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Original article

Antimicrobial, anticoagulant, and cytotoxic evaluation of multidrug resistance of new 1,4-dihydropyridine derivatives

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ABSTRACT

A new series of 1,4-dihydropyridine derivatives (**2a–h, 3a–e,** and **4a–e)** were systematically designed and synthesized via ultrasound irradiation methods with easy work-up and good yields. Compounds structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. The synthesized compounds were screened for both antimicrobial and anticoagulant activities. Compound **2e** (MIC: 0.25 μg/mL) was highly active against *Escherichia coli* and compound **2c** (MIC: 0.5 μg/mL) was also highly active against *Pseudomonas aeruginosa* compared with ciprofloxacin. (MIC: 1 μg/mL) The antifungal activity of **2c** (MIC: 0.5 μg/mL) against *Candida albicans* was high relative to that of clotrimazole (MIC: 1 μg/mL). Anticoagulant activity was determined by activated partial thromboplastin time (APTT) and prothrombin time (PT) coagulation assays. Compound 4-(4-hydroxyphenyl)-2,6-dimethyl-N^3,N^5-bis(5-phenyl-1,3,4-thiadiazol-2-yl)-1,4-dihydropyridine-3,5-dicarboxamide **3d** (>1000 s in APTT assays) was highly active in anticoagulant screening compared with the reference of heparin.

Cytotoxicity was evaluated using HepG2 (liver), HeLa (cervical), and MCF-7 (breast) cancer cell lines, with high toxicities observed for **2c** (GI_{50} = 0.02 μm) against HeLa cell line and **2e** (GI_{50} = 0.03 μm) equipotant against MCF-7 cell line. Therefore, the compounds **2e, 2c** and **3d** can serve as lead molecules for the development of new classes of antimicrobial and anticoagulant agent.

1. Introduction

The development of multidrug resistance is a major obstacle in pharmacology, requiring the constant development of new therapeutics. 1,4-Dihydropyridine derivatives display a broad spectrum of pharmacological activities such as antitumor (Boer and Gekeler, 1995), antihypertensive (Wenzel et al., 2000), anticonvulsant (Surendra Kumar et al., 2010), cytotoxic (Miri et al., 2011), and significant of analgesic activities (Agudoawu et al., 1999; Warren and Knaus ,1981; Ulloora et al., 2013). Fig. 1 shows that other important calcium channel blockers of 1,4-dihydropyridine derivatives such as efonidipine, benidipine, barnidipine, azelnidipine, nicardipine, and manidipine. A large number of organic reactions were carried out in higher yield, shorter reaction time or milder conditions under ultrasonic irradiation (Balaji et al., 2014; Palakshi Reddy et al., 2015; He et al., 2015), for example recently reported that 1,4-dihydropyridine for ultrasound reactions and other important ultrasound reaction (Safari et al., 2015).

Several methods have been described for the synthesis of 1,4-dihydropyridines (Tsuruo et al., 1983; Wan et al., 2009) and previous report of 1,4-dihydropyridine with derivatives modify at the 3- and 5-positions was exhibited significant anticoagulant and antimicrobial activities (Surendra Kumar et al., 2011a).

Thiazole, thiadiazole, and oxadiazole derivatives are also display a wide range of pharmacological activities such as anaesthetic (Geronikaki and Theophilidis, 1992) and anti-inflammatory (Giridhar et al., 2001) properties. Oxadiazole and thiadiazole derivatives have been evaluated and proved for a wide range of pharmacological and clinical uses (Tawfeeq et al., 2012), particu-

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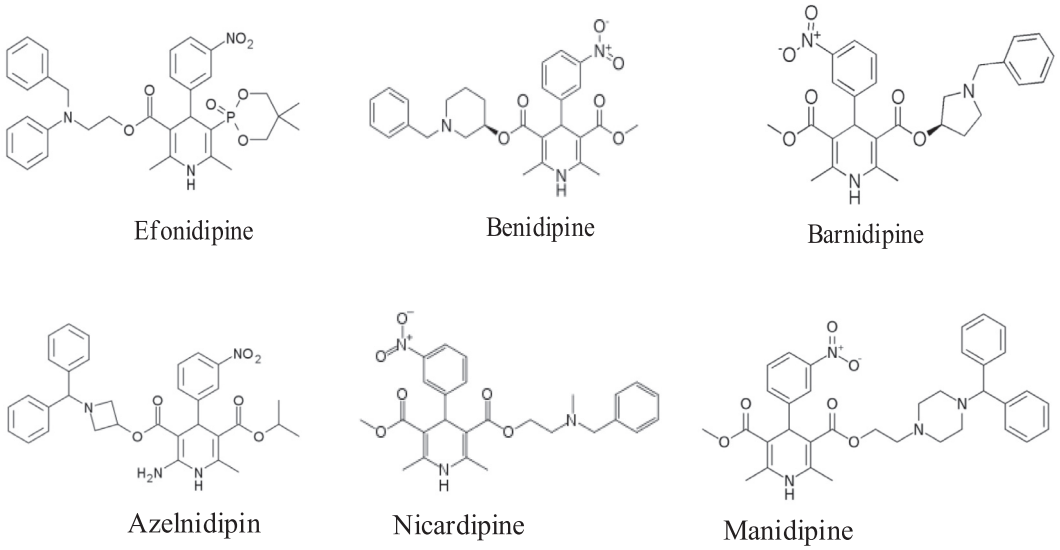


Fig. 1. Multidrug calcium channel blockers.

larly the activity focused by antibacterial, antifungal (Liu et al., 2008), and analgesic (Almasirad et al., 2014) activities.

Thiazoles are useful structural units in the field of medicinal chemistry and have reported antifungal (Tsuruoka et al., 1998) and analgesic (Argyropoulou et al., 2009) activities. The thiazole nucleus appears frequently in the structure of various natural products and biologically active compounds, like thiamine (vitamin-B), also in some antibiotics drugs like penicillin, micrococcin (Rogers et al., 1966), and many metabolic products of fungi and primitive marine animal etc. Based on above literature collections, the current study describes the ultrasound irradiation synthesis of novel 1,4-dihydropyridine connected with thiazole, thiadiazole, and oxadiazole compounds and their evaluation of anticoagulant, antimicrobial, and cytotoxicity activities.

2. Experimental section

2.1. Chemistry

All the chemicals were synthetic grade and commercially procured from Sigma Aldrich. The melting point was determined in an open capillary tube and it is uncorrected. The IR spectra were recorded in KBr on a shimadzu 8201pc (4000–400 cm^{-1}). The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker. The elemental analysis (C, H and N) was recorded using an elemental analyzer model (Varian EL III). The purity of the compounds was checked by thin layer chromatography (TLC) with silica gel plates.

2.1.1. General method for preparation of 4-(furan-2-yl)-2,6-dimethyl-N^3,N^5-bis(4-phenyl thiazol-2-yl)-1,4-dihydropyridine-3,5-dicarboxamide (2a)

A mixture of compound, diethyl-4-(furan-2-yl)-2,6-dimethyl-1 ,4-dihydropyridine-3,5-dicarboxylate **1a** (0.01 mol, 3.19 g) and 2-amino-4-phenylthiazole (0.02 mol, 3.52 g) were added in ethanol solvent stirred by 5 min on ultrasound irradiation. After 5 minute the reaction was completed, the product was confirmed by TLC. The product was washed with distilled water and recrystallized by ethyl acetate to give pure product. The above procedure was followed for the synthesis of compounds **2b**–**h**.

Pale yellow solid; Yield 86%; mp 201–205 °C; IR (cm^{-1}): 3174 (NH), 3074 (CHstr), 3034 (Ar-H), 1652 (OCNH), 1488 (C=N), 738 (C-S-C); ^1H NMR (300 MHz, DMSO-d_6): δ 13.60 (s, 2H, -CONH), 8.84 (s, 1H, NH), 7.89–7.68 (m, 10H, Ar-H), 7.48 (s, 2H, thiazole-2-yl, H-3), 7.29 (d, 1H, J = 6.77 Hz, furan), 6.45 (d, 1H, J = 6.98 Hz, furan), 6.28 (dd, 1H, J = 6.70 Hz, J = 6.92 Hz, furan), 5.85 (s, 1H, 4-CH), 2.50 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 164.2 (2C, thiazol-2-yl, C-1), 163.1 (2C, C=O), 152.5 (1C, furan-C-1), 150.2 (2C, thiazol-2-yl, C-4), 149.8 (2C, 1,4-dihydropyridine, C-2, C-6), 143.2 (1C, furan-C-4), 129.1 (2C, Ar-C′-4), 128.2 (4C, Ar-C′-3, C′-5), 126.9 (4C, Ar-C′-2, C′-6), 126.3 (2C, Ar-C′-1), 113.6 (1C, furan-C-3), 106.4 (1C, furan-C-2), 105.1 (2C, thiazol-2-yl, C-3), 102.8 (2C, 1,4-dihydropyridine, C-3, C-5), 32.4 (1C, 1,4-dihydro pyridine, C-4), 18.0 (2C, 1,4-dihydropyridine, C-2-$\underline{C}H_3$, C-6-$\underline{C}H_3$); EI-MS: m/z 579.23 (M^+,10%), 503.59, 427.49 (100%); 397.45, 367.40, 343.38, 315.32, 289.32, 261.27, 231.24, 203.19. Elemental analysis**:** Calcd. for $C_{31}H_{25}N_5O_3S_2$: C, 64.23%; H, 4.35%; N, 12.08%; S, 11.06%. Found: C, 64.27%; H, 4.40%; N, 12.14%; S, 11.10%.

