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Short communication

# Synthesis and anticoagulant activity of a new series of 1,4-dihydropyridine derivatives

R. Surendra Kumar [a], A. Idhayadhulla [a], A. Jamal Abdul Nasser [a,*], J. Selvin [b]

[a] P.G & Research Dept. of chemistry, Jamal Mohamed College, Tiruchirappalli 620020, Tamil Nadu, India
[b] Dept. of Microbiology, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India

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ABSTRACT

A series of 1,4-dihydropyridine derivatives (**1a**–**g**) were prepared from three compounds condensation reaction of ethylacetoacetate, aromatic aldehyde and ammonium hydroxide. A new series of compounds (**2a**–**g**) were prepared from compounds (**1a**–**g**) via reaction with thiosemicarbazide using the condensation method. The synthesized compounds were confirmed by IR, $^{1}$H NMR, $^{13}$C NMR, mass spectral and elemental analyses. The synthesized compounds (**1a**–**g**) and (**2a**–**g**) were also screened for anticoagulant properties.



## 1. Introduction

1,4-Dihydropyridine derivatives are of interest because of their potential biological activity and use in therapeutics such as antihypertensive [1–4], hypnotic, anti-inflammatory [5], antihypoxic and antiischemic drugs [6] and calcium channel modulators of the nifedipine type [7]. Several methods have been described for the synthesis of 1,4-dihydropyridine [8–12]. The presence of ester groups at the 3- and 5-position on the 1,4-dihydropyridine ring is of crucial importance for its pharmacological effects. Recently, some new 3, 5-substituted 1,4-dihydropyridine derivatives were synthesized which exhibit pharmacological effects such as antihypertensive, bronchodilatory and antitubercular agent [13–15]. In view of these observations, we decided to compare the activity levels of compounds (**1a**–**g**) with compounds (**2a**–**g**). Thiosemicarbazide also has significant biological activities, ranging from anti-tumour, fungicidal, bactericidal and anti-inflammatory and antiviral activities [16–19]. The reactions of primary amines and hydrazines with esters have been studied by several methods [20–23]. The present study synthesized a new series of 1,4-dihydropyridine derivatives and screened their anticoagulant activities.

## 2. Chemistry

A series of diethyl 2,6-dimethyl-(4-substitutedphenyl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives (**1a**–**g**) were prepared as base compounds by following the method previously described in the literature [24]. The 2,6-dimethyl-4-substituted phenyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives (**1a**–**g**) were reacted with thiosemicarbazide to give 2,2′-{[4-(4-substitutedphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diyl]dicarbonyl}dihydrazinecarbothioamide compounds **(2a**–**g)**, shown in Scheme 1.

## 3. Results and discussion

### 3.1. Spectral discussion

The physicochemical characteristics of the synthesized compounds are presented in Table 1. The IR spectra of compound (**1a**) shows an absorption band at 3349 cm$^{-1}$ due to the NH present in the pyridine ring, and another absorption band at 1745 cm$^{-1}$ due to the carbonyl stretching of the ester groups. Compound (**1b**) shows an absorption band for the Cl–C group at 837 cm$^{-1}$ and compound (**1c**) shows an absorption band for the HO–C group at 1447 cm$^{-1}$. Compound (**1d**) shows an absorption band at 1536 cm$^{-1}$ which corresponds to ($O_2$N–C), other absorptions are summarized in the Experimental section.

* Corresponding author. Tel.: +919865560226/+919944549026/+919994265115.
*E-mail address:* jamal_abdulchem@ymail.com (A.J. Abdul Nasser).

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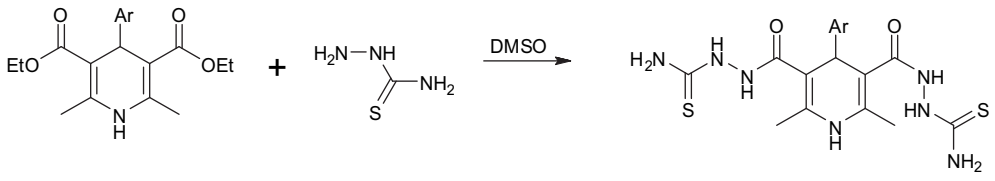



**Scheme 1.** Synthesis route of compounds (**2a**–**g**).

The $^1$H NMR spectra of compound (**1a**) show a singlet at $\delta$ 8.20, which was attributed to the NH proton of the 1,4-dihydropyridine ring, and another important singlet at $\delta$ 4.72, which was attributed to the CH at $C_4$ the 1,4-dihydropyridine ring. The other $^1$H NMR resonances are summarized in the Experimental section.

