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Research article

One pot synthesis of 5-hydroxyalkylated thiadiazine thiones: Implication in pain management and bactericidal properties

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ABSTRACT

The synthesis of a new series of thiadiazine thiones including 5-(2-hydroxyethyl)-3-alkyl/aryl-1, 3, 5-thiadiazine-2-thiones (**1**–**5**), 5-(2-hydroxypropyl)-3-alkyl/aryl-1, 3, 5-thiadiazine-2-thiones (**6**–**8**), 3,5-dipropyl-1, 3, 5-thiadiazine-2-thione (**9**) and (2-(5-alkyl/aryl-6-thioxo-1, 3, 5-thiadiazine-3-yl) alkyl acetate/benzoate) (**10**–**17**) was accomplished *via* one pot reaction. The structures of the synthesized compounds were characterized through NMR and Mass spectrometry. The anti-nociceptive activity of compounds was performed on BALB/C mice by hot plate method, where compounds **3**, **5** (50 μg/kg), and **8** (50, 100 μg/kg) exhibited significant effect ($P < 0.01$, $P < 0.05$) in latency time of 15, 30, and 60 min, while compounds **6** and **16** (100 μg/kg) exhibited significant effect ($P < 0.01$, $P < 0.05$) in latency time interval of 15 and 30 min. Compounds **1**, **12–13**, and **15** showed moderate activity. Among the tested hits, compounds **5** (17.3 ± 2.2), **11** (16.2 ± 2.1), and **8** (16.1 ± 2.1) showed significant anti-nociceptive potential. Molecular docking studies on the most active anti-nociceptive hits indicated that the activity might be attributed to the ability of the compounds to target μ-opioid receptor (μOR) effectively. Furthermore, compounds **14** and **11** showed anti-bacterial activity against *Pseudomonas aeruginosa* and *MSRA* with MIC of 40.97 and 54.77 μg/mL, respectively. In addition, the predicted ADMET profile of **5**, **9**, and **11** indicates that these molecules follow the drug-likeness criteria, and their activity can be enhanced through structural optimization.

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1. Introduction

Anti-microbial drugs resistance is not a newly raised problem, however, the number of resistant organisms, topographical zones affected by drug resistance, and the extent of resistance in a single organism are rising swiftly [1]. Currently, a broad spectrum of antibiotics such as, fluoroquinolones [2], penicillin [3], ampicillin [4], sulfonamide, methicillin, vancomycin, and macrolides are available in the market. The increasing antimicrobial drug resistance leads to the use of high drug dosage, higher toxic medications, long term treatment and consequently leading to a drastic increase in fatality rate [5], estimated to be ~0.7 million people annually [6].

The speed at which pathogenic bacteria are producing drug resistance and the lack of success in the improvement of latest anti-biotics, demands the design of new molecules with high anti-bacterial potency at low dose [7]. Therefore, the treatment of microbial infections is an important therapeutic challenge which needs to be addressed on urgent basis [8]. Microbial infections causes swelling and inflammation in the body [9]. Inflammation is a biological response which protects body from infections and tissue damage [10, 11]. Commonly, analgesic, anti-inflammatory and anti-microbial drugs are recommended simultaneously [12,13]. In the last few decades, non-steroidal anti-inflammatory drugs (NSAIDs) became well-established, however, their chronic use causes various side effects such as gastro-intestinal lesions, bleeding and nephrotoxicity [14]. Therefore, discovering novel and safer anti-inflammatory and antinociceptive drugs is a challenging goal and a dire need.

For the last several decades, 3,5-disubstituted-tetrahydro-2*H*-1, 3, 5-thiadiazine-thione (THTT) and its analogues have gained attention of the medicinal chemist community due to their diverse biological activities [15–20]. These molecules has shown anti-microbial [21], anti-parasitic [22], anti-cancer [23], anti-epileptic [24], anti-tubercular [25], anti-malarial [26], antioxidant [27], anthelmintic, anti-fibrinolytic [28], anti-proliferative [29], and anti-leishmanial [30] properties (Fig. 1a–g). Moreover, these molecules also find use in the field of agriculture, for instance, milneb and dazomet are well-known fungicidal and nematocidal drugs, respectively [31]. Recently, promising herbicidal potential has also been reported for dazomet [32]. The anti-microbial activity of these compounds is generally attributed to the isothiocyanates and dithiocarbamic acid species produced in the biosystem upon hydrolysis [33].

Inspired by the potent pharmacological potential of 3,5-disubstituted-tetrahydro-2*H*-1, 3, 5-thiadiazine-thione and our interest in the synthesis of interesting bioactive molecules [34], we endeavored to design new thiadiazine thiones. These were subsequently converted into ester analogues to assess their anti-microbial and anti-nociceptive potency and selectivity, aiming for reduced toxic effects.

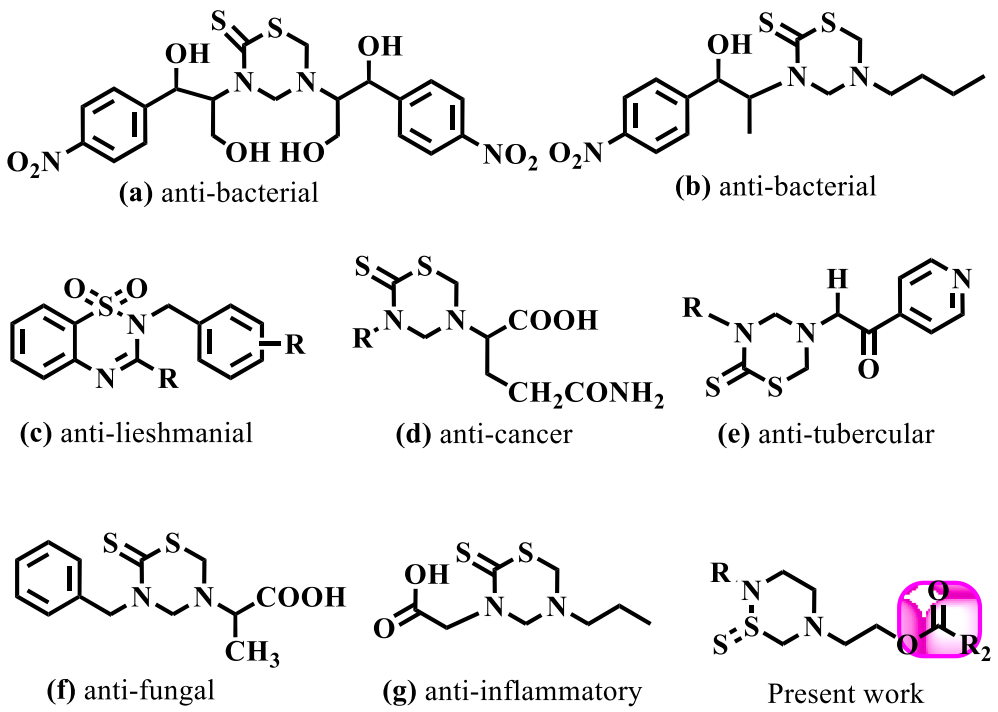


Fig. 1. Rational design of our current work with precious work. The chemical structures of thiadiazine-thione derivatives with different properties are shown (**a**–**g**).

2. Experimental section

2.1. General information

The synthesis of molecules was achieved by utilizing analytical grade starting materials, reagents, and solvents. The course of reactions was examined using thin layer chromatography on pre-coated silica gel aluminum sheets (layer thickness 0.2 mm, 60 HF-254 nm). Digital Gallen Kamp apparatus was used to determine melting point. The structures of the synthesized molecules were determined through the Bruker Vector FTIR spectrometer. Bruker Avance 400 and 600 MHz spectrophotometers were used for ^{1}H while ^{13}C analysis analysis was carried out on 100 and 150 MHz spectrophotometers. HRMS analysis was performed on LC/MS time of flight (TOF) spectrometer using electrospray ionization (ESI) technique.

2.1.1. General procedure for the synthesis of Tetrahydro-1,3,5-thiadiazine-2-thiones (1–9)

The compounds were synthesized by dropwise addition of carbon disulfide (20 mmol) to aqueous solution of primary alkyl/aryl amines (20 mmol) in basic media and stirred for 3–4 h. Formaldehyde (30%, 40 mmol) was added to the reaction mixture and stirred further for 1 h and then filtered. The filtrate was added to phosphate buffer solution (20 mL) of primary amines (hydroxyl amines and propyl amines). The reaction mixture was then stirred for 2–3 h, filtered, and cooled overnight, followed by acidification with 15 % hydrochloric acid to obtain pure THTT product.