2.1.2. 2,6-Dimethyl-4-phenyl-N^3,N^5-bis(4-phenylthiazol-2-yl)-1,4-dihydropyridine-3,5-dicar boxamide (2b)

Yellow solid; Yield 60%; mp:238–241 °C; IR (cm^{-1}): 3162 (NH), 3045 (CHstr), 3034 (Ar-H), 1612 (OCNH), 1485 (C=N), 734 (C-S-C); ^1H NMR (300 MHz, DMSO-d_6): δ 13.62 (s, 2H, -CONH), 8.80 (s, 1H, NH), 7.88–7.64 (m, 10H, Ar-H), 7.46 (s, 2H, thiazole-2-yl, H-3), 7.30 (d, 2H, J = 7.09 Hz, Ar-C-3, C-5), 7.22 (d, 1H, J = 7.12 Hz, Ar-C-4), 7.21 (d, 2H, J = 7.17 Hz, Ar-H-2, H-6), 5.81 (s, 1H, 4-CH), 2.47 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 164.8 (2C, thiazole-2-yl, C-1), 163.9 (2C, C=O), 150.7 (2C, thiazole-2-yl, C-4), 149.2 (2C, 1,4-dihydro pyridine, C-2, C-6), 144.4 (1C, Ar-C-1), 129.6 (2C, Ar-C′-4), 128.9 (2C, Ar-C-3, C-5), 128.7 (4C, Ar-C′-3, C′-5),127.6 (2C, Ar-C-2, C-6), 126.8 (4C, Ar-C′-2, C′-6), 126.0 (2C, Ar-C′-1), 125.2 (1C, Ar-C-4), 105.4 (2C, thiazole-2-yl, C-3),103.1 (2C, 1,4-dihydropyridine, C-3, C-5), 32.7 (1C, 1,4-dihydro pyridine, C-4), 18.2 (2C, 1,4-dihydropyridine, C-2-$\underline{C}H_3$, C-6-$\underline{C}H_3$); EI-MS: m/z 589.72; Elemental analysis: Calcd. For ($C_{33}H_{27}N_5O_2S_2$): C, 67.21%; H 4.61%; N, 11.88%; S, 10.87%. Found: C, 67.25%; H, 4.67%; N, 11.94%; S, 10.91%.

2.1.3. 4-(4-Chlorophenyl)-2,6-dimethyl-N^3,N^5-bis(4-phenylthiazol-2-yl)-1,4-dihydro pyridine-3,5-dicarboxamide (2c)

Yellow solid; Yield 63%; mp:113–145 °C; IR (cm^{-1}):3174 (NH), 3074 (CHstr), 3031 (Ar-H), 1642 (OCNH), 1480 (C=N), 827 (C-Cl), 736 (C-S-C); ^1H NMR (300 MHz, DMSO-d_6): δ 13.58 (s, 2H, -CONH), 8.89 (s, 1H, NH), 7.91–7.66 (m, 10H, Ar-H), 7.48 (s, 2H,

thiazole-2-yl, H-3), 7.30 (d, 2H, *J* = 6.98 Hz, Ar-H-3, H-5), 7.21 (d, 2H, *J* = 6.87 Hz, Ar-H-2, H-6), 5.89 (s, 1H, 4-CH), 2.44 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 163.8 (2C, C=O), 163.1 (2C, thiazole-2-yl, C-1), 151.1 (2C, thiazole-2-yl, C-4), 148.5 (2C, 1,4-dihydropyridine, C-2, C-6), 142.4 (1C, Ar-C-1), 131.2 (1C, Ar-C-4), 130.6 (2C, Ar-C-2, C-6), 129.3 (2C, Ar-C′-4), 128.3 (4C, Ar-C′-3, C′-5), 126.7 (2C, Ar-C-3, C-5), 126.3 (2C, Ar-C′-1), 126.2 (4C, Ar-C′-2, C′-6), 105.1 (2C, thiazole-2-yl, C-3), 102.2 (2C, 1,4-dihydropyridine, C-3, C-5), 32.4 (1C, 1,4-dihydropyridine, C-4), 18.4 (2C, 1,4-dihydropyridine, C-2-$\underline{C}H_3$, C-6-$\underline{C}H_3$); EI-MS: *m*/*z* 624.17, Elemental analysis: Calcd. for ($C_{33}H_{26}ClN_5O_2S_2$): C, 63.50%; H, 4.20%; N, 11.22%; S, 10.27%. Found: C, 63.55%; H, 4.28%; N, 11.27%; S, 10.31%.

2.1.4. 4-(4-Hydroxyphenyl)-2,6-dimethyl-N^3,N^5-bis(4-phenylthiazol-2-yl)-1,4-dihydro pyridine-3,5-dicarboxamide ***(2d)***

Light yellow solid; Yield 69%; mp:207–210 °C; IR(cm^{-1}): 3164 (NH), 3066 (CHstr), 3028 (Ar-H), 1660 (OCNH), 1472 (C-OH), 1470 (C=N), 737 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.55 (s, 2H, -CONH), 9.32 (s, 1H, –OH), 8.81 (s, 1H, NH), 7.84–7.61 (m, 10H, Ar-H), 7.45 (s, 2H, thiazole-2-yl, H-3), 7.01 (d, 2H, *J* = 6.57 H z, Ar-C-2, C-6), 6.75 (d, 2H, *J* = 6.88 Hz, Ar-H-3,5 Ar-C-3, C-5), 5.80 (s, 1H, 4-CH), 2.56 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 164.6 (2C, thiazole-2-yl, C-1), 163.2 (2C, C=O), 155.2 (1C, Ar-C-4), 149.2 (2C, thiazole-2-yl, C-4), 149.1 (2C, 1,4-dihydropyridine, C-2, C-6), 137.2 (1C, Ar-C-1), 131.1 (2C, Ar-C-2, C-6), 128.2 (2C, Ar-C′-4), 129.1 (4C, Ar-C′-3, C′-5), 127.6 (4C, Ar-C′-2, C′-6), 126.5 (2C, Ar-C′-1), 116.7 (2C, Ar-C-3, C-5), 105.3 (2C, thiazole-2-yl, C-3), 102.4 (2C, 1,4-dihydropyridine, C-3, C-5), 32.4 (1C, 1,4-dihydropyridine, C-4), 17.6 (2C, 1,4-dihydropyridine, C-2-$\underline{C}H_3$, C-6-$\underline{C}H_3$); EI-MS:*m*/*z* 605.72 Elemental analysis: Calcd. For ($C_{33}H_{27}N_5O_3S_2$): C, 65.43%; H, 4.49%; N, 11.56%; S, 10.59%. Found: C, 65.49%; H, 4.52%; N, 11.61%; S, 10.65%.

