**/**8d-e** (1 mmol), $SOCl_2$ (1.5 equiv), R^2OH (1.5 mL), 0-4 ºC, 2 h. [b] Isolated yields.

and was recorded as percent growth inhibition. Amphotericin B as a control drug with minimum inhibitory concentration (MIC) of 20.7 µg/mL was used for *A. niger* while miconazole was used with MIC values of 97.8, 113.1, 98.1 and 73.5 µg/mL for *T. rubrum*, *C. albicans*, *M. canis* and *F. lini*, respectively. Sabouraud dextrose agar (SDA) was used as media with pH 5.5–5.6. Initial testing of THTT analogues was performed at 400 µg/mL concentration before final screening at 200 µg/mL. A 4 mm diameter piece from a 7 days old culture fungus was inoculated in tubes then incubation period was kept for 7 days at 27 ± 1 ºC. Growth was determined by measuring linear growth (mm) and growth inhibition percentage (GI%) calculated with reference to the negative control. Three (03) replicate testings with same procedure were performed and the results are summarized in Table 4 for comparison purposes.

Table 4. Antifungal activities of THTT analogues (200 μg/mL) as % growth inhibition zone (± SEM)[a]

Entry	Compound	Fungi				
		Trichphyton rubrum	*Candida albicans*	*Aspergillus niger*	*Microsporum canis*	*Fusarium lini*
1.	**8a**	100	100	–[b]	90.5 ± 0.5	100
2.	**8b**	–	80.2 ± 1.2	–	40.8 ± 2.4	50.2 ± 1.5
3.	**8c**	–	10.8 ± 1.6	–	–	30.5 ± 2.0
4.	**8d**	50.6 ± 1.4	100	–	60.5 ± 1.0	30.8 ± 1.2
5.	**8e**	100	100	100	100	100
6.	**8f**	30.2 ± 2.0	100	–	50.6 ± 1.6	80.2 ± 1.5
7.	**8g**	–	40.5 ± 2.5	–	–	20.5 ± 1.8
8.	**8h**	–	–	–	–	–
9.	**8i**	–	50.6 ± 1.2	–	–	90.4 ± 0.2
10.	**8j**	–	50.8 ± 1.0	–	–	–
11.	**9b**	–	–	–	–	–
12.	**9c**	–	–	–	–	80.6 ± 0.8
13.	**9d**	70.6 ± 0.8	20.2 ± 2.2	–	–	–
14.	**9g**	–	40.4 ± 1.6	–	–	10.4 ± 2.2
15.[c]	Miconazole	100*1	100*2	–	100*3	100*4
16.[d]	Amphotericin B			100		

[a] Result represents as mean of triplicate ± standard error of mean (SEM); testing incubation period 7 days at 27 ± 1 ºC. [b] No inhibition observed. [c] MIC for miconazole; *1 = 97.8 μg mL^{-1}, *2 = 113.1 μg mL^{-1}, *3 = 98.1 μg mL^{-1}, *4 = 73.5 μg mL^{-1}. [d] MIC for amphotericin B = 20.7 μg mL^{-1}.

It pleased us that the parent THTT analogues **8a-f** with N-3 carboxylic functionality (entry 1–6, Table-4) showed very significant potential against all tested pathogens in general, however, as anticipated ester analogues (**9b-d**, **g**) showed lower or no activity against the same pathogens (entry 11–14, Table 4). For instance, on comparing the antifungal activity of compound–**8b** which showed very significant response against *C. albicans* (GI% = 80.2) while its methyl and ethyl ester analogues (**9b-c**) were found in-active (entry 2 *vs* 11-12, Table 4). In addition, its benzyl ester (**9d**) showed weak activity against the same pathogen (GI% = 20.2; entry 13, Table 4). The suppressed activity of esters did not surprise us as ester prodrug are known to activate under biological conditions by converting back to carboxylic group *via* the

action of ubiquitous esterases. Same pattern was identified for compound–**8d** which showed a potent/significant activity for *C. albicans* (GI% = 100) while its methyl ester (**9g**) found weakly active (GI% = 40.4; entry 4 *vs* 14, Table 4). These reduced activity trends showed the prodrug criterion of ester derivatives and suggested a good source for the production of improved antifungal prodrugs against *C. albicans* (ATCC 2091). The antifungal results in Table 4 also explained the impact of hydrophilic groups at N-3 and lipophilic groups at N-5 positions of THTT nucleus. For instance, it is noteworthy that compound–**8a** with N-3 carboxylic and N-5 ethyl substituent, showed significant activities against all organisms tested (GI% = 90.5–100) except *A. niger*. On the other hand, compound–**8g** with same functionality but with altered positions found almost inactive (GI% = 0–40.5; entry 1 vs 7, Table 4). The same trend was observed in comparison of compound–**8b** with **8h** and compound–**8d** with **8i** (entry 2 vs 8; entry 4 vs 9, Table 4).