2.1.2. General method for synthesis of 2-(5-alkyl/aryl-6-thioxo-1, 3, 5-thiadiazine-3-yl) alkyl acetate/benzoate (10–17)

Esterification of the synthesized THTTs (series A) was carried out on a 2–3 mmol scale. The selected THTTs were dissolved in dry pyridine upon stirring for 10–15 min, the reaction mixture was cooled to -4-0 °C and then successive addition of selected acid halides were carried out and stirred further for 2–3 h in ice bath. The reaction mixture was then filtered and diluted with ice cold water. The aqueous layer of pyridine was removed, and the reaction mixture was extracted three times with dichloromethane (DCM). DCM solution was again washed with ice-cold dilute HCl solution. Crystals of the synthesized products were obtained after evaporating DCM. The crystals were washed with water until free from pyridine and dried.

1. 5-(2-Hydroxyethyl)-3-methyl-1,3,5-thiadiazinane-2-thione

This compound was prepared from methylamine, KOH, CS_2, formaldehyde, and ethanolamine as per reported protocol [35]. Yield: 67 % (0.26 g); m.p: 130–132 °C; solubility: DCM.

2. 5-(2-Hydroxyethyl)-3-propyl-1,3,5-thiadiazinane-2-thione

This compound was prepared from propylamine, KOH, CS_2, formaldehyde, and ethanolamine as per reported protocol [35]. Yield: 85 % (0.37 g); m.p: 110 °C; solubility: DCM.

3. 3-Butyl-5-(2-hydroxyethyl)-1,3,5-thiadiazinane-2-thione

This compound was prepared from butylamine, KOH, CS_2, formaldehyde, and ethanolamine as per reported protocol [35]. Yield: 75 % (0.36 g), m.p: 77 °C; solubility: DCM.

4. 3-Benzyl-5-(2-hydroxyethyl)-1,3,5-thiadiazinane-2-thione

This compound was prepared from benzylamine, KOH, CS_2, formaldehyde, and ethanolamine as per reported protocol [35]. Yield: 80 % (4.29 g), m.p: 64 °C; solubility: DCM.

5. 5-(2-Hydroxyethyl)-3-phenyl-1,3,5-thiadiazinane-2-thione

Yield: 80 % (0.43 g); m.p: 142 °C; solubility: ethanol; **FT-IR** (cm^{-1}): 3310 (O–H), 3021 (aromatic C–H), 2853 (aliphatic C–H), 1474 (C═S); **^{1}H-NMR** ($CDCl_3$, 400 MHz), δ (ppm): 7.38–7.42 (m, 5H, C_6H_5), 5.22 (s, 4H, ring), 4.22 (t, 2H, J = 8 Hz, CH_2CH_2OH), 3.62 (t, 2H, J = 4 Hz, CH_2CH_2OH).

6. 5-(2-Hydroxypropyl)-3-propyl-1,3,5-thiadiazinane-2-thione

Yield: 78 % (0.37 g); m.p: 82 °C; solubility: DMSO; solubility: DCM; **FT-IR** (cm^{-1}): 3310 (O–H), 2855 (aliphatic C–H), 1500 (C═S); **^{1}H-NMR** ($CDCl_3$, 400 MHz),.δ(ppm): 4.44 (m, 2H, THTT ring), 4.34 (m, 2H, THTT ring), 3.93 (m, 2H, $CH_3CH_2CH_2$), 3.05 (m, 1H, $CH_2CH(OH)CH_3$), 2.73, 2.53 (m, 2H, CH_2CHCH_3OH), 2.33 (s, 1H, OH), 1.70 (m, 2H, $CH_3CH_2CH_2$), 1.20 (d, 3H, J = 8 Hz, CH_2CHCH_3OH), 0.95 (t, 3H, J = 8 Hz, $CH_3CH_2CH_2$); **^{13}C-NMR** (DMSO, 150 MHz) δ (ppm): 190.77 (C═S), 70.86 (C-4), 65.16 (C-11), 59.48 (C-6), 57.69 (C-10), 53.29 (C-7), 21.94 (C-12), 19.85 (C-8), 11.48 (C-9); **HRMS (ESI):** calculated for $C_9H_{18}N_2OS_2$ $[M+H]^+$: calcd. 235.0933: found 235.0930.

7. 5-(2-Hydroxypropyl)-3-phenyl-1,3,5-thiadiazinane-2-thione

Yield: 80 % (0.43 g); m.p: 152–153 °C; solubility: $CHCl_3$; **FT-IR** (cm^{-1}): 3310 (O–H), 3025 (aromatic C–H), 2855 (aliphatic C–H), 1500 (C═S); **^{1}H-NMR** ($CDCl_3$, 600 MHz), δ(ppm): 7.50 (t, 1H, $J = 8$ Hz, CH phenyl ring), 7.41 (m, 2H, 2CH phenyl ring), 7.23.(m, 2H, 2CH phenyl ring), 5.38 (t, 1H, $J = 12$ Hz, OH), 4.74 (d, 2H, $J = 24$ Hz, THTT ring), 4.62 (dd, 2H, $J = 24$, 18 Hz, THTT ring), 4.03 (m, 1H, $CH_2CH(OH)CH_3$), 3.34, 2.76 (m, dd, 2H, $J = 18$, 12Hz, $CH_2CH(OH)CH_3$), 1.30 (s, 3H, $CH_2CH(OH)CH_3$); **^{13}C-NMR** ($CDCl_3$, 150 MHz), δ(ppm): 192 (C═S), 144.18 (C-7), 129.97 (C-8), 128.49 (C-9), 126.41 (C-10), 74.22 (C-4), 64.77 (C-12), 58.90 (C-6), 58.47 (C-11), 20 (C-13); **HRMS (ESI)**: calculated for $C_{12}H_{16}N_2OS_2$ $[M+H]^+$: calcd: 269.0777 found 269.0765.

8. 3-(Furan-2-ylmethyl)-5-(2-hydroxypropyl)-1,3,5-thiadiazinane-2-thione

Yield: 70 % (0.35 g); m.p: 107 °C; solubility: $CHCl_3$; **FT-IR** (cm^{-1}): 3310 (OH), 3025 (aromatic C–H), 2855 (aliphatic C–H), 1500 (C═S); **^{1}H-NMR** ($CDCl_3$, 400 MHz), δ(ppm): 7.36 (m, 1H, CHCHCHCO furfuryl ring), 6.46 (m, 1H, CHCHCHCO), 6.36 (m, 1H, CHCHCHCO), 5.14, 5.46 (d, 2H, $J = 12$ Hz, Ar-CH_2), 4.35–4.56 (m, 4H, $2CH_2$ ring), 3.77.(m, 1H, $CH_2CH(OH)CH_3$), 2.85, 2.35 (m, 2H, $CH_2CH(OH)CH_3$), 1.10.(d, 3H, $J = 12$ Hz, $CH_2CH(OH)CH_3$); **^{13}C-NMR** (100 MHz, $CDCl_3$), δ (ppm): 192.5 (C═S), 148.5 (C-8), 142.5 (C-11), 110.95 (C-10), 110.65 (C-9), 69.35 (C-4), 64.40 (C-6), 59.16 (C-13), 58.12 (C-12), 47 (C-7), 20.(C-14); **HRMS (ESI):** calculated for $C_{11}H_{16}N_2O_2S_2$ $[M+H]^+$: calcd: 273.0726 found 273.0722.

9. 3,5-Dipropyl-1,3,5-thiadiazine-2-thione

Yield: 80 % (0.35g); m.p: 82 °C; solubility: DCM; FT-IR (cm^{-1}): 2855 (aliphatic C–H), 1500 (C=S); ^{1}H-NMR ($CDCl_3$, 600 MHz), δ(ppm): 4.37 (d, 4H, $J = 18$ Hz, 2CH2), 3.93 (t, 2H, $J = 12$Hz, $CH_2CH_2CH_3$), 2.78 (t, 2H, $J = 12$Hz, $CH_2CH_2CH_3$), 1.70 (m, 2H, CH_3CH_2), 1.58 (m, 2H, $CH_2CH_2CH_3$), 0.95 (t, 6H, $J = 12$Hz, $2CH_3$); ^{13}C-NMR (DMSO-*d*6, 100 MHz), δ(ppm): 190.91 (C=S), 62.01 (C-4), 54.77 (C-6), 50 (C-10), 47.65 (C-9), 21.35 (C-11), 21.35 (C-8), 18.61 (C-12), 18.61 (C-7); HRMS (ESI): calculated for $C_9H_{18}N_2S_2$ [M+H]+: calcd: 219.3800 found 219.3800.