2.1.5. 4-(4-Nitrophenyl)-2,6-dimethyl-N^3,N^5-bis(4-phenylthiazol-2-yl)-1,4-dihydropyri dine-3,5-dicarboxamide ***(2e)***

Yellow solid; Yield 71%; mp:193–195 °C; IR (cm^{-1}): 3172 (NH), 3074 (CHstr), 3041 (Ar-H), 1652 (OCNH), 1530 (C-NO_2), 1479 (C=N), 738 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.60 (s, 2H, -CONH), 8.84 (s, 1H, NH), 8.15 (d, 2H, *J* = 6.78 Hz, Ar-H-3, H-5), 7.91–7.72 (m, 10H, Ar-H), 7.43 (s, 2H, thiazole-2-yl, H-3), 7.41 (d, 2H, *J* = 6.17 Hz, Ar-C-2, C-6), 5.85 (s, 1H, 4-CH), 2.42 (s, 6H, 2,6-$\underline{C}H_3$); ^{13}C NMR (75 MHz, DMSO-d_6): 163.6 (2C, thiazole-2-yl, C-1), 163.1 (2C, C=O), 149.1 (2C, thiazole-2-yl, C-4), 148.5 (2C, 1,4-dihydropyridine, C-2, C-6), 150.4 (1C, Ar-C-1), 143.2 (1C, Ar-C-4), 135.0 (2C, Ar-C′-1), 130.6 (4C, Ar-C′-2, C′-6), 129.5 (4C, Ar-C′-3, C′-5), 128.8 (2C, Ar-C′-4), 126.1 (2C, Ar-C-2, C-6), 123.7 (2C, Ar-C-3, C-5), 105.1 (2C, thiazole-2-yl, C-3), 103.6 (2C, 1,4-dihydropyridine, C-3, C-5), 32.0 (1C, 1,4-dihydropyridine, C-4), 17.5 (2C, 1,4-dihydropyridine, C-2-$\underline{C}H_3$, C-6-$\underline{C}H_3$); EI-MS: *m*/*z* 634.72 (M+, 22%), Elemental analysis: Calcd. For ($C_{33}H_{26}N_6O_4S_2$): C, 62.44%; H, 4.13%; N, 13.24%; S, 10.10%. Found: C, 62.49%; H,4.20%; N, 13.26%; S, 10.15%.

2.1.6. 4-(4-Methoxyphenyl)-2,6-dimethyl-N^3,N^5-bis(4-phenylthiazol-2-yl)-1,4-dihydro pyrid ine-3,5-dicarboxamide ***(2f)***

White solid; Yield 65%; mp 231 °C; IR (cm^{-1}): 3154 (NH), 3070 (CHstr), 1642 (OCNH), 1482 (C=N), 808 (Ar-H), 728 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.56 (s, 2H, -CONH), 8.82 (s, 1H, NH), 7.79–7.60 (m, 10H, Ar-H), 7.45 (s, 2H, thiazole-2-yl, H-3), 7.16 (d, 2H, *J* = 6.70 Hz, Ar-H-2, H-6), 6.81 (d, 2H, *J* = 6.75 Hz, Ar-H-3, H-5), 5.81 (s, 1H, 4-CH), 3.81 (s, 3H, -OCH_3), 2.47 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 164.6 (2C, thiazole-2-yl, C-1), 163.4 (2C, C=O), 150.6 (2C, thiazole-2-yl, C-4), 157.8 (1C, Ar), 148.6 (2C, 1,4-dihydropyridine, C-2, C-6), 136.2 (1C, Ar-C-1), 133.2 (2C, Ar- C′-1), 130.2 (2C, Ar-C-2, C-6), 129.8 (4C, Ar- C′-3, C′-5), 128.4 (2C, Ar- C′-4), 127.2 (4C, Ar- C′-2, C′-6),113 (2C, Ar-C-3, Ar-C-6), 105.1 (2C, thiazole-2-yl, C-3), 103.1 (2C, 1,4-dihydro pyridine, C-3, C-5), 32.4 (1C, 1,4-dihydropyridine, C-4), 17.9 (2C, 1,4-dihydropyridine, C-2-$\underline{C}H_3$, C-6-$\underline{C}H_3$); EI-MS: *m*/*z* 619.75 (M+, 20%); Elemental analysis: Calcd. For ($C_{34}H_{29}N_5O_3S_2$) C, 65.89%; H, 4.72%; N, 11.30%; S, 10.35%. Found: C, 65.95%; H, 4.78%; N, 11.37%; S, 10.40%.

2.1.7. 4-(4-(Dimethylamino)phenyl)-2,6-dimethyl-N^3,N^5-bis(4-phenylthiazol-2-yl)-1,4-dihydro pyridine-3,5-dicarboxamide ***(2g)***

Pale yellow solid; Yield 60%; mp:175–178 °C; IR (cm^{-1}): 3170 (NH), 3069 (CHstr), 1652 (OCNH), 1481 (C=N), 810 (Ar-H), 738 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.08 (s, 2H, -CONH), 8.90 (s, 1H, NH), 7.87–7.65 (m, 10H, Ph), 7.55 (s, 2H, thiazole-2-yl, H-3), 7.14 (d, 2H, *J* = 7.54 Hz, Ar-H), 7.12 (d, 2H, *J* = 7.45 Hz, Ar-H), 5.10 (s, 1H, 4-CH), 3.01 (s, 6H, -$N(CH_3)_2$), 2.25 (s, 6H, 2,6-C-$C\underline{H}_3$); ^{13}C NMR (75 MHz, DMSO-d_6): 165.7 (2C, thiazol-2-yl, C-1), 164.5 (2C, C=O), 150.4 (2C, thiazol-2-yl, C-4), 144.5 (1C, Ar-C-1), 135.2 (1C, Ar-C-4), 134.3 (2C, Ar- C′-1), 129.1 (4C, Ar- C′-3, C′-5), 128.9 (2C, Ar-C-2,6), 128.7 (2C, Ar-C-3,5), 128.0 (2C, Ar- C′-4), 127.0 (4C, Ar- C′-2, C′-6), 105.6 (2C, thiazol-2-yl, C-3), 103.4 (2C, 3,5-C in 1,4-dihydropyridine), 102.9 (2C, 2,6-C in 1,4-dihydropyridine), 21.2 (2C, Ph-$N(\underline{C}H_3)_2$, 43.0 (1C, 1,4-dihydropyridine in C-4), 17.9 (2C, 2,6-CH_3); EI-MS: *m*/*z* 632.15 (M^+, 34%), Elemental analysis: Calcd. For ($C_{35}H_{32}N_6O_2S_2$) C, 66.43%; H, 5.10%; N, 13.28%; S, 10.13%. Found: C, 66.40%; H, 5. 13%; N, 13.22%; S, 10.15%.

2.1.8. 2,6-Dimethyl-N^3,N^5-bis(4-phenylthiazol-2-yl)-4-p-tolyl-1,4-dihydropyridine-3,5-dicarbo xamide ***(2h)***

White Powder; Yield 62%; mp:184–185 °C; IR (cm^{-1}): 3180 (NH), 3072 (CHstr), 1662 (OCNH), 1445 (C=N), 816 (Ar-H), 742 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.03 (s, 2H, -CONH), 8.90 (s, 1H, NH), 7.87–7.63 (m, 10H, Ph), 7.46 (s, 2H, thiazole-2-yl, H-3), 7.14 (d, 2H, *J* = 7.54 Hz, Ar-H), 7.12 (d, 2H, *J* = 7.45 Hz, Ar-H), 5.12 (s, 1H, 4-CH), 2.34 (s, 3H, Ar-$C\underline{H}_3$), 2.22 (s, 6H, 2,6-C-$C\underline{H}_3$); ^{13}C NMR (75 MHz, DMSO-d_6): 165.1 (2C, thiazol-2-yl, C-1), 164.1 (2C, C=O), 150.3 (2C, thiazol-2-yl, C-4), 144.5 (1C, Ar-C-1), 128.9 (2C, Ar-C-2,6), 128.7 (2C, Ar-C-3,5), 135.2 (1C, Ar-C-4), 134.3 (2C, Ar-C′-1), 129.1 (4C, Ar-C′-3, C′-5), 128.0 (2C, Ar-C′-4), 127.0 (4C, Ar-C′-2, C′-6), 105.0 (2C, thiazol-2-yl, C-3), 104.2 (2C, 1,4-dihydropyridine, C-3, C-5), 102.3 (2C,1,4-dihydropyridine, C-2, C-6), 43.0 (1C, 1,4-dihydropyridine-C-4), 21.1 (1C, Ph-$\underline{C}H_3$), 18.4 (2C, 2,6-CH_3); EI-MS:*m*/*z* 603.76 (M^+, 29%), Elemental analysis: Calcd. For ($C_{34}H_{29}N_5O_2S_2$) C, 67.64%; H, 4.84%; N, 11.60%; S, 10.62%. Found: C, 67.38%; H,4.49%; N,11.95%; S, 10.94%.

2.1.9. General method for preparation of 4-(furan-2-yl)-2,6-dimethyl-N^3,N^5-bis(5-phenyl-1,3,4-thiadiazol-2-yl)-1,4-dihydropyridine-3,5-dicarboxamide ***(3a)***

A mixture of compound, (diethyl-4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) **1a** (0.01 mol, 3.19 g), and 5-phenyl-1,3,4-thiadiazol-2-amine (0.02 mol, 3.54 g) were added in ethanol solvent stirred by 5 min on ultrasound irradiation. After 8 minute the reaction was completed, the product was confirmed by TLC. The product was washed with water and recrystallized by ethyl acetate to give pure product. The above procedure was followed for the synthesis of compounds **3b–e**.