In conclusion, THTT analogues–**8a** and **8d–f** were found to be more potent, and thus, can be used for the development of improved antifungal agents. Moreover, their methyl ester analogues can be investigated as potential prodrugs. In this context, more biological studies are in progress.

Antibacterial activity. All the synthesized THTT analogues (**8-9**) were also tested for their in vitro antibacterial activity against *Escherichia coli* (ATCC 25922), *Shigella flexenari* (clinical isolate), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853) by using Microplate ALAMAR Blue Assay (MABA) literature protocols.[27] The samples including standard drug ofloxacin were used in concentration of 50 μg/mL in DMSO for comparison purposes. Antibacterial activity was recorded as percent inhibition and compared with the standard drug ofloxacin. Mueller Hinton media was used for organism's growth. Inoculums were adjusted to 0.5 McFarland turbidity index. The 96 well plates were incubated for the period of 18–20 h. Alamar Blue Dye was dispensed in each well and shaken at 80 rpm in a shaking incubator for 2–3 h. Absorbance was recorded at 570 and 600 ηm by the ELISA reader and the results with three (03) replicate testings were summarized in Table 5.

It is noticed that all the new THTT derivatives (**8-9**) exhibited no response against *E. coli*, *P. aeruginosa* and very weak response of compound–**8f**, **8j** and **9b** against *S. flexenari* was observed (GI% = 1.85–6.88; entry 6, 10–11, Table 5). However, the evidence of antibacterial activity was found against *S. aureus* within 3 to 48 percent growth inhibition range for the whole series.

Table 5. Antibacterial activities of THTT analogues (50 μg mL^{-1})[a]

Entry	Compound	Growth inhibition zone (%)			
		Escherichia coli	*Shigella flexenari*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
1.	**8a**	–[b]	–	20.07 ± 0.5	–
2.	**8b**	–	–	22.37 ± 0.2	–
3.	**8c**	–	–	27.75 ± 0	–
4.	**8d**	–	–	4.77 ± 0.8	–
5.	**8e**	–	–	48.34 ± 0.8	–
6.	**8f**	–	3.51 ± 0.8	31.03 ± 0	–
7.	**8g**	–	–	–	–
8.	**8h**	–	–	4.27 ± 0.1	–
9.	**8i**	–	–	17.30 ± 0.4	–
10.	**8j**	–	6.88 ± 0.2	24.36 ± 0.5	–
11.	**9b**	–	1.84 ± 0.9	7.59 ± 0.1	–
12.	**9c**	–	–	16.25 ± 0.2	–
13.	**9d**	–	–	3.32 ± 0.3	–
14.	**9g**	–	–	7.78 ± 0.1	–
15.	ofloxacin[c]	83.79 ± 0.8	85.24 ± 0.5	88.05 ± 0.6	82.45 ± 0.7

[a] Result represents as mean of triplicate ± standard error of mean (SEM). [b] No inhibition observed.

[c] Standard drug ofloxacin (50 μg/mL) is used.

CONCLUSION

A successful catalyst free approach to prepare a diverse set of alkyl and cycloalkyl ester derivatives of 3,5-disubstituted tetrahydro-2*H*-1,3,5-thiadiazine-thiones (THTT) was developed. This efficient and smooth protocol employed THTT acidic precursors and various alcohols in the presence of thionyl chloride to deliver good to excellent yields. All the synthesized compounds were screened for their antifungal and antibacterial potential. The THTT nucleus with N-3 carboxylic acid showed very promising potential against all tested fungi in general. However, ester analogues showed suppressed or no activity against the same fungi, thus can be used as a template study for the development of improved antifungal agents/prodrugs. Preliminary studies showed that the ester derivatives were indeed hydrolysable by esterase. Currently, a more detailed biological investigation is in progress at our laboratories.

EXPERIMENTAL

General experimental details. NMR spectra were recorded on Bruker AV-III, Bruker AV-500 and Bruker AV-400 instruments. One-dimensional (1D) ^{1}H NMR spectra were acquired using a 300, 400 and 500 MHz spectrometers while ^{13}C NMR at 75, 100, and 125 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard.