10 2-(5-Propyl-6-thioxo-1,3,5-thiadiazinan-3-yl) ethyl acetate

Yield: 83 % (0.43 g); m.p: 98 °C; solubility: Methanol; **FT-IR** (cm^{-1}): 2958-2873 (aliphatic C–H), 1746 (C═O), 1495 (C═S); **^{1}H-NMR** ($CDCl_3$, 400 MHz), δ(ppm): 4.38 (d, 4H, $J = 4$ Hz, THTT ring), 4.24 (t,.2H, $J = 4$ Hz, $CH_2CH_2O(CO)CH_3$), 3.92 (t, 2H, $J = 4$ Hz, $CH_3CH_2CH_2$), 3.06 (t, 2H, $J = 8$ Hz, $CH_2CH_2O(CO)CH_3$), 2.06 (s, 3H, $COCH_3$), 1.70 (m, 2H, $CH_3CH_2CH_2$), 0.95 (t, 3H, $J = 8$ Hz, $CH_3CH_2CH_2$); **^{13}C-NMR** ($CDCl_3$, 100 MHz), δ (ppm): 191.45 (C═S), 170 (C═O), 70.28 (C-4), 61.55 (C-6), 58.47 (C-11), 53.93 (C-7), 49.45 (C-10), 20.95 (C-12), 20.05 (C-8),11.20 (C-9); **HRMS (ESI):** calculated for $C_{10}H_{18}N_2O_2S_2$ $[M+H]^+$: calcd: 263.0888 found 263.0888.

11 2-(5-Propyl-2-thioxo-1,3,5-thiadiazinan-3-yl) ethyl benzoate

Yield: 80 % (0.52 g); m.p: 113–115 °C; solubility: DCM; **FT-IR** (cm^{-1}): 3128 (aromatic C–H), 2865–2996 (aliphatic C–H), 1746 (C═O), 1475 (C═S); **^{1}H-NMR** (DMSO, 600 MHz), δ(ppm): 7.97 (m, 2H, 2CH, Ar), 7.65 (m, 2H, 2CH, Ar), 7.53 (m, 1H, CH, Ar), 4.56 (s, 2H, CH_2 ring), 4.46 (s, 2H, CH_2 ring), 4.45 (t, 2H, $J = 6$ Hz, $CH_2CH_2O(CO)C_6H_5$), 3.89 (t, 2H, $J = 6$ Hz, $CH_2CH_2O(CO)C_6H_5$), 3.10 (t, 2H, $J = 6$ Hz, $CH_3CH_2CH_2$), 1.62 (m, 2H, $CH_3CH_2CH_2$), 0.85 (t, 3H, $J = 6$ Hz, $CH_3CH_2CH_2$); **^{13}C-NMR** (DMSO, 100 MHz), δ (ppm): 190.64 (C═S), 167.78 (C═O), 133.87 (C-15), 131.31 (C-12), 129.72 (C-14), 129.72 (C-13), 70.32 (C-4) 62.91 (C-11), 58.39 (C-6), 53.31 (C-7), 49.14 (C-10), 11.42 (C-9), 19.84 (C-8); **HRMS (ESI):** calculated for $C_{15}H_{20}N_2O_2S_2$ $[M+H]^+$: calcd: 325.1000 found 325.1000.

12 2-(5-Butyl-6-thioxo-1,3,5-thiadiazinan-3-yl) ethyl acetate

Yield: 80 % (0.44 g); m.p: 57 °C; solubility DCM; **FT-IR** (cm^{-1}): 2958-2873 (aliphatic C–H), 1745 (C═O), 1465 (C═S); **^{1}H-NMR** (DMSO, 600 MHz), δ: 4.58 (d, 4H, $J = 12$ Hz ring H), 4.32 (t, 2H, $J = 6$ Hz, $CH_2CH_2O(CO)CH_3$), 3.91 (t, 2H, $J = 6$ Hz, $CH_3CH_2CH_2CH_2$), 2.94 (t, 2H, $J = 6$ Hz, $CH_2CH_2O(CO)CH_3$), 2.03 (s, 3H, $CH_2CH_2O(CO)CH_3$), 1.57 (m, 2H, $CH_3CH_2CH_2CH_2$), 1.28 (m, 2H, $CH_3CH_2CH_2CH_2$), 0.90 (t, 3H, $J = 6$ Hz, $CH_3CH_2CH_2CH_2$); **^{13}C-NMR** (DMSO, 150 MHz), δ (ppm): 190.43 (C═S), 170.82 (C═O), 70.10 (C-4), 62.42 (C-12), 58.35 (C-6), 51.51 (C-11), 48.90 (C-7), 28.46 (C-8), 21.21 (C-14), 19.94 (C-9), 14.17 (C-10); **HRMS (ESI):** calculated for $C_{11}H_{20}N_2O_2S_2$ $[M+H]^+$: calcd: 277.1044 found 277.1044.

13 2-(5-Butyl-6-thioxo-1,3,5-thiadiazinan-3-yl) ethyl benzoate

Yield: 78 % (0.53 g); m.p: 80 °C; solubility: Methanol; **FT-IR** (cm^{-1}): 3135 (aromatic C–H), 2865–2990 (aliphatic C–H), 1748 (C═O), 1475 (C═S); **^{1}H-NMR** ($CDCl_3$, 400 MHz), δ (ppm): 7.45–8.12 (m, 5H, C_6H_5), 4.51 (t, 2H, $J = 4$ Hz, $CH_2CH_2O(CO)C_6H_5$), 4.46 (s, 2H, THTT ring), 4.39 (s, 2H, THTT ring), 3.97 (t, 2H, $J = 12$ Hz, $CH_3CH_2CH_2CH_2$), 3.21 (t, 2H, $J = 8$ Hz, $CH_2CH_2O(CO)C_6H_5$), 1.65 (m, 2H, $CH_3CH_2CH_2CH_2$), 1.35 (m, 2H, $CH_3CH_2CH_2CH_2$), 0.93 (t, 3H, $J = 12$ Hz, $CH_3CH_2CH_2CH_2$); **HRMS (ESI):** calculated for $C_{16}H_{22}N_2O_2S_2$ $[M+H]^+$: calcd: 339.1200 found 339.1192.

14. 2-(5-Phenyl-6-thioxo-1,3,5-thiadiazinan-3-yl) ethyl acetate

Yield: 77 % (0.45 g); m.p: 145 °C; solubility: methanol; **FT-IR** (cm^{-1}): 3110.(aromatic C–H), 2870–2995 (aliphatic C–H), 1746 (C═O), 1473 (C═S); **^{1}H-NMR** ($CDCl_3$, 600 MHz), δ (ppm): 7.94 (m, 2H, 2CH), 7.48 (m, 2H, 2CH), 7.34 (m, 1H, CH), 4.36 (t, 2H, $J = 6$ Hz, THTT ring), 4.32 (t, 2H, $J = 6$ Hz, THTT ring), 4.14 (t, 2H, $J = 6$ Hz, $CH_2CH_2O(CO)CH_3$), 4.04 (t, 2H, $J = 6$ Hz, $CH_2CH_2O(CO)CH_3$), 1.10 (s, 3H, $CH_2CH_2(CO)CH_3$); **^{13}C-NMR** ($CDCl_3$, 150 MHz), δ(ppm): 191 (C═S), 164 (C═O), 140 (C-7), 134.85 (C-9), 134 (C-8), 130 (C-10), 90 (C-4), 71 (C-12), 70 (C-6), 44 (C-11), 20 (C-13); **HRMS (ESI):** calculated for $C_{13}H_{16}N_2O_2S_2$ $[M+H]^+$: calcd: 297.0731 found 297.0718.

15. 2-(5-Phenyl-6-thioxo-1,3,5-thiadiazinan-3-yl) ethyl benzoate

Yield: 75 % (0.56 g); m.p: 139–140 °C; solubility: methanol; **FT-IR** (cm^{-1}): 3130 (aromatic C–H), 2860–2990 (aliphatic C–H), 1746 (C═O), 1475 (C═S); **^{1}H-NMR** ($CDCl_3$, 400 MHz), δ(ppm): 7.20–7.94 (d, m, 10H, $J = 8$ Hz, $2C_6H_5$), 4.69 (s, 2H, THTT ring), 4.60 (s, 2H, THTT ring), 4.55 (t, 2H, $J = 4$ Hz, $CH_2CH_2O(CO)C_6H_5$), 3.43 (t, 2H, $J = 4$ Hz, $CH_2CH_2O(CO)C_6H_5$); **^{13}C-NMR** ($CDCl_3$, 100 MHz), δ (ppm): 193.9 (C═S), 166.37 (C═O), 144.23 (C-7), 133.31 (C-16), 129.94 (C-13), 129.64 (C-8/14), 128.53 (C-15), 128.46 (C-9),126.47 (C-10), 74.43 (C-4), 62.44 (C-12), 59.11 (C-6), 49.81 (C-11); **HRMS (ESI):** calculated for $C_{18}H_{18}N_2O_2S_2$ $[M+H]^+$, calcd: 359.0888 found 359.0874.