The IR spectrum of compound (**2a**) shows an absorption band at 3370 cm$^{-1}$ due to the NH group present the in 1,4-dihydropyridine ring, another absorption band at 3192 cm$^{-1}$ which was due to the NH—CO str and an absorption band for the C═S group was observed at 1263 cm$^{-1}$ respectively, which confirmed the compound as (**2a**). The above IR absorptions absent from the spectra of the other compounds (**2b**–**g**), which confirmed the other compounds (**2b**–**g**). The IR spectra are summarized in the Experimental section.

The $^1$H NMR spectrum (Fig. 1) of compounds (**2a**) shows that singlet at $\delta$ 8.46, which was attributed to NH protons present in the1,4-dihydropyridine ring. The 4CH, CONH, NH—CS and NH protons resonated as singlets at $\delta$ 5.15, 8.12, 2.14 and 9.64 respectively. The above $^1$H NMR values confirmed the compound as (**2a**). The $^1$H NMR spectra of the remaining compounds (**2b**–**g**) are summarized in the Experimental section.

The $^{13}$C NMR spectrum (Fig. 2) of compound (**2a**) shows that peaks at $\delta$ 168.66 (C═O), 182.10(C═S), 38.70 ($C_4$ of pyridine ring) and 18.72($CH_3$). The $^{13}$C NMR spectra of the remaining compounds (**2b**–**g**) are summarized in the Experimental section.

The mass spectrum (Fig. 3) of the compound (**2a**) shows that molecular ion peaks, which confirms the molecular mass of the compound. Scheme 2 shows the fragmentation pattern of compound (**2a**), the mass spectrum of the other compounds (**2b**–**g**) is summarized in the Experimental section.

### 3.2. Anticoagulant screening

We examined the anticoagulant activity of these compounds on human plasma using the APTT and PT coagulant assays. Two concentrations were required to achieve the relative clotting times of compounds (**1a**–**g**) and (**2a**–**g**) in human plasma (*in vitro*).

**Table 1**
Physicochemical characterization of the compounds (**1a**–**g**), (**2a**–**g**).

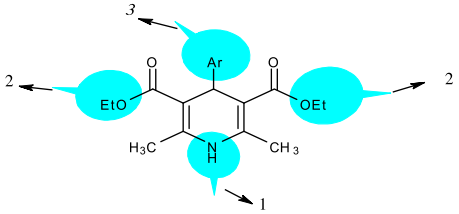
| Compounds no | *R* | M.P | Yield (%) |
|---|---|---|---|
| **1a** | -furyl | 147 | 74 |
| **1b** | -Ph | 153 | 66 |
| **1c** | 4-Cl$C_6H_4$ | 242 | 47 |
| **1d** | 4-OH$C_6H_4$ | 240 | 56 |
| **1e** | 4-$NO_2C_6H_4$ | 197 | 72 |
| **1f** | 4-$CH_3OC_6H_4$ | 229 | 69 |
| **1g** | 4-$(CH_3)_2NC_6H_4$ | 227 | 56 |
| **2a** | -furyl | 187 | 61 |
| **2b** | -Ph | 192 | 53 |
| **2c** | 4-Cl$C_6$H4 | 194 | 68 |
| **2d** | 4-OH$C_6H_4$ | 201 | 74 |
| **2e** | 4-$NO_2C_6H_4$ | 195 | 76 |
| **2f** | 4-$CH_3OC_6H_4$ | 210 | 57 |
| **2g** | 4-$(CH_3)_2NC_6H_4$ | 205 | 61 |

Compounds (**1a**–**g**) did not exhibit anticoagulant activities but compounds (**2a**–**g**) show some significant anticoagulant activity.

The APTT coagulation assay performed on compound (**2f**) showed that this compound had a low anticoagulant activity compared with the other compounds and that compound (**2a**) had a highly active coagulation time of 720.35 s at a concentration of 30 μg/mL compared with the other compounds. Heparin was used as the standard at the same concentration 30 μg/mL. These values are summarized in Table 2. Fig. 4 indicates that the APTT assay against compounds performance was measured the variation of clotting time by two concentrations. The PT coagulation assay performed using compound (**2c**) had a low active compared with the other compounds and that compound (**2a**) had a high relative clotting factor of 240.42 s at concentrations of 30 μg/mL and 60 μg/mL compared with the other compounds. These values are summarized in Table 3. Fig. 5 indicates that the PT assays against compounds performance was measured the variation of clotting time by two concentrations. The results of these two clotting time tests (APTT and PT) were compared in order to determine the inhibition pathways of compounds (**2a**–**g**) at concentration of 60 μg/mL. These results are summarized in Table 4. Based on the APTT and PT Index, the compound **2a** was considered to be having highest relative clotting potency.

### 3.3. Structural activity relationship

From the results of anticoagulant activity of the synthesized 1,4-dihydropyridine derivatives (**1a**–**g**), the following structure activity relationships can be derived:




Compounds (**1a-g**)

Ar = **1a** - furyl, **1b** - Ph, **1c** - 4-Cl-C6H4, **1d** -4-OH-C6H4, **1e** -4-NO2C6H4, **1f** - 4-CH3OC6H4, **1g** -4-(CH3)2NC6H4.