Yellow powder; Yield 80%; mp:192–195 °C; IR (cm^{-1}): 3171 (NH), 3065 (Ar-CHstr), 1641 (OCNH), 1471 (C=N), 828 (Ar-H), 730 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.21 (s, 2H, -CONH), 8.73 (s, 1H, NH), 8.26–7.43 (m, 10H, Ar-H), 7.27 (d, 1H, *J* = 6.70 H z, furan), 6.41 (d, 1H, *J* = 6.93 Hz, furan), 6.24 (dd, 1H, *J* = 6.72 Hz,

J = 6.95 Hz, furan), 5.24 (s, 1H, 4-CH), 2.42 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 174.2 (2C, thiadiazol-2-yl, C-3), 162.2 (2C, C=O), 152.5 (1C, furan, C-1), 152.4 (2C, thiadiazol-2-yl, C-1), 149.1 (2C, 1,4-dihydropyridie,C-2, C-6), 143.2 (1C, furan, C-4), 106.4 (1C furan, C-2), 129.1 (2C, Ar-C′-4), 128.2 (4C, Ar-C′-3, C′-5), 126.5 (4C, Ar-C′-2, C′-6), 126.3 (2C, Ar-C′-1), 113.3 (2C, furan, C-3), 103.6 (2C, 1,4-dihydropyridie, C-3, C-5), 32.4 (1C, 1,4-dihydropyridie, C-4), 18.4 (2C, 2,6-CH_3); EI-MS: *m*/*z* 581.36 (M^+, 24%); Elemental analysis: Calcd. for $C_{29}H_{23}N_7O_3S_2$: C, 59.88%; H, 3.99%; N, 16.86%; S, 11.03%. Found: C, 59.82%; H, 3.97%; N, 16.82%; S, 11.05%.

2.1.10. 2,6-Dimethyl-4-phenyl-N^3,N^5-bis(5-phenyl-1,3,4-thiadiazol-2-yl)-1,4-dihydro pyridine-3,5-dicarboxamide ***(3b)***

White powder; Yield 69%; mp: 192–195 °C; IR (cm^{-1}): 3174 (NH), 3065 (CHstr), 1644 (OCNH), 1474 (C=N), 832 (Ar-H), 728 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.24 (s, 2H, -CONH), 8.70 (s, 1H, NH), 8.21–7.41 (m, 10H, Ar-H), 7.30 (d, 2H, *J* = 7.09 H z, Ar-C-3, C-5), 7.22 (t, 1H, *J* = 7.12 Hz, Ar-C-4),7.21 (d, 2H, *J* = 7.1 7 Hz, Ar-C-2, C-6), 5.22 (s, 1H, 4-CH), 2.41 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 174.0 (2C, thiadiazol-2-yl, C-3), 162.1 (2C, C=O), 151.8 (2C, thiadiazol-2-yl, C-1), 149.1 (2C, 1,4-dihydropyridie-C-2, C-6), 144.4 (1C, Ar-C-1), 129.6 (2C, Ar-C′-4), 128.9 (2C, Ar-C-3, C-5), 128.7 (4C, Ar-C′-3, C′-5), 127.6 (2C, Ar-C-2, C-6), 126.8 (4C, Ar-C′-2, C′-6), 126.0 (2C, Ar-C′-1), 125.2 (1C, Ar-C-4), 103.5 (2C, 1,4-dihydropyridie, C-3, C-5), 32.1 (1C, 1,4-dihydropyridie, C-4), 18.8 (2C, 2,6-CH_3); EI-MS: *m*/*z* 591.36 (M^+, 43%); Elemental analysis: Calcd. for $C_{31}H_{25}N_7O_2S_2$: C, 62.93%; H, 4.26%; N, 16.57%; S, 10.84%. Found: C, 64.27%; H, 4.40%; N, 16.14%; S, 11.10%.

2.1.11. 4-(4-Chlorophenyl)-2,6-dimethyl-N^3,N^5-bis(5-phenyl-1,3,4-thiadiazol-2-yl)-1,4-dihydropyridine-3,5-dicarboxamide ***(3c)***

Yellow solid; Yield 58%; mp:201–205 °C; IR (cm^{-1}): 3175 (NH), 3068 (Ar-CHstr), 1648 (OCNH), 1480 (C=N), 827 (C-Cl), 812 (Ar-H), 736 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.20 (s, 2H, -CONH), 8.72 (s, 1H, NH), 7.56–7.43 (m, 10H, Ar-H), 7.26 (d, 1H, *J* = 6.78 Hz, Ar-H), 6.41 (d, 1H, *J* = 6.91 Hz, Ar-H), 6.29 (t, 2H 1H, *J* = 6.89 Hz, *J* = 6.97 Hz, Ar-H-3,5), 5.20 (s, 1H, 4-CH), 2.40 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 174.1 (2C, thiadiazol-2-yl, C-3), 162.5 (2C, C=O), 152.3 (2C, thiadiazol-2-yl, C-1), 148.1 (2C, 1,4-dihydropyridie-C-2, C-6), 142.4 (1C, Ar-C-1), 131.2 (1C, Ar-C-4), 130.6 (2C, Ar-C-2, C-6), 128.3 (4C, Ar-C′-3, C′-5), 129.3 (2C, Ar-C′-4), 126.7 (2C, Ar-C-3, C-5), 126.5 (2C, Ar-C′-1), 126.2 (4C, Ar-C′-2, C′-6), 103.2 (2C, 1,4-dihydropyridie-C-3,5), 32.8 (1C, 1,4-dihydropyridie-C-4), 18.2 (2C, 2,6-CH_3); EI-MS: *m*/*z* 626.98 (M^+, 30%); Elemental analysis: Calcd. for $C_{31}H_{24}ClN_7O_2S_2$: C, 64.27%; H, 4.40%; N, 12.14%; S, 11.10%. Found: C, 64.35%; H, 4.51%; N, 12.20%; S, 11.15%.

2.1.12. 4-(4-Hydroxyphenyl)-2,6-dimethyl-N^3,N^5-bis(5-phenyl-1,3,4-thiadiazol-2-yl)-1,4-dihydpyridine-3,5-dicarboxamide ***(3d)***

Pale yellow solid; Yield 63%; mp:165–168 °C; IR (cm^{-1}): 3178 (NH), 3070 (CHstr), 1645 (OCNH), 1477 (C-OH), 1475 (C=N), 815 (Ar-H), 733 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.21 (s, 2H, -CONH), 9.32 (s, 1H, –OH), 8.72 (s, 1H, NH), 7.60–7.43 (m, 10H, Ar-H), 7.01 (d, 2H, *J* = 6.57 Hz, Ar-C-2, C-6), 6.75 (d, 2H, *J* = 6 .88 Hz, Ar-C-3, C-5), 5.28 (s, 1H, 4-CH), 2.41 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 174.6 (2C, thiadiazol-2-yl, C-3), 162.8 (2C, C=O), 155.2 (1C, Ar-C-4), 152.1 (2C, thiadiazol-2-yl, C-1), 148.6 (2C, 1,4-dihydropyridie-C-2, C-6), 137.2 (1C, Ar-C-1), 131.1 (2C, Ar-C-2, C-6), 129.1 (4C, Ar-C′-3, C′-5), 128.2 (2C, Ar-C′-4), 127.6 (4C, Ar-C′-2, C′-6), 126.5 (2C, Ar-C′-1), 116.7 (2C, Ar-C-3, C-5), 102.9 (2C, 1,4-dihydropyridie-C-3,5), 32.0 (1C, 1,4-dihydropyridie-C-4), 18.1 (2C, 2,6-CH_3); EI-MS: *m*/*z* 607.12 (M^+, 13%); Elemental analysis: Calcd. for $C_{31}H_{25}N_7O_3S_2$: C, 64.27%; H, 4.40%; N, 12.14%; S, 11.10%. Found: C, 64.35%; H, 4.50%; N, 12.19%; S, 11.19%.