16 2-(5-Benzyl-6-thioxo-1,3,5-thiadiazinan-3-yl) ethyl acetate

Yield: 82 % (0.51 g); m.p: 110 °C; solubility: methanol; **FT-IR** (cm^{-1}): 3130 (aromatic-C-H), 2870–2995 (aliphatic-C-H), 1745 (C═O), 1470 (C═S); **^{1}H-NMR** ($CDCl_3$, 600 MHz), δ(ppm): 7.30–7.42 (m, 5H, $CH_2C_6H_5$), 5.35 (s, 2H, $CH_2C_6H_5$), 4.56 (s, 2H, CH_2 ring), 4.46 (s, 2H, CH_2 ring), 3.94 (t, 2H, $J = 6$ Hz, $CH_2CH_2O(CO)CH_3$), 2.80 (t, 2H, $J = 6$ Hz, $CH_2CH_2O(CO)CH_3$), 1.96 (s, 3H, $CH_2CH_2O(CO)CH_3$); **^{13}C-NMR** ($CDCl_3$, 150 MHz), δ (ppm): 192.32 (C═S), 137.93 (C-8), 129.15 (C-10), 128.57 (C-9), 128.16 (C-11), 85.16 (C-4), 69.14 (C-13), 61.52 (C-6), 58.83 (C-12), 53.52 (C-7), 21.12 (C-14); **HRMS (ESI):** calculated for $C_{14}H_{18}N_2O_2S_2$ $[M+H]^+$: calcd: 311.0882 found 311.0875.

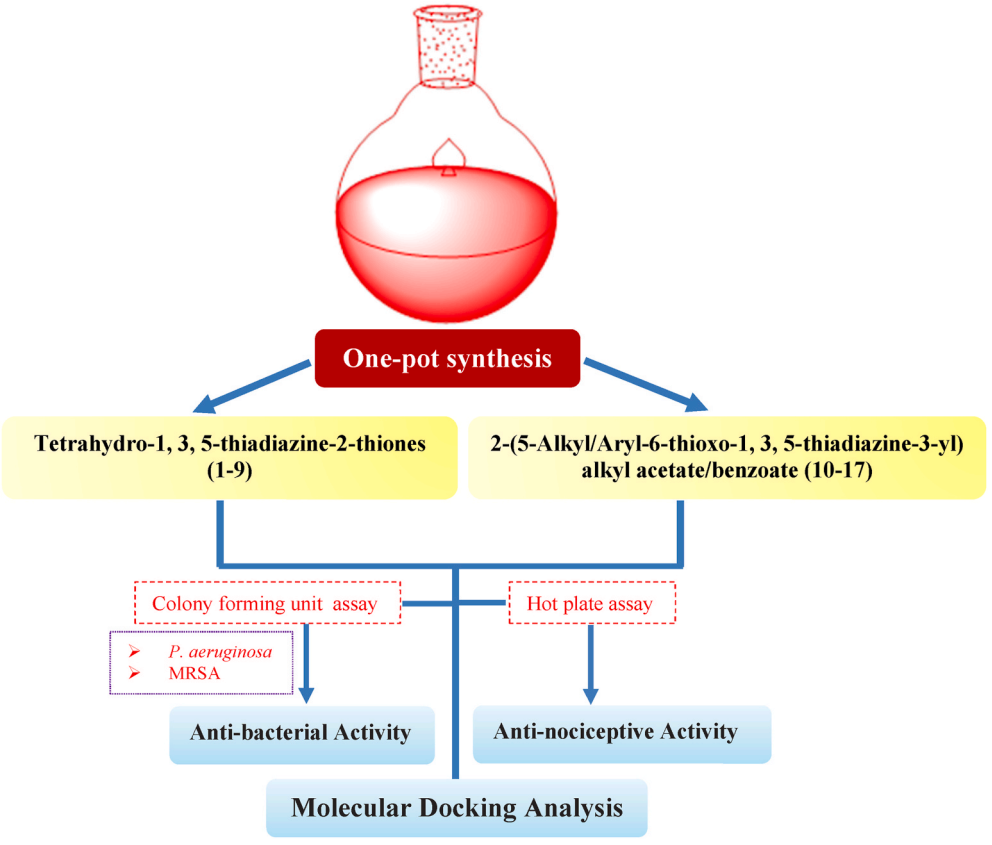


Fig. 2. Work-flow diagram of this research work.

17. 2-(5-Benzyl-6-thioxo-1,3,5-thiadiazinan-3-yl) ethyl benzoate

Yield: 77 % (0.58 g), m.p: 115–117 °C; solubility: methanol; **FT-IR** (cm^{-1}): 3103 (aromatic-C-H), 2865–2990 (aliphatic-C-H), 1746 (C═O), 1473 (C═S); **^{1}H-NMR** ($CDCl_3$, 400 MHz), δ(ppm): 8.1 (d, 2H, J = 8 Hz, 2CH), 7.93 (d, 2H, J = 8 Hz, 2CH), 7.55 (m, 1H, CH), 7.29–7.48 (m, 5H, $2C_6H_5$), 5.34 (s, 2H, CH_2–C_6H_5), 4.44 (s, 2H, THTT ring), 4.33 (s, 2H, THTT ring), 4.15 (t, 2H, J = 8 Hz, $CH_2CH_2O(CO)C_6H_5$), 2.97 (t, 2H, J = 8 Hz, $CH_2CH_2O(CO)C_6H_5$); **^{13}C-NMR** (DMSO, 100 MHz), δ(ppm): 193.9 (C═S), 166.02 (C═O), 136.22 (C-8), 133.85 (C-17), 132.9 (C-14), 129.64 (C-16), 129 (C-15), 129.21 (C-10), 129.05 (C-9), 128.16 (C-11), 69.27 (C-4), 62.49 (C-13), 58.95 (C-6), 53.52 (C-7), 49.17 (C-12); **HRMS (ESI):** calculated for $C_{19}H_{20}N_2O_2S_2$ $[M+H]^+$: calcd: 373.1039 found 373.1032. The work-flow of this research is given in Fig. 2.

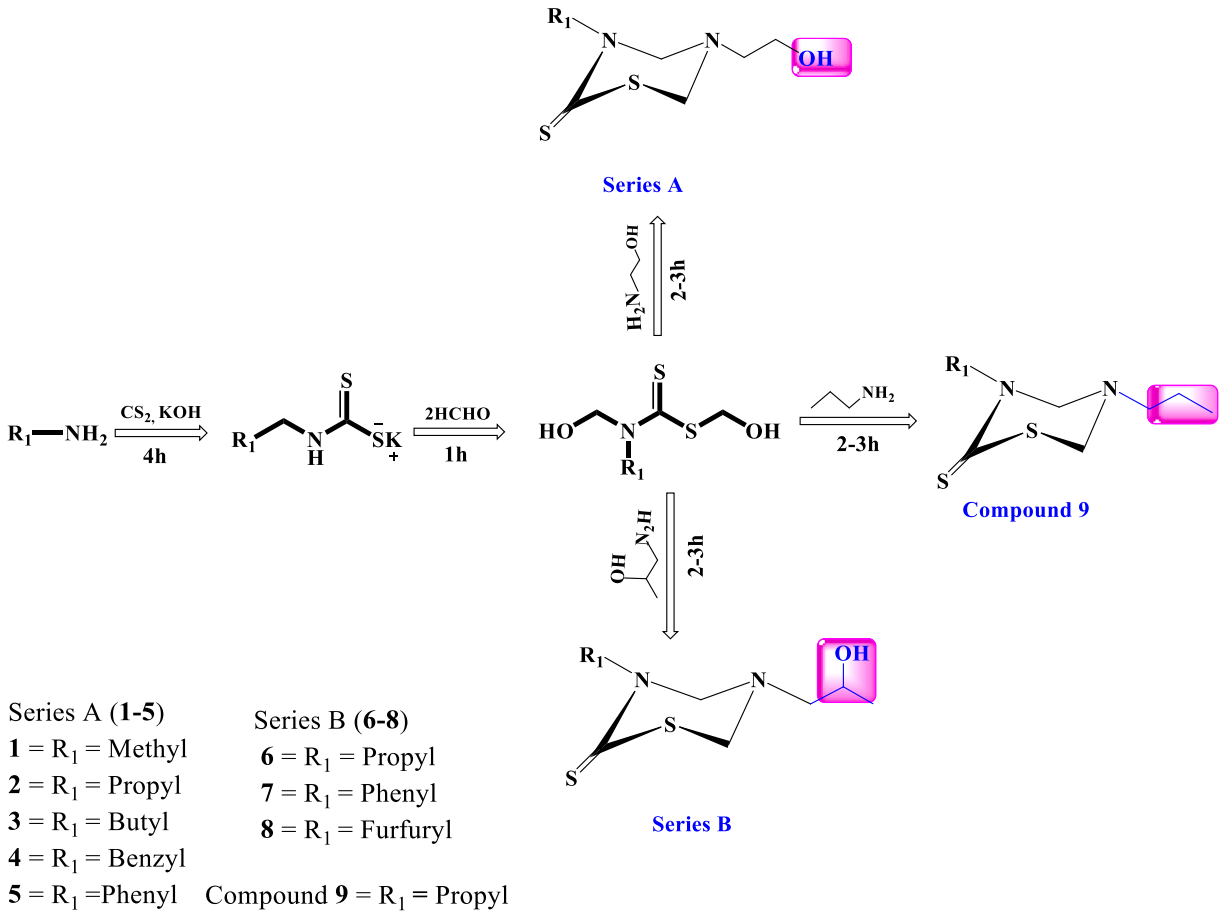
3. Biological evaluation

Ethical approval

The study was approved by the Ethical Committee of the National Institute for Biotechnology and Genetic Engineering (NIBGE), P. O. Box 577, Jhang Road, Faisalabad-3800, Animals models (mice) used in this study were taken from the Animal House Facility after approval from Institutional Bioethics Committee, ref. no NIBGE/Bioethics/2014/02.

