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# Preparation and Antimicrobial Evaluation of *3*, 5-Disubstituted Tetrahydro-2*H*-1, *3*, 5-Thiadiazine-Thione Derivatives

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**Key words:** 3, 5-disubstituted THTT, Antimicrobial activity, Disc diffusion method

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**Abstract**

Fourteen derivatives of tetrahydro-2*H*-1, 3, 5-thiadiazine-2-thiones with different functional groups were synthesized and evaluated for their antibacterial activity against gram negative and positive bacteria by utilizing disc diffusion method and recorded as zone of inhibition in milli meter and compared with standard drug Streptomycin. The results showed that two compounds namely 6d and 7a having 2-phenyl ethyl at N-3 position had significant antibacterial activity against *Salmonella typhi*, similar to the standard drug streptomycin however all other synthesized compounds were found weakly active towards different tested bacterias.

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## Introduction

Tetrahydro-2H-1, 3, 5-thiadiazine-thione (THTT) compounds are gaining importance in the medicinal field since its first synthesis in 1848 (Bermello *et al.,* 2011). Thiadiazine compounds embraces of a wide range of biological activities including antimicrobial (Rodríguez H *et al.,* 2012), anticancer (Radwan *et al.,* 2012), and antitubercular activities (Katiyar *et al.,* 2003) (Fig. 1). Moreover several fragments like amino acids, peptides, and drug molecules are also tried to attach with the THTT nucleus with the aim to improve pharmacokinetic profile of the drug molecules.

Drugs molecules comprising THTT core are documented as bio-labile prodrugs (El-Shorbagi *et al.,* 1994), because of its high lipid solubility and enzymatic conversion (Irfanullah *et al.,* 2021), under physiological conditions that provide the biologically active drug metabolite. The produrg therapy has been considered as a popular approach for treating illnesses as it offers improved pharmacokinetics, less side effects, less amount of dose and controlled drug delivery targets. Further this nucleus also graced with the advantage of being stable under the gastric pH and thus it promotes the absorption through stomach in non-ionized form when administered orally (Hortensia Rodríguez *et al.,* 2012). Many compounds from this class have been employed as prodrugs for treating human illnesses; for example, 5-amino-2-(5-cyclopentyl-6-thioxo-1, 3, 5-thiadiazinan-3-yl)-5-oxopentanoic acid (2. Fig. 1) is used as anti-cancer agent (Rodríguez H *et al.,* 2012) and antiepileptic prodrug. Generally these prodrugs have advantage of higher availability of the active form of the drug to its pharmaceutical target (El-Shorbagi *et al.,* 1994), reduced toxicity, higher pharmacological efficiency and reduced side effects (Ettmayer *et al*., 2004).

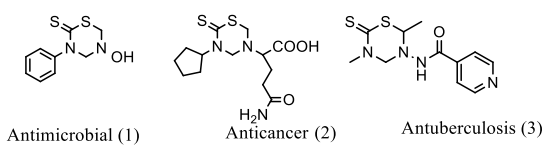



**Fig. 1.** Important drug molecules containing THTT skeleton.

The importance of the nature and position of the substituents at 3-N or 5-N and related antimicrobial activity and toxicity is well documented in literature (Abdel Moty SG 2005). To obtained the optimum pharmacological effects of these compounds, lipophilic groups at 3-N position while hydrophilic groups at the 5-N position of the THTT nucleus are attached**.** Inspired by the marvelous biological properties of THTT nucleus we prepared 14 THTT derivatives having variety of substituents and studied it antimicrobial potential. Herein we report the antibacterial activity of fourteen (14) synthesized THTT compounds having variety of substituent attached at 3-N and 5-N position by disc diffusion method against seven pathogens *named as Salmonella typhi. aureus, Klebsiella pneumonia, Escherichia coli, Proteus Mirabilis, Salmonella typhi, Salmonella typhi. Cerevisiae and Pseudomonas aeruginosa*. The antibacterial activity of these compounds by disc diffusion method along with the synthesis and characterization of one new compound is being first time reported in this paper.

## Materials and methods

The synthesis of fourteen compounds were performed as (shown in scheme 1) by following literature protocol (Bermello *et al.,* 2011, Arshad *et al.,* 2018) and isolated in excellent yields via flash chromatography by using using EtOAc and hexane as eluent solvent. The compounds were characterized through $^1$H NMR (400 MHz, $CD_3OD$) and mass spectroscopy. At NMR spectrum, the characteristic peaks related to the 4 $CH_2$ and 6 $CH_2$ of thiadiazine nucleus were appeared in all compounds between the ranges of δ value 3.90 to 4.50 ppm as multiplet that confirmed the presence of THTT nucleus. The spectral data of all fourteen compounds were found in good agreement with the reported data (Jorgensen *et al.*, 2007). However as an example one characteristic complete spectral data of the most active compound (6d) is shown below.