2.1.13. 2,6-Dimethyl-4-(4-nitrophenyl)–N^3,N^5-bis(5-phenyl-1,3,4-thiadiazol-2-yl)-1,4-dihydro pyridine-3,5-dicarboxamide ***(3e)***

White powder; Yield 69%; mp:150–152 °C; IR (cm^{-1}): 3180 (NH), 3070 (Ar-CHstr), 1641 (OCNH), 1530 (C-NO_2), 1478 (C=N), 820 (Ar-H), 736 (C-S-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.21 (s, 2H, -CONH), 8.72 (s, 1H, NH), 8.15 (d, 2H, *J* = 6.78 Hz, Ar-C-3, C-5), 7.58–7.40 (m, 10H, Ar-H), 7.41 (d, 2H, *J* = 6.17 Hz, Ar-C-2, C-6), 5.25 (s, 1H, 4-CH), 2.46 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 174.9 (2C, thiadiazol-2-yl, C-3), 162.0 (2C, C=O), 154.3 (2C, thiadiazol-2-yl, C-1), 150.4 (1C, Ar-C-1), 148.4 (2C, 1,4-dihydropyridie-C-2, C-6), 143.2 (1C, Ar-C-4), 135.0 (2C, Ar-C′-1), 130.6 (4C, Ar-C′-2, C′-6), 129.5 (4C, Ar-C′-3, C′-5), 128.8 (2C, Ar-C′-4), 126.1 (2C, Ar-C-2, C-6), 123.7 (2C, Ar-C-3, C-5), 103.61 (2C, 1,4-dihydropyridie-C-3,5), 32.1 (1C, 1,4-dihydropyridie-C-4), 18.5 (2C, 2,6-CH_3); EI-MS: *m*/*z* 636.36 (M^+, 27%); Elemental analysis: Calcd. for $C_{31}H_{24}N_8O_4S_2$: C, 64.27%; H, 4.40%; N, 12.14%; S, 11.10%. Found: C, 64.31%; H, 4.45%; N, 12.20%; S, 11.13%.

2.1.14. General method for preparation of 4-(furan-2-yl)-2,6-dimethyl-N^3,N^5-bis(5-phenyl-1,3,4-oxadiazol-2-yl)-1,4-dihydropyridine-3,5-dicarboxamide ***(4a)***

A mixture of compound, (diethyl-4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) **1a** (0.01 mol, 3.19 g), and 5-phenyl-1,3,4-oxadiazol-2-amine (0.02 mol, 3.22 g) were added in ethanol solvent stirred by 5 min on ultrasound irradiation. After 9 minute the reaction was completed, the product was confirmed by TLC. The product was washed with water and recrystallized by ethyl acetate to give pure product. The above procedure was followed by synthesis of remaining compounds **4b–e.**

Pale yellow solid; Yield 60%; mp:195–198 °C; IR (cm^{-1}): 3170 (NH), 3054 (CHstr), 1642 (OCNH), 1470 (C=N), 812 (Ar-H), 712 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.21 (s, 2H, -CONH), 8.75 (s, 1H, NH), 7.78–7.42 (m, 10H, Ar-H), 7.22 (d, 1H, *J* = 6.65 H z, furan), 6.45 (d, 1H, *J* = 6.97 Hz, furan), 6.27 (dd, 1H, *J* = 6.75 Hz, *J* = 6.91 Hz, furan), 5.25 (s, 1H, 4-CH), 2.43 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 164.8 (2C, oxadiazol-2-yl, C-3), 164.3 (2C, oxadiazol-2-yl, C-1), 162.2 (2C, C=O), 152.5 (1C, furan-C-1), 148.3 (2C, 1,4-dihydropyridie-C-2, C-6), 143.2 (1C, furan-C-4), 129.1 (2C, Ar-C′-4), 128.2 (4C, Ar-C′-3, C′-5), 126.5 (2C, Ar-C′-2, C′-6), 126.3 (2C, Ar-C′-1), 113.0 (2C, furan-C-3), 106.4 (2C, furan-C-2), 103.3 (2C, 1,4-dihydropyridie-C-3,5), 32.4 (1C, 1,4-dihydropyridie-C-4), 18.9 (2C, 2,6-CH_3); EI-MS: *m*/*z* 549.36 (M^+, 56%); Elemental analysis: Calcd. for $C_{29}H_{23}N_7O_5$: C, 63.38%; H, 4.22%; N, 17.84%; Found: C, 63.30%; H, 4.21%; N, 17.85%.

2.1.15. 2,6-Dimethyl-4-phenyl-N^3,N^5-bis(5-phenyl-1,3,4-oxadiazol-2-yl)-1,4-dihydropy ridine-3,5-dicarboxamide ***(4b)***

Pale yellow solid; Yield 60%; mp:207–210 °C; IR (cm^{-1}): 3175 (NH), 3066 (CHstr), 1644 (OCNH), 1475 (C=N), 821 (Ar-H), 708 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.22 (s, 2H, -CONH), 8.72 (s, 1H, NH), 7.69–7.41 (m, 10H, Ar-H), 7.30 (d 2H, *J* = 7.09 H z, Ar-C-3, C-5), 7.22 (d, 1H, *J* = 7.12 Hz, Ar-C-4), 7.20 (d, 2H, *J* = 7. 17 Hz, Ar-C-2, C-6), 5.25 (s, 1H, 4-CH), 2.42 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 163.6 (2C, oxadiazol-2-yl, C-3), 165.8 (2C, oxadiazol-2-yl, C-2), 162.2 (2C, C=O), 148.4 (2C, 1,4-dihydropyridie, C-2, C-6), 144.4 (1C, Ar-C-1), 129.6 (2C, Ar-C′-4), 128.7 (2C, Ar-C-3, C-5), 128.5 (4C, Ar-C′-3, C′-5), 127.6 (2C, Ar-C-2, C-6), 126.8 (4C, Ar-C′-2, C′-6), 126.0 (2C, Ar-C′-1), 125.2 (1C, Ar-C-4), 102.5 (2C, 1,4-dihydropyridie, C-3, C-5), 32.2 (1C, 1,4-dihydropyridie, C-4), 17.1 (2C, 2,6-CH_3); EI-MS: *m*/*z* 559.36 (M^+,

46%); Elemental analysis: Calcd. for $C_{31}H_{25}N_7O_4$: C, 66.54%; H, 4.50%; N, 17.52%; Found: C, 64.27%; H, 4.40%; N, 17.14%.

2.1.16. 4-(4-Chlorophenyl)-2,6-dimethyl-N^3,N^5-bis(5-phenyl-1,3,4-oxadiazol-2-yl)-1,4-dihydr opyridine-3,5-dicarboxamide ***(4c)***

Yellow solid; Yield 64%; mp: 241–244 °C; IR (cm^{-1}): 3171 (NH), 3065 (CHstr), 1641 (OCNH), 1471 (C=N), 827 (C-Cl), 823 (Ar-H), 730 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.21 (s, 2H, -CONH), 8.71 (s, 1H, NH), 7.74–7.51 (m, 10H, Ar-H), 7.30 (d, 2H, J = 6.98 Hz, Ar-H-3, H-5), 7.21 (d, 2H, J = 6.87 Hz, Ar-H-2, H-6), 5.20 (s, 1H, 4-CH), 2.40 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 165.2 (2C, oxadiazol-2-yl, C-3), 163.6 (2C, oxadiazol-2-yl, C-1), 163.2 (2C, C=O), 148.2 (2C, 1,4-dihydropyridie-C-2, C-6), 142.4 (1C, Ar-C-1), 131.2 (1C, Ar-C-4), 130.6 (2C, Ar-C-2, C-6), 129.3 (2C, Ar-C′-4), 128.3 (4C, Ar-C′-3, C′-5), 126.7 (2C, Ar-C-3, C-5), 126.3 (2C, Ar-C′-1), 126.2 (4C, Ar-C′-2, C′-6), 102.3 (2C, 1,4-dihydropyridie-C-3,5), 33.2 (1C, 1,4-dihydropyridie-C-4), 17.5 (2C, 2,6-CH_3); EI-MS: m/z 594.25 (M^+, 19%); Elemental analysis: Calcd. for $C_{31}H_{24}ClN_7O_4$: C, 62.68%; H, 4.07%; N, 16.51%; Found: C, 62.27%; H, 4.15%; N, 16.14%.