3.1. Anti-nociceptive activity

Hot plate (Harvard apparatus, USA) test was performed to evaluate anti-nociceptive activity of the synthesized compounds on BALB/C mice (18–22 g, either sex). Temperature was kept at 55.0 ± 0.1 °C and their thermally induced nociception responses (hind paw-lifting, licking, jumping, or flicking) were noted. 20 s were considered as cut-off time to avoid tissue damage. As a control group, phosphate buffer saline (PBS) (10 ml/kg) was used, tramadol (50 μg/kg) as standard group and the synthesized compounds (50, 100



Scheme 1. Synthesis of thiadiazine thiones from different amines, series A **(1–5)**, series B **(6–8)** and compound **9**. Reaction Conditions: H_2O (30 mL), KOH (1.12 mg), CS_2 (1.2 mL), 3–4 h stirring, 2HCHO (3.01 mL), 1h stirring, Phosphate buffer (20 mL, pH 7.8), 2–3 h stirring.

μg/kg) were taken as test group and injected into mice [36] and their response was noted at 15, 30, 60 and 90 min, post-administration [37].

3.2. Anti-bacterial studies

The anti-bacterial activities of the synthesized compounds were determined against *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) by colony forming unit (c.f.u) method (described earlier) [7]. Before the experiment, bacterial species were grown in Luria-Bertani (LB) broth at 37 °C overnight. The optical density (OD) of the overnight grown bacteria was adjusted to 0.22 at 600 nm using spectrophotometer (Infinite® 200 PRO, TECAN, Switzerland). Subsequently, bacteria (1×10^6 c.f.u/mL) were exposed to 50 μg/mL of the synthesized compounds and the final assay volume was raised up to 200 μL using PBS [38]. The compound-treated bacteria were incubated for 2 h at 37 °C and subsequently serially diluted (ten-fold) from 10^{-1} to 10^{-6}. Dilution factors (10^{-3} – 10^{-6}) were plated on freshly prepared nutrient agar plates, maintained for 24 h at 37 °C. Next day, cfu/mL was determined by enumerating the viable bacterial growth. All the experiments were performed three times in duplicates. Gentamicin (50 μg/mL) was used as positive control while, bacteria cultured in PBS was used as negative control [38].

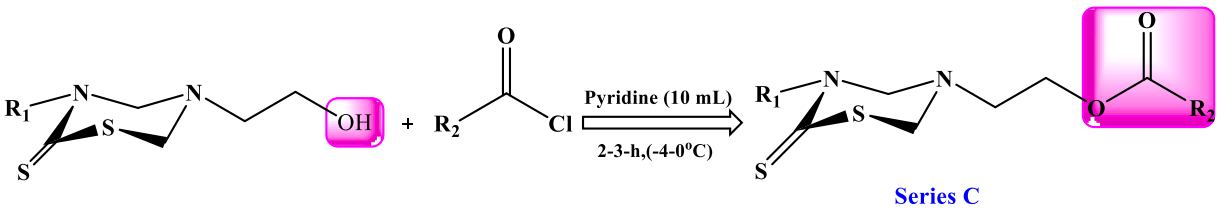
3.3. Docking studies

Molecular Operating Environment (MOE) suite 2020.09 [39] was used in docking studies of compounds **5**, **9**, and **11** in the binding sites of μ-Opioid receptor (μ-OR, PDB Code 5C1M) [40]. The receptor file was set up for docking by adding protons and partial charges by protonate 3D of MOE with AMBER12:EHT force field. The structures of ligands (**5**, **9**, and **11**) were prepared by MOE with MMFF94x charges, and energy minimized with default parameters of MOE (MMFF94x force field, RMS gradient = 0.1 kcal/mol/Å2). The compounds were docked with Alpha Triangle placement method, London dG scoring function, and 30 docked poses of compounds were refined by GBVI/WSA dG rescoring.

4. Results and discussion

4.1. Synthesis

Different methods are reported for the synthesis of THTT scaffold like solid and liquid phase synthesis [41,42] from isothiocyanates salts [43], and the most common method proceeds *via* diathiocarbamate salt formation [44]. Herein, we report the simple and convenient method to synthesize the targeted compounds, involving reaction of primary amines, such as methyl, propyl, butyl, benzyl, furfuryl amines, and aniline, with CS_2 in the presence of KOH, to yield their respective diathiocarbamate as intermediate salts. Later formaldehyde was added to diathiocarbamate salts, followed by cyclocondensation with appropriate amines i.e., ethanolamine and amino-2-propanol in buffer (pH 7.8), affording the respective substituted thiadiazine-2-thione analogues in moderate to excellent yields (Scheme 1). This method is flexible and afforded large number of THTTs with different substitution pattern [45]. In thisarticle, two series of compounds have been disclosed, in which one series has been attributed to different substituted thiadiazine thiones analogues. This includes, 3, 5-disubstituted-tetrahydro-2*H*-1, 3, 5-thiadiazine-6-thione with *N*-3 alkyl/aryl and *N*-5 hydroxyethyl (Series A), *N*-3 alkyl/aryl and *N*-5 tethered with 2-propanol-1-yl group (series B), and compound **9**.



Series C (**10-17**)

10 = R_1 = Propyl, R_2 = Methyl
11 = R_1 = Propyl, R_2 = Phenyl
12 = R_1 = Butyl, R_2 = Methyl
13 = R_1 = Butyl, R_2 = Phenyl
14 = R_1 = Phenyl, R_2 = Methyl
15 = R_1 = Phenyl, R_2 = Phenyl
16 = R_1 = Benzyl, R_2 = Methyl
17 = R_1 = Benzyl, R_2 = Phenyl

Scheme 2. Synthetic outline for the synthesis of **Series C** compounds **(10–17)**. Reaction conditions: THTT (2–3 mmol), Acetyl chloride, benzoyl chloride (1.3equiv), adding dropwise stirring for 2–3 h, temp (-4-0 °C).

Esterification is one of the most extensively used reactions and considered as the most vital chemical transformation in synthetic organic chemistry. Since after Fischer esterification, vast experimental research has been attributed to this and ample synthetic methodologies have been established. However, esters are most conveniently synthesized by the action of activated carboxylic acid chlorides on alcohols [46].

In this article, we further extended the synthesis of ester analogues of our ethanolamine substituted 3, 5-disubstituted-thiadiazine-2-thiones (series A) to form series C. Therefore, these alcohols affixed THTTs were reacted with acetyl chloride and benzoyl chloride in dry pyridine in ice bath. Upon reaction completion, the reaction was quenched with water, extracted with DCM and the pure product was obtained as white crystalline solids (Scheme 2).

In these reactions, pyridine served as solvent, HCl as scavenger and more importantly as acylating reagent (nucleophilic activation) [46]. Moreover, these reactions have also been tried in Et_3N, however, THTT insolubility, poor yields and the gummy products were some of the problems. Also, the reaction needs to be done in freshly distilled pyridine as the yield of esters reduces due to the competing reactions of water with the alcohol to react with acetyl chloride and benzoyl chloride to produce acetic acid and benzoic acid, respectively.

During acylation reactions, we observed that acetyl chloride reacts more rapidly with a given THTT alcohol than benzoyl chloride and the yields were high for the former. This reflects that in benzoyl chloride, the positive charge of the carbonyl carbon atom is considerably dropped by the nearby phenyl moiety *via* resonance, whereas, in acetyl chloride, the carbonyl has no aromatic nucleus in conjugation. Since acid chlorides were used instead of acids for esterification, this offered several advantages, like the reactions proceeded to completion due to no reversible equilibrium, the reactions were faster and completed at much lower temperatures [47].