5-(2-hydroxyethyl)-3-(1-phenylethyl)-1,3,5-thiadiazinane-2-thione (6d). White solid; 2.43gm (86%); Rf (60% EtOAc/hexane) 0.22; 1H NMR (400 MHz, $CD_3OD$) δ 1.59 (d, J = 7.2 Hz, 3H), 2.37–2.43

(m, 1H), 2.71–2.77 (m, 1H), 3.13–3.19 (m, 1H), 3.31–3.34 (m, 1H), 3.98 (d, J = 13.6 Hz, 1H), 4.23 (d, J = 12.0 Hz, 1H), 4.36 (d, J = 13.6 Hz, 1H), 4.59 (d, J = 12.0 Hz, 1H), 7.31–7.47 (m, 6H); MS (positive EI) m/z = 282.

*Antibacterial Activity by Disc Diffusion Method*

*Test Microorganisms*

Pure culture strains were obtained from Department of Microbiology University of Karachi, Pakistan. The cultures were maintained on Nutrient and Luria-Bertani (L.B) media.

*Gram negative bacteria*

Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Salmonella typhi, Klebsiella pneumonia and Staphylococcus aureus.

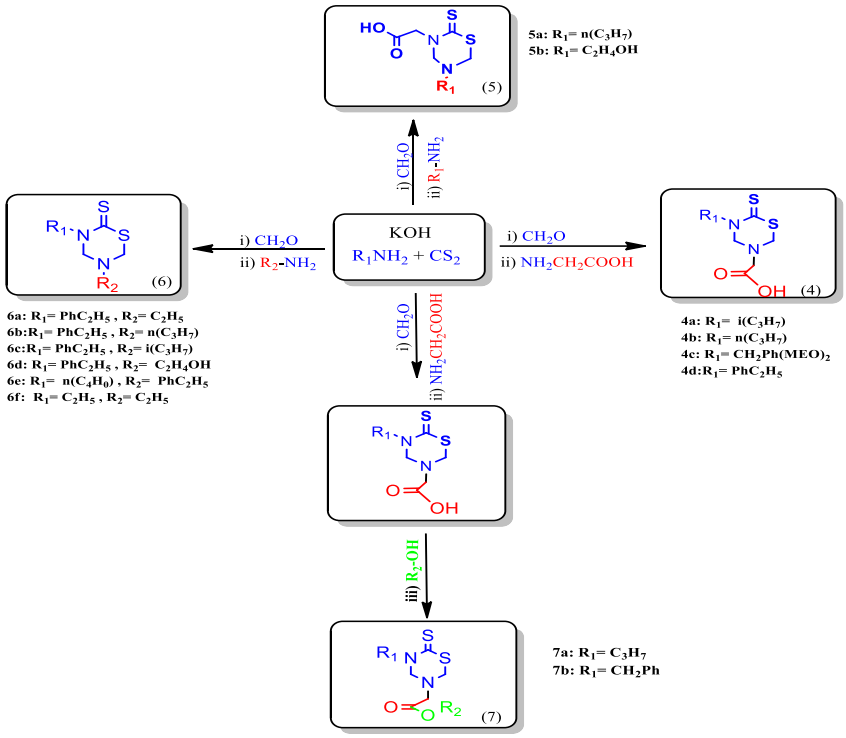
*Gram positive bacteria/Yeast*

*Streptococcus faecalis*, *Micrococcus* sp., *Saccharomyces cerevisiae.*

*Antibacterial Testing*

Antibacterial activity was determined by the method of disc diffusion (Jorgensen and Turnidge 2007, Nostro *et al.,* 2000, Ross *et al.,* 2011). ThemcFarland Index was prepared and 0.5 standard was used for testing method (Coro *et al.,* 2008). Briefly, an inoculums of test organism was cultured into 5ml of Luria-Bertani (LB) broth.

The LB agar plates were inoculated to make an evenly microbial lawn using sterile cotton swabs. 1mm solutions were prepared of test samples in Methanol.