2.1.17. 4-(4-Hydroxyphenyl)-2,6-dimethyl-N^3,N^5-bis(5-phenyl-1,3,4-oxadiazol-2-yl)-1,4-dihy dropyridine-3,5-dicarboxamide ***(4d)***

White powder; Yield 86%; mp 195–198 °C; IR (cm^{-1}): 3174 (NH), 3061 (CHstr), 1643 (OCNH), 1475 (C=N), 1472 (C-OH), 820 (Ar-H), 732 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.23 (s, 2H, -CONH), 9.41 (s, 1H, –OH), 8.70 (s, 1H, NH), 7.74–7.42 (m, 10H, Ar-H), 7.01 (d, 2H, J = 6.57 Hz, Ar-H-2, H-6), 6.75 (d, 2H, J = 6.88 Hz, Ar-C-3, C-5), 5.24 (s, 1H, 4-CH), 2.44 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 164.8 (2C, oxadiazol-2-yl, C-2), 164.4 (2C, oxadiazol-2-yl, C-4), 162.1 (2C, C=O), 155.2 (1C, Ar-C-4), 149.6 (2C, 1,4-dihydropyridie, C-2, C-6), 137.2 (1C, Ar-C-1), 131.1 (2C, Ar-C-2, C-6), 129.1 (4C, Ar-C′-3, C′-5), 128.2 (2C, Ar-C′-4), 127.6 (4C, Ar-C′-2, C′-6),126.5 (2C, Ar-C′-1), 116.7 (2C, Ar-C-3, C-5), 103.4 (2C, 1,4-dihydropyridie, C-3, C-5), 32.4 (1C, 1,4-dihydropyridie, C-4), 18.1 (2C, 2,6-CH_3); EI-MS: m/z 575.10 (M^+, 33%); Elemental analysis: Calcd. for $C_{31}H_{25}N_7O_5$: C, 64.27%; H, 4.40%; N, 12.14%; S, 11.06%. Found: C, 64.18%; H, 4.45%; N, 12.18%; S, 11.15%.

2.1.18. 2,6-Dimethyl-4-(4-nitrophenyl)–N^3,N^5-bis(5-phenyl-1,3,4-oxadiazol-2-yl)-1,4-dihydro pyridine-3,5-dicarboxamide ***(4e)***

White solid; Yield 79%; mp:210–212 °C; IR (cm^{-1}): 3173 (NH), 3065 (CHstr), 1530 (C-NO_2), 1641 (OCNH), 1471 (C=N), 818 (Ar-H), 730 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.20 (s, 2H, -CONH), 8.72 (s, 1H, NH), 7.68–7.42 (m, 10H, Ar-H), 8.15 (d, 2H, J = 6.78 Hz, Ar-H-3, H-5), 7.41 (d, 2H, J = 6.17 Hz, Ar-H-2, H-6), 5.23 (s, 1H, 4-CH), 2.46 (s, 6H, 2,6-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 164.8 (2C, oxadiazol-2-yl, C-3), 165.2 (2C, oxadiazol-2-yl, C-1), 162.0 (2C, C=O), 150.4 (1C, Ar-C-1), 149.3 (2C, 1,4-dihydro pyridie, C-2, C-6), 143.2 (1C, Ar-C-4), 135.0 (2C, Ar-C′-1), 130.6 (4C, Ar-C′-2, C′-6), 129.5 (4C, Ar-C′-3, C′-5), 128.8 (2C, Ar-C′-4),126.1 (2C, Ar-C-2, C-6), 123.7 (2C, Ar-C-3, C-5), 102.6 (2C, 1,4-dihydropyridie, C-3, C-5), 32.1 (1C, 1,4-dihydropyridie-C-4), 17.8 (2C, 2,6-CH_3); EI-MS: m/z 604.76 (M^+, 17%); Elemental analysis: Calcd. for $C_{31}H_{24}N_8O_6$: C, 64.27%; H, 4.00%; N, 18.53%; Found: C, 64.30%; H, 4.48%; N, 12.14%.

2.2. Biological evaluation

2.2.1. Anticoagulant activity

The anticoagulant study was carried out according to the method described in previous literature (De-Zoysa et al., 2008).

2.2.2. Antibacterial activity

Compounds **2a–h**, **3a–e** and **4a–e** were evaluated for their in *vitro* antibacterial activity against *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853), *Staphylococcus aureus* (ATCC-25923), *Staphylococcus epidermidis,* and *Klebsiella pneumoniae* (recultured) by disc diffusion method (Wayne, 2003) with performed using Mueller-Hinton agar (Hi-media) each compounds. Each compound was tested at a concentration of 100 μg/mL in DMSO. Ciprofloxacin was used as the standard. The zone of inhibition was measured after 24 h incubation at 37 °C. The minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited the growth on agar plates.

2.2.3. Antifungal screening

The compounds were evaluated for their *in-vitro* antifungal activity against *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, and *Microsporum audouinii* using an agar dilution method (Gillespie, 1994) with sabouraud's dextrose agar (Hi-Media). Each compound was tested at a concentration of 100 μg/mL in DMSO and samples were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. Clotrimazole was used as the standard. The plates were then incubated for 24 h at 37 °C, and the diameter of the growth inhibition zones was measured and recorded.

2.2.4. Determination of the minimum inhibitory concentration (MIC)

Selected synthesized compounds **2c**, **2e**, **3b** and **4b** where determined by the minimum inhibitory concentration (MIC) at a concentration of 64 μg/mL. The two fold dilutions of the solution were prepared (64, 32. . ., 0.5 μg/mL). The microorganism suspensions at 106 (CFU/mL, colony forming unit/mL) concentrations were inoculated to the corresponding wells. The plates were incubated at 36 °C at 24 h. The (MIC) values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms.

2.2.5. Cytotoxic activity

The newly synthesized compounds **(2a–h), (3a–e),** and **(4a–e)** were screened for their anticancer activity according to a previously published procedure (Surendra Kumar et al., 2017). Compounds (100 μM) were incubated in a microtiter plate with three different cell lines for 72 h, and cell viability was assessed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The three cell lines were HepG2 (liver), MCF-7 (breast), and HeLa (cervical). The percentage of growth of the treated cells compared to that of the untreated control cells was calculated. Compounds that reduced the growth of a cell line by 32% or more were considered to have antitumor activity.

The measured 0.1 mL of the cell suspension (containing 5×10^6 cells/100 μL) and 0.1 mL of the test solution (6.25–100 μg 1% DMSO such that the final concentration of DMSO in media was less than 1%) were added to the 27 well plates and kept in a 5% CO_2 incubator at 37 °C for 72 h. The blank contained only cell suspension and control wells contained 1% DMSO and cell suspension. After 72 h, 20 μL of MTT was added and kept in the CO_2 incubator for 2 h followed by addition of 100 μL propanol. The plate was covered with aluminum foil to protect from light. Then the 27 well plates were kept in a rotary shaker for 10–20 min. After 10–20 min, the 27 well plates were processed on an ELISA reader for absorption at 562 nm.

3. Results and discussion

3.1. Chemistry

We reported that 1,4-dihydropyridine derivatives **1a–h** using the Hantzsch method (Hadizadeh et al., 2002). Compounds **2a–h, 3a–e,** and **4a–e** were prepared via amination method (Scheme 1).

To recognize the optimization of the reaction conditions, the reaction was studied by employing ethanol medium in ultrasound irradiation the hope to maximize the product good yield in short reaction times than other solvents and conventional method.

The IR spectrum of **2a–h** displayed absorption bands range at 3154–3180, 1612–1662, 728–742, 1445–1488, and 3045–3074 cm^{-1}, corresponding to the -NH, -HNCO, -C-S-C, -C=N, and aromatic CH groups, respectively. The ^{1}H NMR spectrum of **2a–h** shows that signals range at δ 13.03–13.62, 8.81–8.90, and 5.10–5.89 ppm, corresponding to the CONH, NH, and 4CH protons, respectively. The ^{13}C NMR spectrum of **2a–h** shows peaks at δ163.1–164.5, 105.0–105.6, and 32.0–43.4 ppm, corresponding to the C=O, CH-thiazole ring, and 4-C carbon atoms present in 1,4-dihydropyridne, respectively. The EI mass spectrum of **2a** showed a molecular ion peak at m/z 579.23 (M^+, 10%), confirming the molecular weight of compound **2a**.

IR spectrum of compounds **3a–e** shows that important characteristics absorption band range at 3171–3180, 1641–1648, 728–736, and 1471–1480 cm^{-1}, corresponding to the -NH, -HNCO, -C-S-C, and C=N group present in 1,3,4-thiadiazol-2-yl, respectively. The ^{1}H NMR spectrum of compounds **3a–e** shows that signals range at δ 13.20–13.24 and 5.20–5.28 ppm, corresponding to the CONH and 4CH proton present in 1,4-dihydropyridine ring, respectively. The ^{13}C NMR spectrum of **3a–e** showed peaks range at δ 162.0–162.8, 174.0–174.9, 151.8–154.3, and 32.0–32.8 ppm, corresponding to the CO, 2C-NH and 4-CH carbon present in 1,4-dihydropyridine, respectively.