The letters, s, d, t, q, and m are used to indicate singlet, doublet, triplet, quartet, and multiplet. Coupling constants (*J* values) are quoted in hertz (Hz).

Electron ionization (EI) and electrospray ionization (ESI) were measured on JEOL (JMS-600H) and Applied Biosystems (QSTAR XL MS/MS System), respectively. High-resolution mass spectra were obtained by ESI method using time-of-flight (TOF) analyzer or EI methods using high-resolution magnetic sector analyzer mass spectrometer. FTIR spectra were recorded on Shimadzu (FTIR-8900) and Bruker (Vector-22). Analytical HPLC analysis was carried out on Shimadzu (LC-20 system).

The synthesized compounds were purified *via* flash chromatography on silica gel or Biotage SP-1 automated flash chromatography system using cartridges packed with KP-SIL, 60 Å (32–63 mm particle size). TLC analyses were performed on precoated (silica gel 60 HF_{254}) plates. All anhydrous solvents (stored over molecular sieves) and chemicals were obtained from standard commercial vendors and were used without any further purification.

General procedure for synthesis of 3,5-disubstituted tetrahydro-2*H*-1,3,5-thiadiazine-6-thione (8a–f).[6b]

Carbon disulfide 3.6 mL (60 mmol, 6 equiv) was added portion wise to a stirred mixture of corresponding alkyl-, cycloalkyl-, or aralkylamine (**7a-f**) (10 mmol) and potassium hydroxide 0.561 g (20%, 10 mmol, 1 equiv) in 10 mL absolute EtOH. Stirring was continued for 3 h at ambient temperature (25 ºC). Then the reaction mixture, which contains the dithiocarbamates (**10a-f**), formaldehyde solution 1.9 mL (35%, 22 mmol, 2.2 equiv) was added slowly, and stirring was continued for a further 1 h. The resulting clear solution of **11a–f** was added portion wise during 15 min to a stirred solution of 0.75 g glycine (10 mmol, 1 equiv) and phosphate buffer (pH 7.8, 20 mL). After stirring for further 1 h at ambient temperature, the reaction mixture was cooled to 0 ºC, acidified with 8% HCl upto pH 2 and stirring was continued for a further 30 min. The resulting precipitates were collected by filtration, recrystallized from EtOH to afford THTT (**8a-f**) as pure white solid which were directly subjected for characterization and esterification step.

(5-Ethyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (8a).[20,6b] White solid; 1.67 g (76%); mp 132–133 ºC (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.20 (t, *J* = 7.2 Hz, 3H), 3.63 (s, 2H), 4.02 (q, *J* = 7.2 Hz, 2H), 4.52 (s, 2H), 4.53 (s, 2H); MS (direct probe, positive EI) *m/z* 221 (1, M + 1), 220 (4, M).

(5-Propyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (8b).[20,6b] White solid; 2.02 g (86%); mp 127–128 ºC (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 0.93 (t, J = 7.4 Hz, 3H), 1.66–1.72 (m, 2H), 3.63 (s, 2H), 3.93 (t, J = 7.8 Hz, 2H), 4.52 (s, 2H), 4.54 (s, 2H); MS (positive EI) *m/z* 235 (3, M + 1), 234 (18, M).

(5-Isopropyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (8c).[6b] White solid; 1.52 g (65%); mp 123–124 ºC (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.19 (d, J = 6.8 Hz, 6H), 3.58 (s, 2H), 4.45 (s, 2H), 4.51 (s, 2H), 6.11–6.16 (m, 1H); MS (direct probe, positive EI) *m/z* 234 (5, M), 201 (3, M – 33); HRMS (EI^+) *m/z* calcd. for $C_8H_{14}N_2O_2S_2$ $[M]^+$: 234.0497, found: 234.0494.

(5-Butyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (8d).[20,6b] White solid; 1.99 g (80%); mp 145–146 ºC (EtOH); ^{1}H NMR (500 MHz, CD_3OD) δ 0.96 (t, J = 7.2 Hz, 3H), 1.32–1.39 (m, 2H), 1.61–1.67 (m, 2H), 3.63 (s, 2H), 3.97 (t, J = 8.0 Hz, 2H), 4.52 (s, 2H), 4.53 (s, 2H); MS (direct probe, positive EI) *m/z* 250 (4, M + 2), 249 (4, M + 1), 248 (32, M), 246 (12, M – 2).