1,4-Dihydropyridines (DHPs) are an important class of drugs which exert potent blocking activities on calcium ($Ca^{2+}$) currents through voltage-dependent L – type channels [25]. Several DHPs are clinically used in the treatment of a number of cardiovascular diseases. Structure activity relationships of this class of compounds have been the subject of several studies [25]. The ester groups in the 3- and 5-positions are of crucial importance for activity. The above structure (**1a**–**g**) showed that NH group (1), —OEt groups (2) and substituted aromatic aldehyde presenting in the compounds (**1a**–**g**) 1,4-dihydropyridine derivatives exhibited no response the anticoagulant activity.

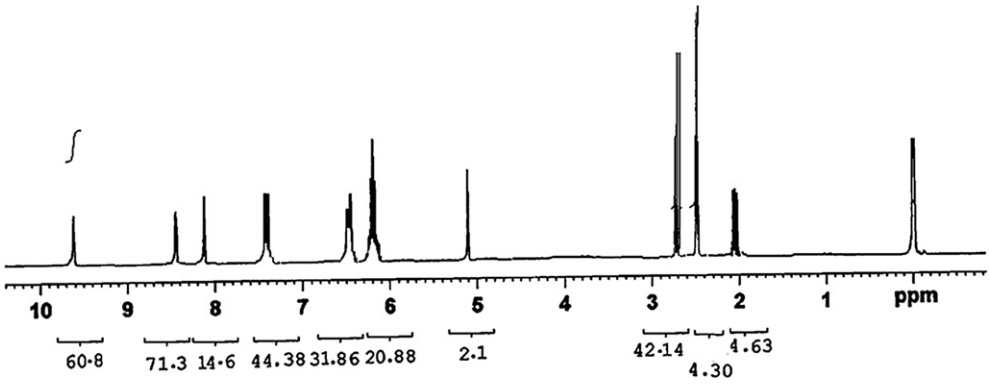



**Fig. 1.** $^{1}$H NMR spectrum of the compound (**2a**).

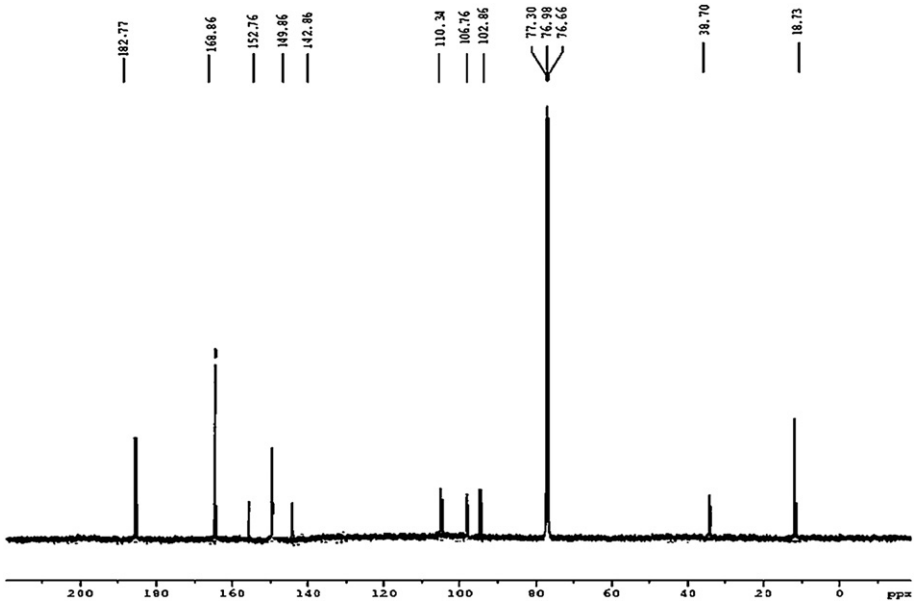
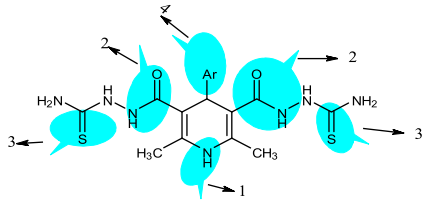



**Fig. 2.** $^{13}$C NMR spectrum of the compound (**2a**).




Compounds (**2a-g**)

Ar = **1a** - furyl, **1b** - Ph, **1c** - 4-Cl-C6H4, **1d** -4-OH-C6H4, **1e** -4-NO2C6H4, **1f** - 4-CH3OC6H4, **1g** -4-(CH3)2NC6H4.

The above structure (**2a**–**g**) showed that NH group (1), CONH group (2), $CSNH_2$ group (3), and substituted aromatic aldehyde (4) presenting in the compounds (**2a**–**g**) 1,4-dihydropyridine derivatives exhibited response the anticoagulant activity.