The important IR spectrum characteristics bonds of compounds **4a–e** shows that the absorption bands at 3170–3175, 1641–1644, 708–732, 1470–1475, and 3054–3066 cm^{-1}, corresponding to the -NH, -HNCO, -C-O-C, -C=N, and aromatic CH groups, respectively. The ^{1}H NMR spectrum of compound **4a–e** showed signals range at δ 13.20–13.23 and 5.20–5.25 ppm, corresponding to the CONH and 4CH protons, respectively. The ^{13}C NMR spectrum of **4a–e** showed peaks range at δ 162.0–163.2, and 32.1–32.4 ppm, corresponding to a C=O group, and the 4-CH carbon atom present in 1,4-dihydropyridine ring, respectively.

3.2. Biological activity

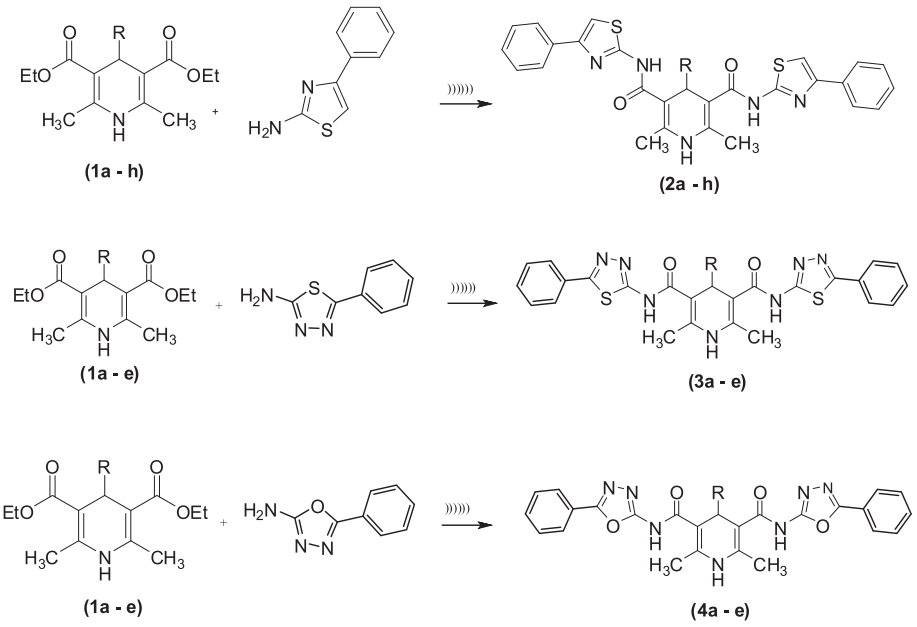
3.2.1. Anticoagulant activity

All of the synthesized compounds **(2a–h), (3a–e),** and **(4a–e)** were screened for their anticoagulant activity, and the data were compared with heparin. The anticoagulant evaluations data of the synthesized compounds were represented in Table 1. The

Table 1
Anti-coagulant activity of compounds **2a–h, 3a–h**, and **4a–h** with standard heparin.

Comp. No	Concentration (60 μg/mL)			
	Clotting time(s) (APTT)	APTT Index	Clotting time(s) (PT)	PT index
2a	424.42	11.65	112.58	5.66
2b	316.81	8.69	110.20	5.54
2c	519.65	14.26	132.62	6.67
2d	662.76	18.19	123.21	6.20
2e	622.41	17.08	141.05	7.10
2f	514.03	14.11	108.52	5.46
2g	458.75	12.59	128.87	6.48
2h	659.92	18.11	142.67	7.18
3a	834.21	22.90	104.30	5.26
3b	578.48	15.88	107.61	5.41
3c	768.95	21.11	131.53	6.62
3d	>1000[a]	27.45	106.62	5.38
3e	675.32	18.54	103.85	5.24
4a	156.30	4.29	37.52	1.88
4b	152.73	4.19	25.21	1.26
4c	158.81	4.36	30.05	1.38
4d	163.40	4.48	27.56	1.36
4e	142.82	3.92	30.78	1.54
Heparin	>1000[a]	27.45	–	–
Control	36.42	1.0	19.86	1.0

Clotting time >1000 s considered as 1000 s to calculate the relative clotting potency. Values are expressed as mean of five trails.
[a] Highly significant index.



Scheme 1. Synthetic route of compounds (**2a–h**, **3a–e** and **4a–e**). R = **2a, 3a, 4a: -**Furyl; **2b, 3b, 4b:** -Ph; **2c, 3c, 4c:** 4-Cl-Ph; **2d, 3d, 4d:** 4-OH-Ph; **2e, 3e, 4e:** 4-NO_2–Ph; **2f:** 4-CH_3O-Ph; **2g**: 4-(CH_3)2N-Ph; **2h:** 4-CH_3-Ph.

in vitro anticoagulant activity of APTT and PT in human plasma was examined. All synthesized compounds (**2a–h), (3a–e),** and **(4a–e)** showed higher APTT and PT values than the vehicle control. Among the synthesized compounds **3d** was highly active against APTT and PT assays for anticoagulant screening. The APTT coagulation assay was performed at 60 μg/mL concentration.

Compounds **4a–e** was low active compared to **2a–h** and **3a–3e** against APTT and PT assays. Particularly, compound **2d** shows low active (662.76 s) in APTT assays compared with compound **3d** (>1000 s) where as compound **2d** was (662.76 s) highly active compared with other compounds (**2a, 2b, 2c, 2e, 2f, 2g,** and **2h**).

The heparin is caused by its complexing with antithrombin 111, which accelerates the formation of a stable 1:1 complex between antithrombin 111 and thrombin (Harpel and Rosenberg, 1976). Hirano et al., reported that sulphur derivatives shows high anticoagulant activities with respect to activated partial thromboplastin time (Hirano et al., 1985). This study was propose that the synthetic sulphur containing derivatives work as anticoagulants in a mechanism same from that of heparin.

3.2.2. In vitro antimicrobial activity

Compound **2e** was highly active against *E. coli* and compounds **3b** and **4b** exhibited moderately antibacterial activities against *E. coli*. The primary screening was performed using the agar disc diffusion method on Müller-Hinton medium. The majority of the synthesized compounds showed varying degrees of inhibition against the microorganisms tested. The *in vitro* antifungal activities of compounds **2a–h**, **3a–e**, and **4a–e** were evaluated at 100 μg/mL against fungal spices. The primary screening was performed using the agar dilution method on Sabouraud's dextrose agar (Hi-Media). Compound **2c** was highly active against *C. albicans* (growth inhibition zones 26 mm) compared with the positive control and compounds **3b** and **4b**, which showed moderate activity against *C. albicans*.

Minimum inhibitory concentrations (MICs): The data in Table 2 shows that the antibacterial and antifungal activities of compounds **2c**, **2e**, **3b**, and **4b** were greater than those of the other 1,4-dihydropyridine derivatives and standards. The antibacterial activity compound **2e** was highly activity MIC: (0.25 μg/mL) were comparable against *E. coli.* compared with positive control MIC: (0.5 μg/mL). The antifungal activity compound **2c** was highly activity (MIC: 0.5 μg/mL) were comparable against *C. albicans* compared with positive control (MIC: 1 μg/mL).

3.2.3. Cytotoxicity screening

All compounds were also screened for anticancer activity against liver, cervical, and breast cancer cell lines. The GI_{50}, tumor growth inhibition (TGI), and LC_{50} values were determined. Compound (**2c**) was highly active against HeLa (GI_{50}: 0.02 μm), and MCF-7 cells (GI_{50}:0.03 μm) compared with the activity of other compounds. Compound (**2e**) were equipotant active against MCF-7 cell (GI_{50}: 0.03 μm) compared with Doxorubicin. The other compounds (**2c, 2e, 3d, 4b**) showed moderate activity against HepG2 and MCF-7 cell line compared to Doxorubicin. The cytotoxicity screening results are summarized in Table 3.

3.2.4. Structure activity relationship

The relationship between the chemical structures and biological activities were validates several assumptions. Fig. 2 shows that the 4-substituted phenyl ring acts as a lipophilic domain, while the C=O and NH groups act as electron donors and hydrogen bonding domains, respectively. Therefore, the 1,4-dihydropyridine moiety with thiazole moiety was an essential pharmacophoric requirement of biological activity.