**Scheme 1.** Synthetic Scheme of Compounds from 4 to 7.

Paper disks of 4mm diameter were placed on the inoculated plates and 10ul sample was loaded on each disk and left to dry. Methanol is used as negative control and antibiotic disks of Streptomycin as positive control. The plates were incubated at 37°C for 24 hours. The samples were run in triplicate.

The positive test samples showing considerable zone of inhibition were then examined for Minimal Inhibitory Concentration (MIC). Stock Solution of 100 ul of 1mm concentration of each test sample was prepared in DMSO/Methanol. Ten two fold dilutions were made. LB agar plates streaked with test organism and borrowed 4mm agar well marked with dilution factor was then loaded with onemL of serially diluted test sample. The plates were incubated at 37°C for 24 hours. The test was conducted in triplicate. The concentration of extract was calculated accordingly.

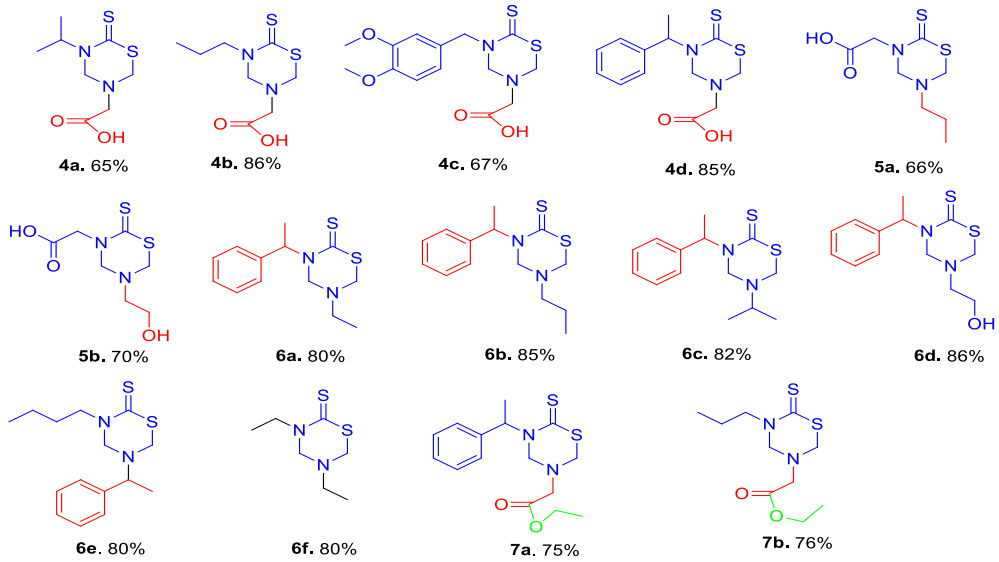
**Results and discussion**

*Chemistry*

Four sets (14 examples) of tetrahydro-2*H*-1, 3, 5-thiadiazine-6-thiones (THTT) (4a-d, 5a-b, 6a-f) along with the ester derivatives of THTT (7a–b) were synthesized in excellent yields (Table 1) by following literature protocol [8]. Reaction of suitable primary amine i.e. alkyl/aralkylamine or glycine with carbon disulphide and base (potassium hydroxide) produces analogous of dithiocarbamate salts [13]. The dithiocarbamate salts without any purification were treated with formaldehyde solution to give intermediates, which were made to react with primary amines or glycine to provide THTT derivatives (4a-d, 5a-b, 6a-f) in high yields (65–86%, Table 1). Variety was successfully produced by introducing various substituted primary amines or glycine at N-3 and N-5 positions of THTT core. Further the compounds 4b and 4d were converted into ethyl esters of type (7).

For the conversion of THTT 4b and 4d into N-3 ester moieties (7a and 7b) thionyl chloride was used in ethanol as solvent. The reaction with thionyl chloride (1.5 equivalent) in ethanol for 2h under ice cooling (0–4°C), provided corresponding esters in good isolated yield after flash chromatography.

**Table 1.** -1,3,5-thiadiazine scaffolds[a,b]




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

*Antibacterial Activity*

For the screening of the antimicrobial activity,all the fourteen (14) compounds (4a-7b) were tested against the gram negative and gram positive bacterias using disc diffusion method (Jorgensen and Turnidge 2007, Nostro *et al.,* 2000, Ross *et al.,* 2011) and antimicrobial potential was recorded as zone of inhibition in millimeters (Table 2). *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumonia* and *Staphylococcus aureus* ware used as gram negative becterias while *Streptococcus faecalis*, *Micrococcus sp* and *Saccharomyces cerevisiae* were tested as gram positive bacterias. Streptomycin was used as reference drug. Compound 6d and 7a displayed a notable antibacterial activity against *Salmonella typhi* with the zone of inhibition values (20 ± 0.73 and 21 ± 0.63) respectively which is similar to the standard drug Streptomycin (21 ± 0.36).