The compounds (**2a**–**g**) have higher anticoagulant activity compared with compounds (**1a**–**g**) because the activity may be due to the sulfur group present in the compounds (**2a**–**g**).

The compound (**2a**) is high relative clotting factor at concentrations (30 and 60 μg/mL) compared with the compounds (**2b**–**g**) and Heparin also in APTT assay, the activity may be the presence of furyl ring in 4-position of 1,4-dihydropyridine ring and 3,5-position of CONH, $CSNH_2$ groups. The compounds (**2a**–**g**) have very low response in PT assay at concentrations (30 and 60 μg/mL).

## 4. Experimental

### 4.1. Chemistry

The melting points were recorded in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr on an FT-IR Shimadzu 8201 pc (4000–400 $cm^{-1}$) and the $^{1}$H NMR and $^{13}$C NMR were recorded on a Bruker DRX-400 MHz. Mass spectra (EI) were recorded on a Jeol JMS D-300 spectro meter operating at 70 eV. Elemental analyses (C, H, N and S) were performed using an Elemental analyzer model vario EL III. The purity of the compounds was checked by thin layer chromatography (TLC) with silica gel plates.

#### 4.1.1. Synthesis of diethyl 4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (*1a*)

A mixture of ethyl acetoacetate (0.2 mol), furfuraldehyde (0.1 mol) and ammonium hydroxide (0.1 mol) was made up in methanol (20 mL). It was then heated and refluxed for 4 h. The