[5-(1-Phenethyl)-6-thioxo-1,3,5-thiadiazinan-3-yl]acetic acid (8e).[6b] White solid; 2.52 g (85%); mp 139–140 ºC (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.56 (d, J = 7.2 Hz, 3H), 3.09 (d, J = 17.6 Hz, 1H), 3.45 (d, J = 17.6 Hz, 1H), 4.05 (d, J = 13.6 Hz, 1H), 4.27 (d, J = 12.4 Hz, 1H), 4.41 (d, J = 13.6 Hz, 1H), 4.56 (d, J = 12.4 Hz, 1H), 7.30–7.44 (m, 5H), 7.50 (q, J = 7.2 Hz, 1H); MS (direct probe, positive EI) *m/z* 296 (2, M).

[5-(3,4-Dimethoxybenzyl)-6-thioxo-1,3,5-thiadiazinan-3-yl]acetic acid (8f).[6b] White solid; 2.29 g (67%); mp 175–177 ºC (EtOH); ^{1}H NMR (400 MHz, DMSO–d_6) δ 3.31 (s, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 4.45 (s, 2H), 4.53 (s, 2H), 5.18 (s, 2H), 6.90 (s, 2H), 6.99 (s, 1H), 12.54 (brs, 1H); MS (positive ESI) *m/z* 345 (3, M + 3), 344 (6, M + 2), (343 (47, M + 1); HRMS (ESI-TOF) *m/z* calcd. for $C_{14}H_{18}N_2O_4S_2$ $[M + H]^+$: 343.0786, found: 343.0774.

General procedure for synthesis of 3,5-disubstituted tetrahydro-2*H*-1,3,5-thiadiazine-2-thione (8g–j).[6b]

Carbon disulfide 3.6 mL (60 mmol, 6 equiv) was added portion wise to a stirred mixture of glycine 0.75 g (10 mmol) and potassium hydroxide 0.561 g (20%, 10 mmol, 1 equiv). Stirring was continued for 3 h at ambient temperature (25 ºC). To the reaction mixture, which contains the dithiocarbamate (**10g-j**), formaldehyde solution 1.9 mL (35%, 22 mmol, 2.2 equiv) was added slowly and stirring was continued for a further 1 h. The reaction mixture was filtered through a filter paper (to separate the excess carbon

disulfide). The resulting clear solution of **11g-j** was added portion wise during 15 min to a stirred solution of the appropriate alkylamine (10 mmol, 1 equiv) in phosphate buffer (pH 7.8, 20 mL). After stirring for 1 h at ambient temperature, the reaction mixture was cooled to 0 ºC and acidified by 8% HCl upto pH 2 and stirring was continued for further 30 min. The resulting precipitates were collected by filtration, recrystallized from EtOH to afford THTT (**8g-j**) as pure white solid which were directly subjected for characterization.

(5-Ethyl-2-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (8g).[3,6b] White solid; 1.43 g (65%); mp 128–129 ºC (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.17 (t, J = 7.2 Hz, 3H), 2.96 (q, J = 7.2 Hz, 2H), 4.55 (s, 4H), 4.74 (s, 2H); MS (direct probe, positive EI) *m/z* 220 (3, M).

5-Propyl-2-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (8h).[3,6b] White solid; 1.55 g (66%); mp 138–139 ºC (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 0.95 (t, J = 7.4 Hz, 3H), 1.55–1.61 (m, 2H), 2.88 (t, J = 7.2 Hz, 2H), 4.53 (s, 4H), 4.74 (s, 2H); MS (direct probe, positive EI) *m/z* 234 (1, M).

(5-Butyl-2-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (8i).[3,6b] White solid; 1.74 g (70%); mp 247–248 ºC (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 0.94 (t, J = 7.4 Hz, 3H), 1.36–1.42 (m, 2H), 1.51–1.56 (m, 2H), 2.92 (t, J = 7.2 Hz, 2H), 4.53 (s, 4H), 4.74 (s, 2H); MS (direct probe, positive EI) *m/z* 248 (3, M).

[5-(2-Hydroxyethyl)-2-thioxo-1,3,5-thiadiazinan-3-yl]acetic acid (8j).[28,6b] White solid; 1.65 g (70%); mp 134–135 ºC (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 3.07 (t, J = 5.4 Hz, 2H), 3.71 (t, J = 5.4 Hz, 2H), 4.57 (s, 4H), 4.75 (s, 2H); MS (positive EI) *m/z* 236 (4, M).