Compounds **2e** and **2c**, both of which bear a 1,4-dihydropyridine moiety with a 4′-substituted group (4′-NO_2, 4-Cl-Ph), shows significant antibacterial and antifungal activities. Compound **2e** was highly active against *E. coli* due to the presence of a NO_2 containing 1,4-dihydropyridine moiety. Compound **2c** was highly active against *C. albicans* compared with clotrimazole. In general, compounds with electron withdrawing groups (4′-Cl) exhibited significantly higher activity than those of compounds containing an electron withdrawing group (4′-NO_2). These observations indicate that 4′-NO_2 and 4-Cl-Ph groups are well tolerate in this series. The cyctotoxicity activity of compound (**2c**) showed highly active against HeLa cells, whereas the compound **2e** had moderate activity against HeLa cells compared with that of doxorubicin. The compound **2c, 2e** were highly activity against MCF-7 and also modarate active against HepG2.

Compound **3b**, which bears a 1,4-dihydropyridine moiety with 1,3,4-thiadiazol-2-yl group showed significant antibacterial activity against *E. coli.* Compound **4b**, which contains 1,4-

Table 2
Antimicrobial activity with minimum inhibitory concentrations (MIC, μg/mL).

Comp. No.	Minimum Inhibitory Concentration (MIC, μg/mL)								
	Antibacterial activity					Antifungal activity			
	E.c	*P.a*	*S.a*	*S.e*	*K.p*	*A. n*	*C. a*	*C. n*	*M. a*
2a	64	1	32	32	–	64	64	>100	>100
2b	32	32	32	32	8	32	32	>100	>100
2c	2	0.5	8	32	>100	32	0.5	64	>100
2d	64	>100	32	64	64	8	64	>100	>100
2e	0.25	64	>100	32	32	>100	>100	>100	64
2f	32	>100	32	8	>100	8	32	>100	>100
2g	32	64	64	64	>100	4	64	>100	>100
2h	64	>100	>100	64	>100	32	32	>100	>100
3a	64	>100	>100	32	64	4	64	>100	16
3b	1	>100	32	>100	8	32	2	8	64
3c	32	32	16	32	4	32	32	16	8
3d	64	32	>100	64	64	8	32	>100	16
3e	64	32	32	64	>100	32	16	64	8
4a	32	8	32	>100	>100	32	>100	>100	8
4b	4	64	64	>100	8	64	1	16	8
4c	32	32	64	16	32	8	>100	16	16
4d	32	64	16	16	8	>100	8	32	64
4e	32	>100	–	32	8	8	>100	>100	32
Ciprofloxacin	0.5	1	0.5	4	2	–	–	–	–
Clotrimazole	–	–	–	–	–	2	1	0.5	0.5

Table 3
Cytotoxic activity of compounds **(2a–h), (3a–e),** and **(4a–e)**.

Compounds	HepG2			MCF-7			HeLa		
	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)
2a	16.2	29.1	>100	22.9	46.8	57.2	11.6	29.4	41.2
2b	13.3	24.8	81.2	20.1	45.1	56.4	11.0	27.2	47.3
2c	08.2	18.1	25.1	0.03	0.64	0.81	0.02	0.48	0.89
2d	12.9	21.6	42.7	25.9	57.4	60.8	12.9	22.5	47.9
2e	03.9	23.9	47.9	0.03	47.6	87.0	09.8	16.0	34.6
2f	16.3	25.3	48.2	15.2	20.1	38.2	18.1	27.1	45.3
2g	15.4	22.5	42.5	13.5	26.9	43.5	16.8	24.7	42.8
2h	11.7	22.1	40.9	12.6	22.5	48.4	19.8	22.6	41.0
3a	17.9	27.9	42.9	10.7	27.9	41.2	19.7	23.2	45.4
3b	14.8	25.8	47.1	16.2	26.4	42.3	13.8	22.4	46.3
3c	11.0	22.1	42.1	14.9	24.9	43.1	11.7	28.9	56.2
3d	01.6	14.8	22.4	01.6	15.0	3.8	12.8	20.4	41.2
3e	18.6	20.3	42.2	11.7	24.8	64.9	17.9	31.0	47.7
4a	17.9	24.8	43.2	11.8	23.8	40.3	12.8	24.9	39.5
4b	05.9	16.9	28.3	07.6	18.9	32.1	18.9	21.9	35.6
4c	16.7	21.0	40.4	18.3	20.2	42.4	13.3	26.0	48.3
4d	14.2	26.8	42.4	15.6	29.3	92.3	13.2	24.0	42.3
4e	16.0	28.6	42.0	19.3	25.2	39.0	15.3	23.2	44.3
Doxorubicin	0.01	0.13	0.58	0.02	0.21	0.74	0.05	0.41	0.88

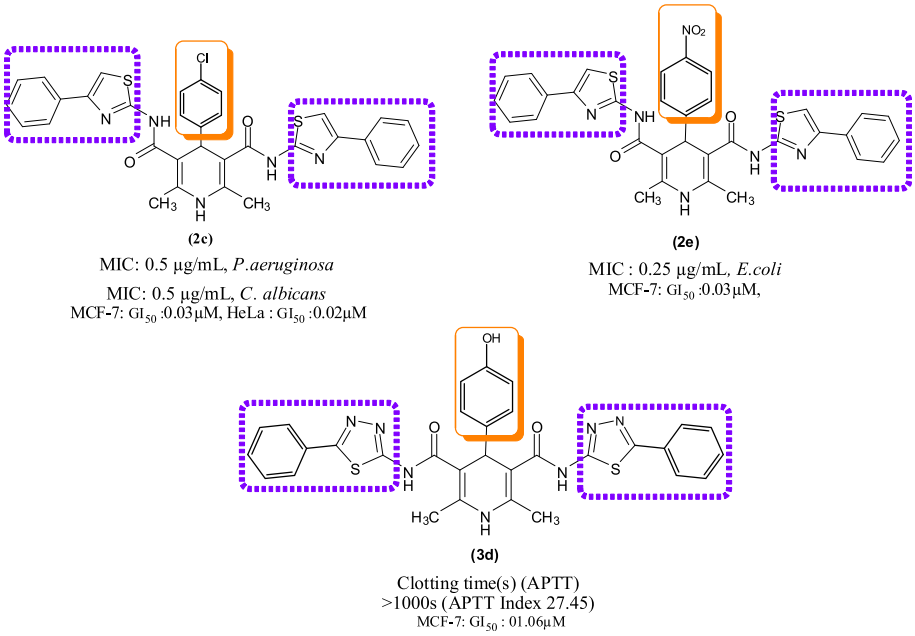


Fig. 2. Structural activity relationship of compound **2c, 2e,** and **3b.**

dihydropyridine moiety with 1,3,4-oxadiazol-2-yl groups exhibited equipotent activity against *C. albicans* and the other fungal strains.

The compounds **2d, 3d,** and **4d** containing para position OH-atoms of phenyl ring shows high potent inhibitors against APTT assay (clotting time values 662.76 s, >1000 s, 163.40 s) and PT assays (clotting time values 123.21 s, 106.62, and 27.56 s).

This emphasizes that the hydrophobic and lipophilic domains in the molecules are responsible for the potent anticoagulant activity. In addition, the effect of electron donating groups on the substituted benzene with 1,4-dihydropyridine moiety.

As a result, synthesized 1,4-dihydropyridine-3,5-dicarboxamide derivatives shows highly anticoagulant activity due to the presence of sulphur group (**2a–h, 3a–e**) compared with compounds **4a–e** in APTT and PT assays. Compound **3d** containing OH group with 1,3,4-thiadiazole shows highly active among the synthesized compounds.

4. Conclusion

A new series of 1,4-dihydropyridine derivatives, **2a–h**, **3a–e**, and **4a–e** were synthesized via ultrasound irradiation and evaluated for antimicrobial and anticoagulant activities. The compound **2e** (1,4-dihydropyridine with thiadiazole) was highly active against *Escherichia coli* and compound **2c** was also highly active against *Pseudomonas aeruginosa* compared with ciprofloxacin. The compound **2c** was highly active against *Candida albicans* compared with clotrimazole. Compound **3d** was also a strong anticoagulant activity compared with heparin. Compound (**2c**) was highly active against HeLa (GI_{50}: 0.02 μm), and MCF-7 (GI_{50}: 0.03 μm) cell lines compared with other compounds, the compound (**2e**) was equipotant active against MCF-7 (GI_{50}: 0.03 μm) cell line compared with that of doxorubicin. Therefore, these new 1,4-dihydropyridine derivatives may serve as lead molecules in the development of a clinically useful and novel class of antimicrobial, and anticoagulant agents.

Conflict of interest

None.

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