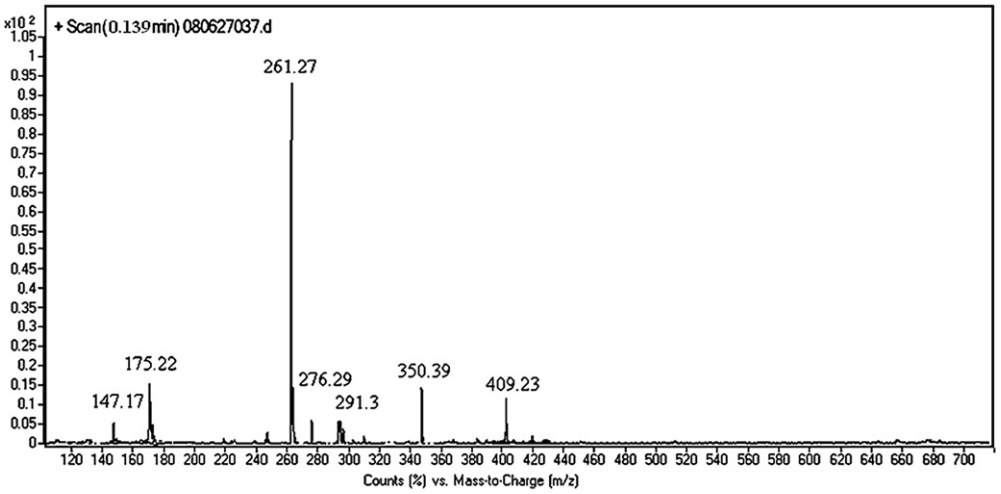


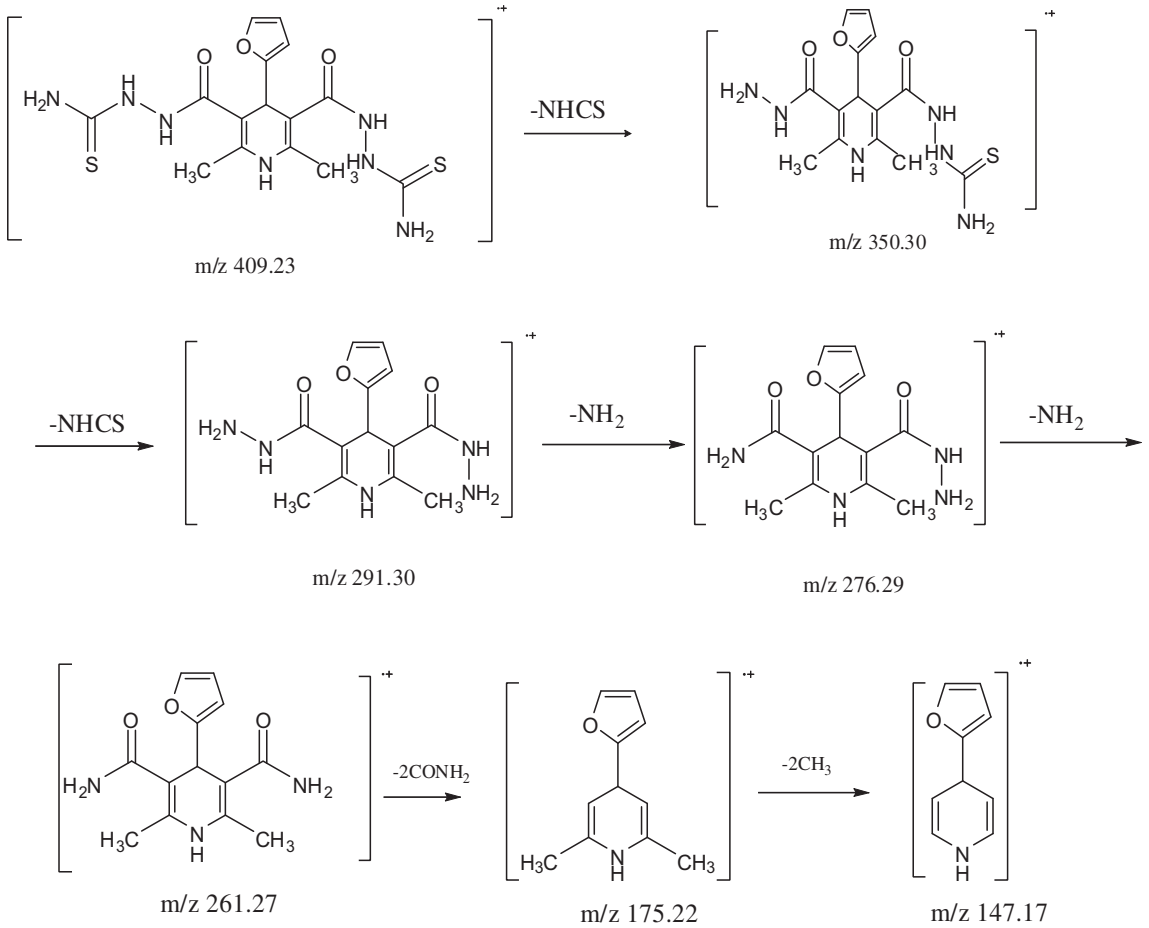

**Fig. 3.** Mass spectrum of the compound (**2a**).

obtained solid was filtered and washed with water. The solid was then re-crystallized using absolute ethanol. The above procedure was followed for the synthesis of compounds (**1b**–**g**).

$C_{17}H_{21}NO_5$; Elemental Analysis: Calculated for: C, 63.94; H, 6.63; N, 4.39; Found: C, 63.84; H, 6.61; N, 4.30%; IR (KBr, $cm^{-1}$): $\nu = 3349$ (N–H str), 3030 (Ar-H), 2940(C–H str of $CH_3$), 1745 (C=O, ester), 812 (Ar-H); $^1$H NMR (DMSO-$d_6$): $\delta = 8.20$ (s, 1H, NH of pyridine ring), 7.27 (s, 5H, Ph-ring), 6.10–6.27 (d, 2H, furyl ring), 4.72 (s, 2H, C4–H), 4.20 (q, 4H, C3–OC<u>H</u>$_2$CH$_3$ and C5–OC<u>H</u>$_2$CH$_3$), 2.31 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 1.34 (t, 6H, C2–OCH$_2$C<u>H</u>$_3$ and C6–OCH$_2$C<u>H</u>$_3$); $^{13}$C NMR (DMSO-$d_6$ , $\delta$/ppm) : 142.1, 110.6, 106.7, 152.5 (furyl ring), 151.8 (C-2,6), 33.2 (C-4), 102.3 (3,5-<u>C</u>OOCH$_2$CH$_3$ ), 61.1(3,5-COO<u>C</u>H$_2$CH$_3$ ), 14.9 (3,5-COOCH$_2$<u>C</u>H$_3$), 18.1 (2,6-$CH_3$).

*4.1.2. Diethyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (**1b**)*

$C_{19}H_{23}NO_4$; C, 69.28; H, 7.04; N, 4.25; Found: C, 69.24; H, 7.07; N, 4.30%; IR (KBr, $cm^{-1}$): $\nu = 3350$ (N–H str), 3034 (Ar-H), 2953 (C–H




**Scheme 2.** Fragmentation pattern of compound (**2a**).

**Table 2**
Comparison of anticoagulant activity compounds (**2a**–**g**) with standard Heparin.

| Compounds no | Concentration (μg/mL) | Clotting time(s) APTT | APTT index |
|---|---|---|---|
| **2a** | 30 | 720.35 | 19.78[a] |
| | 60 | >1,000[a] | 27.47[a] |
| **2b** | 30 | 87.5 | 2.40 |
| | 60 | 182.4 | 5.01 |
| **2c** | 30 | 90.7 | 2.49 |
| | 60 | 200.4 | 5.50 |
| **2d** | 30 | 400.8 | 11.0[a] |
| | 60 | 882.7 | 24.25[a] |
| **2e** | 30 | 321.2 | 8.82 |
| | 60 | 721.8 | 19.82[a] |
| **2f** | 30 | 64.7 | 1.85 |
| | 60 | 120.2 | 3.30 |
| **2g** | 30 | 221.8 | 6.09 |
| | 60 | 480.0 | 13.18[a] |
| Heparin | 30 | 185.0 | 5.08 |
| | 60 | >1.000[a] | 27.47[a] |
| Control | 0 | 36.4 | 1.0 |

Values are expressed as mean of five trails.
[a] Shows highly significant index.