All the structures from chemical synthesis were established by spectroscopic analyses. FT-IR data displayed hydroxyl (O–H) and carbonyl (CO) absorption band in the region of 3310-3300 cm^{-1} and 1747-1745 cm^{-1} while, C═S band appeared around 1500-1465 cm^{-1}. Aliphatic stretches of C–H bond have been observed in the range 2865-2850 cm^{-1} while, that of aromatic C–H stretches has been observed in the range of 3025-3021 cm^{-1}. For ester derivatives, aliphatic stretches for C–H bonds occurred in the region of 2990-2860

Table 1
Antinociceptive effects of Compounds on the response latency in seconds (mean ± S.D).

Compounds	Treatment (dose/kg body weight)	Number of mice	Time after Drug Injection (min ±S.D)			
			15 min	30 min	60 min	90 min
	Placebo	6	13.9 ± 3.1	14.2 ± 1.1	15.3 ± 3.0	14.9 ± 2.8
	Tramadol (50 μg)	6	16 ± 2.1**	19.3 ± 3.1**	24.3 ± 1.20***	23.4 ± 2.20***
1	50 μg	6	12.9 ± 2.8	14.1 ± 3.0*	15.1 ± 1.1**	14.8 ± 2.5*
	100 μg	6	14.6 ± 2.2*	14.9 ± 2.2*	15.5 ± 2.4**	13.4 ± 2.1
2	50 μg	6	11.6 ± 1.8	15.5 ± 2.3**	14.9 ± 1.0*	15.5 ± 1.4**
	100 μg	6	13 ± 2.0	14.3 ± 2.9*	13.3 ± 1.2	11.2 ± 1.6
3	50 μg	6	14.1 ± 3.0*	14.8 ± 2.5*	15.1 ± 1.0**	14.3 ± 2.1*
	100 μg	6	13.3 ± 2.2	13.4 ± 2.1	15.3 ± 2.5**	16.4 ± 2.6**
4	50 μg	6	14.8 ± 2.3*	16.5 ± 2.1**	15.9 ± 2.8**	13.8 ± 2.5
	100 μg	6	13.4 ± 1.2	14.3 ± 2.6	15.5 ± 2.9**	16.1 ± 2.5**
5	50 μg	6	15.5 ± 2.1**	15.9 ± 1.9**	16.1 ± 3.0**	14.3 ± 1.2*
	100 μg	6	16.3 ± 2.6**	16.9 ± 2.1**	17.3 ± 2.2**	14.3 ± 2.8*
6	50 μg	6	12.6 ± 1.3	14.5 ± 2.3*	16.9 ± 1.0**	15.3 ± 1.7**
	100 μg	6	14 ± 2.0*	15.3 ± 2.9**	14.3 ± 1.2*	16.2 ± 1.6**
7	50 μg	6	11.3 ± 2.1	12.8 ± 2.1	13.7 ± 2.7	14.6 ± 3.6*
	100 μg	6	14.1 ± 2.2*	13.9 ± 2.2	13.2 ± 1.9	14.6 ± 2.5*
8	50 μg	6	14.8 ± 2.5*	14.3 ± 3.0	12.9 ± 2.8	13.6 ± 2.5
	100 μg	6	13.4 ± 2.1*	16.1 ± 2.1**	15.1 ± 2.2**	14.9 ± 2.6*
9	50 μg	6	13.5 ± 1.3*	14.8 ± 2.0*	14.1 ± 1.3	13 ± 2.9
	100 μg	6	12.1 ± 1.0	15.1 ± 2.3*	15.6 ± 1.7**	14.6 ± 2.2*
10	50 μg	6	12.4 ± 1.9	14.7 ± 1.0*	13.4 ± 1.0	15.2 ± 1.1
	100 μg	6	16.2 ± 2.1**	16.1 ± 2.5**	14.3 ± 2.4*	13.9 ± 1.2
11	50 μg	6	12.2 ± 1.3	12.8 ± 2.5	13.5 ± 1.0	13.7 ± 2.5
	100 μg	6	13.7 ± 1.8	14.4 ± 2.2*	13.9 ± 2.5	14.6 ± 2.6*
12	50 μg	6	11.4 ± 2.9	11.9 ± 2.0	13.7 ± 1.3	14.1 ± 2.2*
	100 μg	6	14.2 ± 1.1*	15.1 ± 3.2**	12.2 ± 1.8	13.5 ± 2.8
13	50 μg	6	14.3 ± 1.5*	14.8 ± 1.3*	12.3 ± 1.9	13.7 ± 1.8
	100 μg	6	12.2 ± 1.1	13.5 ± 1.2	11.5 ± 1.1	12.1 ± 2.1
14	50 μg	6	12.5 ± 1.0	13.6 ± 2.0	12.5 ± 1.1	14.2 ± 1.2*
	100 μg	6	11.3 ± 1.7	12.1 ± 1.4	12.6 ± 2.1	13.9 ± 1.3
15	50 μg	6	10.1 ± 2.2	11.9 ± 1.7	10.1 ± 1.4	12.2 ± 2.1
	100 μg	6	13.4 ± 2.7	14.1 ± 2.8*	14.3 ± 2.4*	15.3 ± 2.6**
16	50 μg	6	12.9 ± 1.7	13.7 ± 3.0	14.3 ± 1.7*	14.9 ± 1.5*
	100 μg	6	13.7 ± 3.1	14.5 ± 1.6*	14.2 ± 2.5*	14.7 ± 2.2*
17	50 μg	6	11.1 ± 1.2	12.7 ± 1.4	13.1 ± 1.0	13.6 ± 2.5
	100 μg	6	10.2 ± 3.1	11.1 ± 1.2	12.3 ± 2.6	12.7 ± 1.5

Each value represents % anti-nociception ± S.D. $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to vehicle (Veh) treated group (one-way ANOVA followed by Tukey's or Dunnett's *post hoc* test, $n = 4$ mice per group).

cm^{-1}. In the ^{1}HNMR spectra, the THTT characteristic peaks were observed as singlets in the range δ 4.12–5.22 ppm in presence of hydroxyethyl group at *N-5* position, while in case of hydroxypropyl group having stereocenter, two separate signals were observed as multiplets in the region, 4.30–4.75 ppm. The splitting of ring methylene group as doublet and multiplet was due to presence of diastereotopic protons at *N*-5 position. Similarly, methylene protons adjacent to the stereocenter (non-equivalent), in hydroxypropyl group at *N*-5 position were split into two separate signals as doublet and multiplet in the range of1.66–3.34 ppm.

In the same way, the ^{13}CNMR spectra of the synthesized compounds showed characteristic signals of THTT carbons i.e., C4 and C6 in the region δ 69.3–90 ppm and 58.3–70 ppm respectively, while signal for C═S group has been observed in the range 190.4–193.9 ppm. The indicative signal for carbonyl group occurred in the region 166.0–170.8 ppm.

5. Biological assay

5.1. Anti-nociceptive activity

Anti-nociceptive activity of all the synthesized thiadiazine thiones was assessed *via* hot plate test on BALB/C mice (18–22 g, either sex). Results (Table 1) indicate that compounds **3**–**6**, **10, 16** (50, 100 μg/kg), **1, 2** (50 μg/kg) and **8** (100 μg/kg) showed remarkable inhibition of thermally induced pain. These compounds demonstrated pronounced effect ($P < 0.01$, $P < 0.05$) in latency time (seconds) as compared to placebo treated animals at 15, 30, and 60 min intervals. Moreover, compounds **3**, **5** (50 μg/kg), and **8** (50, 100 μg/kg) displayed notable increase ($P < 0.01$, $P < 0.05$) in latency time at 15, 30 and 60 min, while compounds **6** and **16** (100 μg/kg) exhibited significantly high ($P < 0.01$, $P < 0.05$) latency at 15- and 30 min interval. Similarly, compound **7** showed major protection ($P < 0.05$) at 15 min interval. However, compound **5** treated group (50 μg/kg) indicated an increase ($P < 0.05$, $P < 0.01$) in latency time at 15, 30, and 60 min interval as compared to placebo treated animals. Compound **10** (100 μg/kg) showed similar latency ($P < 0.01$) at 15–60 min intervals. Compounds **1**, **12**, **13** and **15** showed less potential at different time intervals, the rest of compounds exhibited no significant effect. The efficacy of all the tested compounds was less than the standard drug.

The limited structure-activity relationship indicates that the most active compound (**5**) in series A have phenyl group at R1 position, which may be involved in the π-π interaction with ligand binding site. The second most active compound (**4**) of this series also has the benzyl group at R1 position, which may have similar patterns of interaction like **5**. Compounds **1**, **2** and **3** have methyl, propyl and butyl groups, respectively, also showed good activity but not like compounds **4** and **5**. Similarly, in series B, compound **6** have propyl group at R1 position showed good activity but not like **4** and **5**. Interestingly, in series C, the most active compound (**16**) also has benzyl group at R1 position, which may have same pattern of interaction like **4** and **5**. In series C, there are also substitution at R2 position, but this substitution has no significant effect on activity. The rest of the compounds (**1–15** and **16**) of this series have almost similar activity with smaller variation. In conclusion it indicates that phenyl and benzyl group at R1 position play key role in the activity.