General procedure for 3,5-disubstituted tetrahydro-2*H*-1,3,5-thiadiazine-6-thione ester derivatives (9a-h).[6b]

To a stirred solution of THTT derivatives (**8a–b/8d-e**) having carboxylic group at N-3 position (1 mmol) in 1.5 mL of corresponding alcohol, under ice cooling condition, was added thionyl chloride 110 μL (1.5 mmol, 1.5 equiv) drop wise over 10 min. After stirring the reaction mixture for 2 h, alcohol was evaporated and 25 mL of water was added. The separated ester analogue was extracted with EtOAc and washed with 10 mL saturated sodium bicarbonate solution. Dried over ($MgSO_4$) and evaporation of EtOAc gave the ester in crude form. Thereafter crude products were purified by flash column chromatography to obtain yellowish gummy THTT ester derivatives (**9a-h**) in pure form for characterization and biological screening.

Ethyl (5-ethyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetate (9a). Yellowish gum; 149.0 mg (60%); R_f (30% EtOAc/hexane) 0.30; IR (KBr) ν_{max} 2960, 2875, 1739, 1498, 1326, 1205, 1107, 773, 673 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H), 3.59 (s, 2H), 4.02 (q, J = 7.2 Hz, 2H), 4.20 (q, J = 7.2 Hz, 2H), 4.43 (s, 2H), 4.45 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.8, 169.3, 69.3, 61.4, 58.8, 51.6, 46.9, 14.1, 11.5; MS (Positive EI) *m/z* 250 (6, M + 2), 249 (10, M + 1), 248 (64, M), 161 (32, M – 87), 129 (31, M – 119); HRMS (EI^+) *m/z* calcd. for $C_9H_{16}N_2O_2S_2$ $[M]^+$: 248.0653, found: 248.0654.

Methyl (5-propyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetate (9b).[6b] Yellowish gum; 194.0 mg (78%); ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (t, J = 7.4 Hz, 3H), 1.64-1.71 (m, 2H), 3.62 (s, 2H), 3.74 (s, 3H), 3.90 (t, J = 8.0 Hz, 2H), 4.43 (s, 2H), 4.44 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.1, 169.8, 69.9, 58.7, 53.8, 52.3, 51.4, 19.9, 11.1; MS (positive EI) *m/z* 248.0 (100, M); HRMS (EI^+) *m/z* calcd. for $C_9H_{16}N_2O_2S_2$ $[M]^+$: 248.0653, found: 248.0626.

Ethyl (5-propyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetate (9c).[6b] Yellowish gum; 199.0 mg (76%); ^1H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H), 1.64–1.68 (m, 2H), 3.60 (s, 2H), 3.89 (t, J = 7.7 Hz, 2H), 4.19 (q, J = 7.2 Hz, 2H), 4.43 (s, 2H), 4.44 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.1, 169.3, 69.9, 61.3, 58.7, 53.7, 51.5, 19.9, 14.1, 11.0; MS (direct probe, positive EI) *m/z* 265 (10, M + 3), 264 (16, M + 2), 263 (100, M + 1), 262 (20, M); HRMS (EI^+) *m/z* calcd. for $C_{10}H_{18}N_2O_2S_2$ $[M]^+$: 262.0810, found: 262.0816.

Benzyl (5-propyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetate (9d).[6b] Yellowish gum; 237.0 mg (73%); ^1H NMR (500 MHz, CD_3OD) δ 0.88 (t, J = 7.5 Hz, 3H), 1.59–1.63 (m, 2H), 3.70 (s, 2H), 3.88 (t, J = 8.0 Hz, 2H), 4.51 (s, 4H), 5.19 (s, 2H), 7.31–7.38 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 193.0, 171.0, 137.1, 129.6 (2C), 129.4 (3C), 70.9, 67.8, 59.5, 54.6, 52.3, 20.8, 11.3; MS (direct probe, positive EI) *m/z* 327 (3, M + 3), 326 (4, M + 2), 325 (24, M + 1), 324 (10, M); HRMS (EI^+) *m/z* calcd. for $C_{15}H_{20}N_2O_2S_2$ $[M]^+$: 324.0966, found: 324.0969.