**Table 3**
Comparison of anticoagulant activity of the compounds (**2a**–**g**).

| Compounds no | Concentration (μg/mL) | Clotting time(s) PT | PT index |
|---|---|---|---|
| **2a** | 30 | 240.42 | 12.14[a] |
| | 60 | 420.21 | 21.22[a] |
| **2b** | 30 | 0 | 0 |
| | 60 | 45.8 | 2.31 |
| **2c** | 30 | 0 | 0 |
| | 60 | 33.8 | 1.24 |
| **2d** | 30 | 45.6 | 2.30 |
| | 60 | 70.8 | 3.5 |
| **2e** | 30 | 24.7 | 8.82 |
| | 60 | 52.8 | 2.66 |
| **2f** | 30 | 45.7 | 2.03 |
| | 60 | 93.4 | 4.71 |
| **2g** | 30 | 21.6 | 1.09 |
| | 60 | 48.2 | 2.43 |
| Control | 0 | 19.8 | 1.0 |

Values are expressed as mean of five trails.
[a] Shows highly significant index.

str of $CH_3$), 1755 (C=O, ester), 802 (Ar-H); $^1$H NMR (DMSO-$d_6$): $\delta = 8.25$ (s, 1H, NH of pyridine ring), 7.33–7.27 (m, 5H, Ph-ring), 4.70 (s, 2H, C4–H), 4.22 (q, 4H, C3–O$\underline{CH_2}$CH$_3$ and C5–O$\underline{CH_2}$CH$_3$), 2.28 (s, 6H, C2–$CH_3$ and C6-$CH_3$), 1.32 (t, 6H, C2–O$\underline{CH_2}$CH$_3$ and C6-O$\underline{CH_2}$CH$_3$); $^{13}$C NMR ( DMSO-$d_6$ , $\delta$/ppm) : 125.1, 128.4, 127.1, 144.8 (Phenyl ring), 150.7 (C-2,6), 101.9 (3,5-$\underline{C}$OOCH$_2$CH$_3$), 62.1 (3,5-COO$\underline{CH_2}$CH$_3$ ), 44.1(C-4), 19.1 (2,6-$CH_3$ ), 15.4 ( 3,5-COOCH$_2$ $CH_3$).

*4.1.3. Diethyl 4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**1c**)*

$C_{19}H_{22}ClNO_4$; C, 62.72; H, 6.09; N, 3.85; Found: C, 62.75; H, 6.07; N, 3.81%; IR (KBr, cm$^{-1}$): $\nu$ = 3332 (N–H str), 3074 (Ar-H), 2942 (C–H str of $CH_3$), 1741 (C=O, ester), 837 (C–Cl), 787 (Ar-H); $^1$H NMR (DMSO-$d_6$): $\delta = 8.31$ (s, 1H, NH of pyridine ring), 7.36–7.19 (m, 5H, Ph-ring), 4.76 (s, 1H, C4–H), 4.18 (q, 4H, C3–O$\underline{CH_2}$CH$_3$ and C5-O$\underline{CH_2}$CH$_3$), 2.21 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 1.34 (t, 6H, C2–O$\underline{CH_2}$CH$_3$ and C6–O$\underline{CH_2}$CH$_3$); $^{13}$C NMR (DMSO-$d_6$ , $\delta$/ppm) : 131.4, 128.1, 130.8, 142.5 (Ph-Cl), 152.5 (C-2,6), 34.6 (C-4), 103.9 (3,5-$\underline{C}$OOCH$_2$CH$_3$), 60.3 (3,5-COO$\underline{CH_2}$CH$_3$), 15.2 (3,5-COOCH$_2$ $CH_3$), 18.6 (2,6-$\underline{C}$H$_3$), 32.1(C-4).

*4.1.4. Diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**1d**)*

$C_{19}H_{23}NO_5$; C, 66.07; H, 6.71; N, 4.06; Found: C, 66.10; H, 6.75; N, 4.01%; IR (KBr, cm$^{-1}$): $\nu$ = 3342 (N–H str), 3024 (Ar-H), 2922 (C–H str of $CH_3$), 1764 (C=O, ester), 1447 (C–OH), 814 (Ar-H); $^1$H NMR (DMSO-$d_6$): $\delta = 9.47$ (s, 1H, COH), 8.41 (s, 1H, NH of pyridine ring), 6.34–7.07 (m, 4H, Ph-ring), 4.67 (s, 1H, C4–H), 4.28 (q, 4H, C3–O$\underline{CH_2}$CH$_3$ and C5–O$\underline{CH_2}$CH$_3$), 2.12 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 1.28 (t, 6H, C2–OCH2C$\underline{H}$3 and C6–OCH2C$\underline{H}$3); $^{13}$C NMR (DMSO-$d_6$ , $\delta$/ppm) : 155.6, 116.2, 131.2138.2 (Ph-OH), 151.4 (C-2,6), 44.9(C-4), 101.4 (3,5-$\underline{C}$OOCH$_2$CH$_3$), 62.3 (3,5-COO$\underline{CH_2}$CH$_3$), 14.1(3,5-COOCH$_2$C$\underline{H}$$_3$), 18.4 (2,6-$\underline{C}$H$_3$).