Note that these compounds contain 2-phenyl ethyl substituent at N-3 position of the THTT skeleton and thus these antibacterial results showed the importance of 2-phenyl ethyl substituent at N-3 position of Thiadiazine skeleton. All other compounds showed weak responses against all the tested bacteria.

**Table 2.** Antibacterial activity of fourteen synthesized compounds against gram negative/positive bacterias.

| SL | Compound | *Zone of Inhibition (mm ± SEM)* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *S. aureus* | *Klebsiella pneumonia* | *Escherichia coli* | *Proteus mirabilis* | *Salmonella typhi* | *S. Cerevisiae* | *Pseudomonas aeruginosa* |
| 1. | $R_1 = n(C_3H_7)$ $R_2 = CH_2COOH$ | - | 3± 0.22 | 1 ± 0.62 | 1± 0.06 | - | - | 1 ± 0.23 |
| 2. | $R_1 = -i(C_3H_7)$ $R_2 = CH_2COOH$ | - | - | 2 ± 0.17 | 2 ± 0.70 | 1± 0.11 | - | 1± 0.75 |
| 3. | $R_1 = CH_2Ph(MEO)_2$ $R_2 = CH_2COOH$ | - | - | 2 ± 0.55 | 2 ± 0.87 | - | - | - |
| 4. | $R_1 = C_2H_5Ph$ $R_2 = CH_2COOH$ | - | - | 1± 0.11 | 2 ± 0.20 | - | - | - |
| 5. | $R_1 = CH_2COOH$ $R_2 = n(C_3H_7)$ | - | 1± 0.76 | 1± 0.27 | 1 ± 0.43 | 1± 0.54 | - | - |
| 6. | $R_1 = CH_2COOH$ $R_2 = C_2H_4OH$ | - | 3± 0.37 | 1.5 ± 0.72 | 2 ± 0.55 | - | - | - |
| 7. | $R_1 = C_2H_5Ph$ $R_2 = C_2H_5$ | - | - | 1± 0.27 | 2 ± 0.29 | - | - | 1± 0.78 |
| 8. | $R_1 = C_2H_5Ph$ $R_2 = n(C_3H_7)$ | - | - | 1 ± 0.87 | - | - | - | - |
| 9. | $R_1 = C_2H_5Ph$ $R_2 = i(C_3H_7)$ | - | 1± 0.08 | 1± 0.21 | - | - | - | 2± 0.27 |
| 10. | $R_1 = C_2H_5Ph$ $R_2 = C_2H_4OH$ | - | 2 ± 0.65 | 2± 0.55 | - | 20± 0.73 | - | - |
| 11. | $R_1 = C_4H_9$ $R_2 = C_2H_5Ph$ | - | 1± 0.77 | 2 ± 0.28 | - | 2± 0.74 | - | - |
| 12. | $R_1 = R_2 = C_2H_5$ | - | 1± 0.54 | - | - | - | - | - |
| 13. | $R_1 = C_2H_5Ph$ $R_2 = -CH_2COOC_2H_5$ | - | 1 ± 0.48 | 1 ± 0.11 | - | 21± 0.63 | - | - |
| 14. | $R_2 = -C_3H_7$ $R_{2=} = -CH_2COOC_2H_5$ | - | 2± 0.35 | 1± 0.80 | 1± 0.23 | 2± 0.32 | - | - |
| 15. | Streptomycin | 20 ± 0.34 | 22 ± 0.18 | 23 ± 0.12 | 22 ± 0.11 | 21 ± 0.36 | 31 ± 0.29 | 25 ± 0.21 |

Values are mean ± SEM of three independent experiments, *Zone of inhibition, including the diameter of the filter paper disk.

## Conclusion

Fourteen derivatives of tetrahydro-2*H*-1, 3, 5-thiadiazine -2-thiones with different functional groups were synthesized and evaluated for their antibacterial activity against gram negative and gram positive bacteria. The results showed that two compounds namely 6d and 7a had significant antibacterial activity against *Salmonella typhi*, similar to the standard drug streptomycin however all other synthesized compounds were weakly active towards the tested bacteria.

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