5.2. Anti-bacterial effects of compounds

Infectious diseases were the major cause of death in the beginning of 20th century and led to >14 million deaths annually [5–7]. The decline in morbidity and mortality from infectious diseases occurred with the discovery of antimicrobial agents [8]. Currently, the emergence of anti-microbial resistance is hazardous to human life and has been reaching to such a critical level that it was the focus of 2011 by the WHO [3]. The growing interest and absolute discovery of new, selective, and promising molecules with best efficacy profile, motivated us to uncover synthetically accessible heterocyclic molecules with enhanced anti-bacterial activity.

Table 2
Antibacterial activities of compounds against Gram-positive and Gram-negative pathogenic bacteria.

Compounds	%Inhibition against MRSA (%±SD)	MIC (μg/mL)	% Inhibition against *P. aeruginosa* (%±SD)	MIC (μg/mL)
Negative control	0.00 ± 0.00	–	0.00 ± 0.00	–
Gentamicin	100.00 ± 0.00	2	100.00 ± 0.00	3
1	30.1 ± 1.15 (*)	250	2.46 ± 1.98	300
2	26.4 ± 1.01 (*)	285	14.42 ± 1.10	>500
3	16.9 ± 1.29 (*)	445	29.21 ± 3.70 (*)	258
4	36.42 ± 2.69 (*)	206	10.40 ± 1.85	>500
5	26.9 ± 2.95 (*)	278	24.85 ± 2.14 (*)	300
6	28.34 ± 1.34	284	14.31 ± 1.10	>500
7	48.27 ± 4.43 (*)	155	12.88 ± 2.05	>500
8	16.77 ± 2.56	445	1.02 ± 0.10	>500
9	43.73 ± 4.64 (*)	140	12.95 ± 4.37	>500
10	44.56 ± 0.43 (*)	170	10.65 ± 2.19	>500
11	54.77 ± 3.63 (*)	54.77 (*)	9.38 ± 1.68	>500
12	49.65 ± 5.12 (*)	152	26.15 ± 0.97 (*)	>500
13	33.74 ± 5.15	220	7.93 ± 3.68	>500
14	40.97 ± 2.86 (*)	40.97 (*)	6.29 ± 3.75	>500
15	6.40 ± 1.53	>500	3.95 ± 2.13	>500
16	51.89 ± 3.28 (*)	145	17.83 ± 0.43 (*)	420
17	20.04 ± 2.05 (*)	375	13.14 ± 4.42	>500

P values were calculated by T-test statistics, (*) is $P \leq 0.05$.

The anti-bacterial activity of the synthesized compounds was tested against *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) *via* colony forming unit (c.f.u) method [7]. Minimum inhibitory concentration (MIC) values were calculated, and gentamycin was used as a standard drug (Table 2). Several compounds exhibited notable anti-bacterial activities ($P \leq 0.05$) against MRSA and moderate activity against *P. aeruginosa*. Compounds **14** and **11** were found to be the most active against MSRA and exhibited best potential ($P \leq 0.05$) with MIC values of 40.97 and 54.77 μg/mL, respectively. Compounds **14** and **11** possess phenyl at R1 and methyl at R2 (**14**), and R1 propyl and phenyl at R2 position (**11**), respectively. Compounds **9** and **16** also possess significant activity (140 and 145 μg/mL) which have propyl and benzyl groups at R1 position, respectively, while compounds **7**, **10**, and **12** exhibited good response with MIC values of 155, 170, 152 μg/mL, respectively, against MRSA. Rest of the compounds showed effectiveness with MIC >500 μg/mL, against MSRA.

Moreover, compound **3** showed efficacy with MIC 258 μg/mL against *P. aeruginosa* while, compounds **1** and **5** exhibited anti-bacterial potential with MIC 300 µg/mL. Rest of the compounds were found to be less effective against *P. aeruginosa*, with MIC >400 μg/mL. The results show that most of the compounds are more active against *MSRA* than *P. aeruginosa*, however, less effective than the standard drug, gentamicin.

5.3. Molecular docking of the most active anti-nociceptive molecules

Compounds **5**, **9**, and **11** exhibited significant anti-nociceptive potential; therefore, these molecules were docked at the ligand binding site of µ-opioid receptor, which is a target of the standard drug, tramadol. We observed an excellent binding of these three

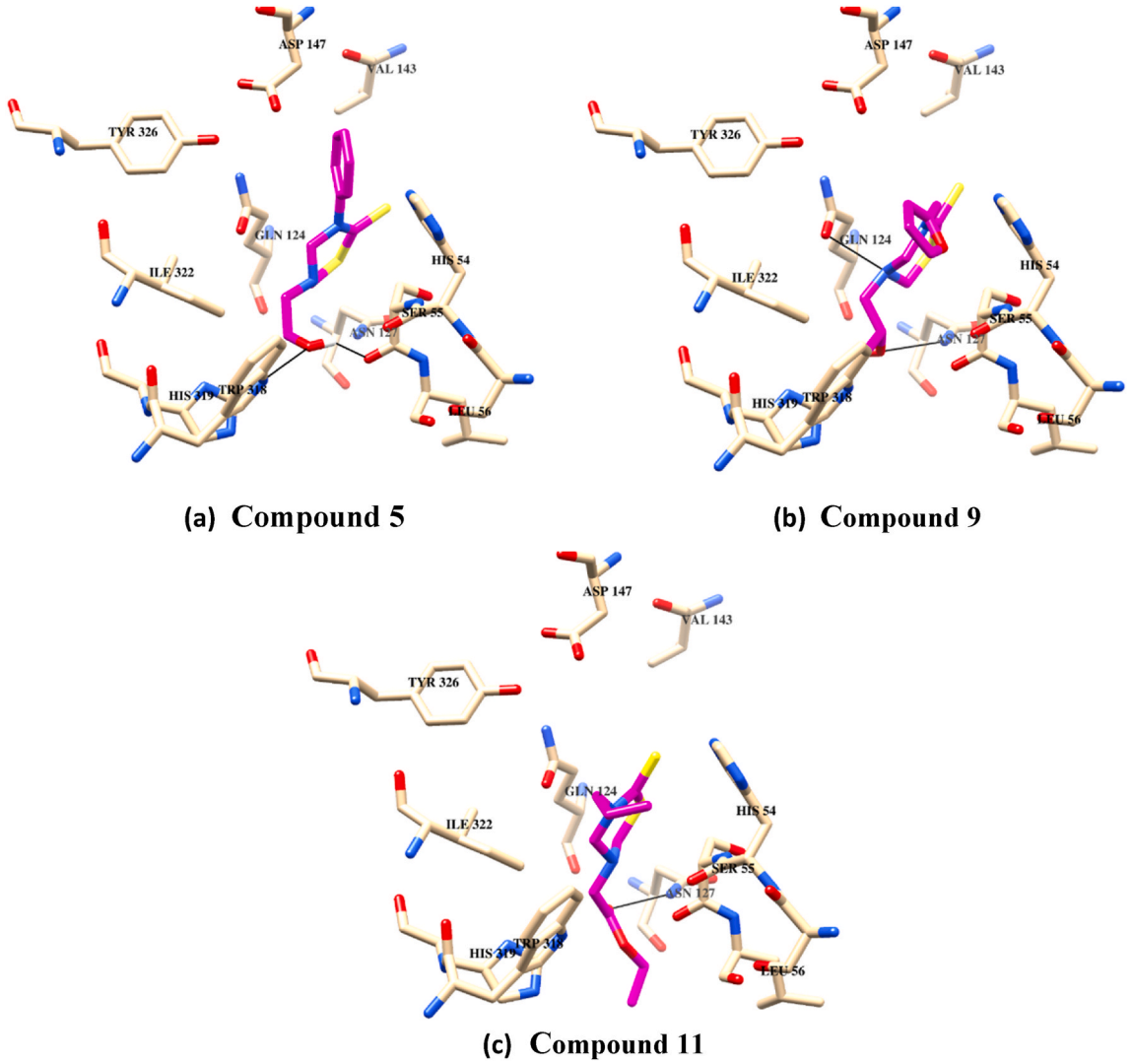


Fig. 3. The binding modes of compounds (**a**) **5**, (**b**) **9**, and (**c**) **11** are shown in the ligand binding site of µ-opioid receptor (µOR). The compounds, residues of µOR, and H-bonds are shown in magenta stick model, tan stick model and in black lines, respectively.

compounds at the ligand binding of μ-opioid receptor (μOR). Compound **5** mediates strong Hydrogen bonds (H-bonds) with Ser55 and Trp318. The –OH of compound **5** interacts with the carbonyl oxygen of Ser55 and the side chain of Trp318 at 2.08 Å and 2.17 Å, respectively (Fig. 3a). Whereas the –OH group of compound **9** formed an H-bond with the side chain of Asn127 at 2.39 Å, while the furan ring of this molecule mediates π-π interaction with the side chain of His54 at 3.45 Å (Fig. 3b). Similarly, the carbonyl oxygen of compound **11** formed a strong H-bond with the side chain of Asn127 at 1.65 Å (Fig. 3c). The binding modes of these compounds are presented in Fig. 3(a–c). Compounds **5**, **9** and **11** exhibited high negative docking scores i.e., −6.27 kcal/mol, −6.93 kcal/mol, and −6.88 kcal/mol, respectively. We also docked the tramadol as a reference drug, which indicates that the –OH group of the standard drug mediates H-bonds with the side chain of Asp147 at 1.83 Å, and the docked molecules exhibits higher docking score (−7.53 kcal/mol) than the rest of the compounds. This *in-silico* docking result indicates that these molecules bind effectively within the active pocket of μOR, however, their conformations are slightly diverse than the docked orientation of tramadol.