*4.1.5. Diethyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxlate (**1e**)*

$C_{19}H_{22}N_2O_6$; C, 60.95; H, 7.48; N, 7.48; Found: C, 60.91; H, 7.42; N, 7.41%; IR (KBr, cm$^{-1}$): $\nu$ = 3354 (N–H str), 3037 (Ar-H), 2973 (C–H str of $CH_3$), 1762 (C=O, ester), 1536 (C–$NO_2$), 812 (Ar-H); $^1$H NMR (DMSO-$d_6$) : $\delta = 8.11$ (s, 1H, NH of pyridine ring), 8.13–7.47 (m, 4H, Ph-ring), 4.79 (s,1H,C4–H), 4.25 (q, 4H, C3–O$\underline{CH_2}$CH$_3$ and C5–O$\underline{CH_2}$CH$_3$), 2.31 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 1.37 (t, 6H, C2–O$\underline{CH_2}$CH$_3$ and C6–O$\underline{CH_2}$CH$_3$); $^{13}$C NMR (DMSO-$d_6$ , $\delta$/ppm) : 144.8, 123.6, 126.9, 151.0 (Ph-$NO_2$), 152.2 (C-2,6), 43.9(C-4), 103.2 (3,5-$\underline{C}$OOCH$_2$CH$_3$), 60.8 (3,5-COO$\underline{CH_2}$CH$_3$), 14.4 (3,5-COOCH$_2$$\underline{C}$H$_3$), 18.8 (2,6-$\underline{C}$H$_3$), 31.4 (C-4).

*4.1.6. Diethyl 4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**1f**)*

$C_{20}H_{25}NO_5$; C, 66.83; H, 7.01; N, 3.90; Found: C, 66.87; H, 7.07; N, 3.97%; IR (KBr, cm$^{-1}$): $\nu$ = 3352 (N–H str), 3026 (Ar-H), 2961(C–H str of $CH_3$), 1742 (C=O, ester), 823 (Ar-H); $^1$H NMR (DMSO-$d_6$):$\delta = 8.21$ (s, 1H, NH of pyridine ring), 6.86–7.17 (m, 5H, Ph-ring), 4.69 (s,1H, C4–H), 4.23 ( q, 4H, C3–O$\underline{CH_2}$CH$_3$ and C5–O$\underline{CH_2}$CH$_3$), 3.84 (s, 3H, –O$\underline{CH_3}$), 2.23 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 1.30 (t, 6H, C2–OCH$_2$C$\underline{H}$3 and C6–OCH$_2$C$\underline{H}$$_3$); $^{13}$C NMR (DMSO-$d_6$ , $\delta$/ppm) : 157.2, 114.6, 129.3, 135.9 (Ph), 158.3(C-2,6), 102.3(3,5-$\underline{C}$OOCH$_2$CH$_3$), 61.5(3,5-COO$\underline{CH_2}$CH$_3$), 55.7 (Ph-O$\underline{CH_3}$), 43.6 (C-4), 14.8 (3,5-COOCH$_2$$\underline{C}$H$_3$), 18.0 (2,6-$\underline{C}$H$_3$).

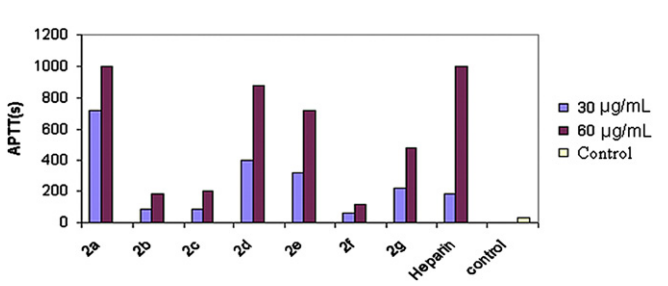



**Fig. 4.** Anticoagulant activity variation of compounds (**2a**–**g**) were used two concentrations at 30 and 60 (μg/mL) by APPT assay.