3-Phenylpropyl (5-propyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetate (9e). Yellowish gum; 212.0 mg (60%); R_f (30% EtOAc/hexane) 0.21; IR (KBr) ν_{max} 2960, 2864, 1737, 1502, 1191, 803, 675 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (t, J = 7.4 Hz, 3H), 1.65–1.70 (m, 2H), 1.93–2.01 (m, 2H), 2.67 (t, J = 7.6 Hz, 2H), 3.58 (s, 2H), 3.90 (t, J = 7.8 Hz, 2H), 4.17 (t, J = 6.4 Hz, 2H), 4.43 (s, 4H), 7.14–7.20 (m, 3H), 7.24–7.29 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.1, 169.4, 140.7, 128.5 (2C), 128.3 (2C), 126.1,

69.9, 64.7, 58.7, 53.8, 51.5, 32.1, 29.9, 19.9, 11.1; MS (positive EI) *m/z* 352 (16, M), 219 (10, M – 133), 147 (5, M – 205), 91 (88, M – 261); HRMS (EI^+) *m/z* calcd. for $C_{17}H_{24}N_2O_2S_2$ $[M]^+$: 352.1279, found: 352.1253.

2-Methylcyclohexyl (5-propyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetate (9f). Yellowish gum; 205.0 mg (62%); R_f (25% EtOAc/hexane) 0.25; IR (KBr) ν_{max} 2943, 2862, 1739, 1512, 1215, 771, 675 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.4 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H), 1.00–1.09 (m, 1H), 1.27–1.33 (m, 2H), 1.43–1.53 (m, 2H), 1.60–1.75 (m, 5H), 1.92–1.94 (m, 1H), 3.61 (d, *J* = 9.2 Hz, 2H), 3.89 (t, *J* = 7.8 Hz, 2H), 4.45 (s, 4H), 4.46–4.48 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.2, 169.0, 79.8, 69.8, 58.8, 53.7, 51.6, 37.1, 33.3, 31.6, 29.6, 24.6, 19.9, 18.4, 11.1; MS (positive EI) *m/z* 331 (4, M + 1), 330 (29, M), 101 (25, M –229), 97 (100, M – 233); HRMS (EI^+) *m/z* calcd. for $C_{15}H_{26}N_2O_2S_2$ $[M]^+$: 330.1436, found: 330.1437.

Ethyl (5-butyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetate (9g).[6b] Yellowish gum; 235.0 mg (85%); ^1H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, *J* = 7.5 Hz, 3H), 1.26 (t, *J* = 7.0 Hz, 3H), 1.30–1.36 (m, 2H), 1.57–1.63 (m, 2H), 3.60 (s, 2H), 3.93 (t, *J* = 8.0 Hz, 2H), 4.20 (q, *J* = 7.0 Hz, 2H), 4.43 (s, 2H), 4.45 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.8, 169.2, 69.7, 61.3, 58.6, 51.9, 51.4, 28.5, 19.8, 14.0, 13.6; MS (positive ESI) *m/z* 279 (9, M + 3), 278 (11, M + 2), 277 (100, M + 1); HRMS (ESI-TOF) *m/z* calcd. for $C_{11}H_{20}N_2O_2S_2$ $[M + H]^+$: 277.1044, found: 277.1041.

Ethyl [5-(1-phenethyl)-6-thioxo-1,3,5-thiadiazinan-3-yl]acetate (9h). Yellowish gum; 243.0 mg (75%); R_f (20% EtOAc/hexane) 0.33; IR (KBr) ν_{max} 2977, 2929, 1743, 1461, 1313, 1207, 1120, 1031, 781, 702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, *J* = 7.2 Hz, 3H), 1.56 (d, *J* = 7.2 Hz, 3H), 3.09 (d, *J* = 17.4 Hz, 1H), 3.41 (d, *J* = 17.7 Hz, 1H), 3.97–4.17 (m, 4H), 4.29 (d, *J* = 13.5 Hz, 1H), 4.54 (d, *J* = 12.3 Hz, 1H), 7.30–7.41 (m, 5H), 7.54 (q, *J* = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.9, 169.0, 138.0, 128.8 (2C), 128.2, 127.6 (2C), 63.9, 61.0, 58.6, 54.8, 50.8, 14.2, 14.0; MS (positive EI) *m/z* 324 (4, M), 129 (9, M – 195), 105 (100, M – 219), 76 (13, M – 248); HRMS (EI^+) *m/z* calcd. for $C_{15}H_{20}N_2O_2S_2$ $[M]^+$: 324.0966, found: 324.0959.

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