5.4. Predicted ADMET of compounds 5, 9, and 11

The physicochemical profile of compounds **5**, **9** and **11** were also predicted to determine their safety profile. The molecular weight, $LogP_{o/w}$ (partition coefficient), and LogS (aqueous solubility) indicates that these are small fragments, with molecular weights in the range of >254 to <325, LogP from 1.73 to 3.33 and less water solubility (−3 to −4.4). They possess 3–5 hydrogen bond acceptor atoms, and only compound 5 has one hydrogen bond donor atom. Moreover, their acute oral toxicity ranges from 1.54 to 2.49 mol/kg (Table 3).

The ADMET profile of compounds indicated that they possess good human intestinal absorption, Caco-2 permeability, and human oral bioavailability. However, these molecules can penetrate the blood brain barrier. These molecules do not possess inhibitory or substrate like property for P-glycoprotein, they have no carcinogenic ability, no skin irritation, and skin sensitivity. Only compound **9** showed AMES mutagenesis ability. Whereas, compounds **5** and **9** have shown hepatotoxicity with low probability. All these molecules can cause respiratory toxicity, while only compound **9** does not have reproductive toxicity. Moreover, these molecules do not possess nephrotoxicity. They are categorized in category III of acute oral toxicity which indicates they may cause acute oral toxicity at very high dose (300 mg/kg). The metabolic profile of these molecules suggests that only compound **11** can act as substrate for CYP3A4, while none of the molecules can act like substrate for CYP2C9 and CYP2D6. Moreover, compounds **9** and **11** cannot inhibit CYP3A4, while compound **5** did not shown CYP2C9 and CYP2D6 inhibitory potential. All the molecules can inhibit CYP2C19 and CYP1A2, while none of the molecules can inhibit CYP2C8. The results (Table 4) demonstrate that these small fragments can have better ADMET profile upon structural and functional optimization.

6. Conclusion

Two series of 3,5-di-substituted-thiadiazine-2-thione and their ester derivatives have been designed and screened for their anti-nociceptive and anti-bacterial potential. The structure-activity relationship revealed that compounds containing aryl groups (phenyl, benzyl) at *N*-3 and hydroxyethyl groups at position *N*-5 (**4** and **5**) showed significant anti-nociceptive activity and significantly increased the latency time ($P < 0.05$, $P < 0.01$). Compound **5** having phenyl group at *N*-3, significantly reduced the thermally induced nociception ($P < 0.01$) at 50 and 100 μg/kg concentrations, while those bearing benzyl groups (**4**) showed prominent potential at 100 μg/kg concentration. Similarly, activity of acetyl ester of propyl containing compound at *N*-3 position (**10**) increased in a dose-dependent manner ($P < 0.01$). Anti-bacterial activity data revealed that compounds containing hydroxypropyl group at *N*-5 position exhibited best anti-bacterial potential as compared to compounds containing hydroxyethyl group at position *N*-5 due to lipophilic group at *N-5* position. Acetate and benzoate ester of aniline and propyl containing compounds (**14**, **11**) possessed significant activity with MIC 40.97 and 54.77 μg/mL.Acetate ester of alkyl containing compound at *N-3* showed best anti-bacterial potential with MIC 145 and 152 μg/mL. All the tested compounds exhibited greater resistance against MRSA than *P. aeruginosa.* The anti-bacterial activity of all the synthesized compounds were found to be enhanced with esterification. Furthermore, molecular docking and predicted ADMET profile of compounds **5**, **9**, and **11** indicated that their biological potency can be improved by further structural optimization. The superior anti-nociceptive activities of compounds **5**, **9**, and **11** and safe drug character, indicate that these compounds can be used as drug candidates for pain management, subject to further investigations.

Table 3
Physicochemical properties of Compounds 5, 9, and 11.

Property	Compound 5	Compound 9	Compound 11
Molecular Weight	254.38	218.39	324.47
LogP	1.73	2.36	3.33
H-Bond Acceptor	4	3	5
H-Bond Donor	1	0	0
Rotatable Bonds	3	4	5
Applicability Domain	In domain	In domain	In domain
Water solubility (LogS)	−3.097	−3.039	−4.381
Plasma protein binding (100 %)	0.653	0.357	0.706
Acute Oral Toxicity [log(1/(mol/kg))]	1.538	2.464	2.486

Table 4
ADMET properties of Compounds 5, 9, and 11.

ADMET profile	Value	Probability	Value	Probability	Value	Probability
	Compound 5		Compound 9		Compound 11	
Human Intestinal Absorption	+	0.9522	+	0.9641	+	0.9830
Caco-2	+	0.8478	+	0.9012	+	0.8153
Blood Brain Barrier	+	0.9750	+	0.9250	+	0.9000
Human oral bioavailability	+	0.6571	+	0.6857	+	0.5857
P-glycoprotein inhibitor	–	0.9898	–	0.9766	–	0.8483
P-glycoprotein substrate	–	0.9006	–	0.8817	–	0.8384
CYP3A4 substrate	–	0.6350	–	0.6199	+	0.5565
CYP2C9 substrate	–	0.8000	–	0.6000	–	0.5873
CYP2D6 substrate	–	0.7203	–	0.7499	–	0.8495
CYP3A4 inhibition	+	0.6167	–	0.7468	–	0.6452
CYP2C9 inhibition	–	0.7145	+	0.6588	+	0.7329
CYP2C19 inhibition	+	0.6149	+	0.8210	+	0.8492
CYP2D6 inhibition	–	0.7031	+	0.7334	–	0.7004
CYP1A2 inhibition	+	0.5176	+	0.7731	+	0.5415
CYP2C8 inhibition	–	0.8784	–	0.9770	–	0.8065
CYP inhibitory promiscuity	+	0.7845	+	0.6537	+	0.9510
Carcinogenicity	–	0.9600	–	0.9600	–	0.8600
Skin irritation	–	0.7425	–	0.6344	–	0.7663
AMES mutagenesis	–	0.5800	+	0.5100	–	0.7000
Human Ether-a-go-go-Related Gene inhibition	–	0.4145	–	0.5944	–	0.4188
Hepatotoxicity	+	0.6035	+	0.5250	–	0.5091
skin sensitization	–	0.8566	–	0.8772	–	0.8208
Respiratory toxicity	+	0.8889	+	0.6333	+	0.7444
Reproductive toxicity	+	0.6556	–	0.6778	+	0.5556
Nephrotoxicity	–	0.7446	–	0.8067	–	0.7288
Acute Oral Toxicity	III	0.6606	III	0.6040	III	0.5541

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Consent for publication

Not applicable.

Ethics approval

The study was approved by the Ethical Committee of the Department of Pharmacy, University of Peshawar (Approval no. 12/EC-17/Pharm) and the experiments were performed following rules of the UK Animals (Scientific Procedures) Act 1986.

Availability of data and materials

All datasets on which the conclusions of the manuscript rely are presented in the paper.

CRediT authorship contribution statement

Asma Gul: Methodology, Writing - original draft, Data curation, Formal analysis. **Sobia Ahsan Halim:** Methodology, Formal analysis. **Ajmal Khan:** Writing – review & editing, Conceptualization. **Rasool Khan:** Supervision, Conceptualization. **P.A.N. Xian-Dao:** Resources, Formal analysis. **Salman Zafar:** Investigation, Data curation, Formal analysis and manuscript editing. **Noor Akbar:** Validation, Software, Methodology, Writing - original draft, Data curation, Formal analysis. **Afnan Jan:** Methodology, Investigation. **Abdullatif Bin Muhsinah:** Project administration, Investigation, Data curation. **Anar Gojayev:** Methodology, Data curation. **Ahmed Al-Harrasi:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Author Ajmal Khan working as a associate editor in section “Pharmaceutical Sciences” of this journal.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30435.

Supporting Information

Figs. S1–S13 have ^{1}H NMR and ^{13}C NMR spectra of compounds **5–17**.

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