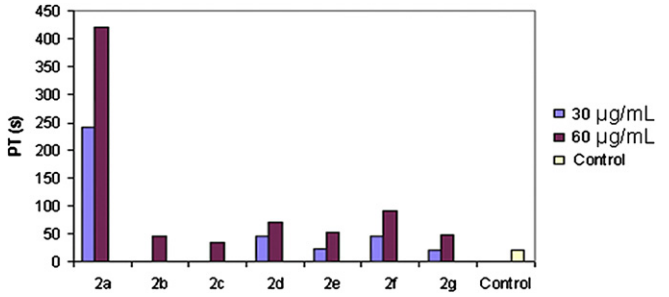



**Fig. 5.** Anticoagulant activity variation of compounds (**2a**–**g**) were used two concentrations at 30 and 60 (μg/mL) **by** PT assay.

**Table 4**
Comparison of the anticoagulant activity of the compounds (**2a**–**g**) by different coagulant assays of APTT, PT.

| Clotting time (s) concentration 60 (μg/mL) | | | | |
|---|---|---|---|---|
| Compounds no | APTT | Relative clotting potency | PT | Relative clotting potency |
| **2a** | >1.000[a] | 27.47 | 240.42 | 72.8 |
| **2b** | 182.4 | 5.01 | 45.8 | 2.31 |
| **2c** | 200.4 | 5.50 | 33.8 | 1.24 |
| **2d** | 882.7 | 24.25 | 70.8 | 3.5 |
| **2e** | 721.8 | 19.82 | 52.8 | 2.66 |
| **2f** | 120.2 | 3.30 | 93.4 | 4.71 |
| **2g** | 480.0 | 13.18 | 48.2 | 2.43 |
| Control | 36.4 | 1.0 | 19.8 | 1.0 |

[a] Clotting time >1000 s considered as 1000 s to calculate the relative clotting potency.

#### 4.1.7. Diethyl 4-(4-(dimethylamino)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbo xylate (**1g**)

$C_{19}H_{23}NO_4$; C, 67.72; H, 7.58; N, 7.52; Found: C, 67.77; H, 7.52; N, 7.58%; IR (KBr, $cm^{-1}$): $\nu = 3348$ (N–H str), 3027 (Ar-H), 2956 (C–H str of $CH_3$) 1761 (C=O, ester), 808 (Ar-H); $^1$HNMR (DMSO-$d_6$): $\delta = 8.37$ (s, 1H, NH of pyridine ring), 7.28–7.21 (m, 5H, Ph-ring), 4.70 (s, 2H, C4–H), 4.22 (q, 4H, C3–O$\underline{CH_2}CH_3$ and C5–O$\underline{CH_2}CH_3$), 3.12 (s, 6H, –N($CH_3$)), 2,2.28 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 1.32 (t, 6H, C2–OCH$_2\underline{CH3}$ and C6–OCH$_2\underline{CH3}$); $^{13}$C NMR (DMSO-$d_6$ , $\delta$/ppm) : 128.3, 112.9, 148.5, 133.9 (Ph), 151.8 (C-2,6), 43.8 (C-4), 102.8 (3,5-$\underline{C}OOCH_2CH_3$), 60.5 (3,5-COO$\underline{C}H_2CH_3$), 40.8 (–N($\underline{C}H_3)_2$), 13.9 (3,5-$\underline{C}OOCH_2\underline{C}H_3$), 18.9(2,6-$\underline{C}H_3$).

#### 4.1.8. Synthesis of 2,2′-{[4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diyl]dicarbonyl}dihydrazinecarbothioamide (**2a**)

A reaction mixture consisting of compound **1a** (0.1 mol), thiosemicarbazide (0.2 mol) dissolved in ethanol (30 mL) and a few drops of DMSO was added. It was then heated under reflux for 10 h. The obtained solid was cooled and then poured in to crushed ice. The solid was collected by filtration, washed with water and recrystallized using ethanol. The above procedure was repeated for the synthesis of compounds (**2b**–**g**).

$C_{15}H_{19}N_7O_3S_2$; C, 48.00; H, 4.68; N, 23.94; S, 15.66; Found: C, 48.64; H, 5.57; N, 23.31; S, 15.34%; IR (KBr, $cm^{-1}$): $\nu = 3370$ (NH), 3221 ($NH_2$), 3192 (NH–C=O), 3037 (Ar-H), 1263 (C=S), 1721 (C=O), 1095 (N–C–N), 811 (Ar-H); $^1$H NMR (DMSO-$d_6$):$\delta = 9.64$ (s, 2H, $NH_2$), 8.46 (s, 1H, NH of pyridine ring), 8.12 (d, 1H, C3–CONH and C5–CONH), 7.22 (s, 5H, Ph-ring), 6.14–6.32 (d, 2H, furyl ring), 5.15 (s, 2H, C4–H), 2.33 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 2.14 (d, 1H, –NHCS); $^{13}$C NMR (DMSO-$d_6$): $\delta = 110.37, 106.79, 142.11, 152.79$ (4C in furyl ring), 102.79 (3,5-C in pyridine ring), 168.66 (C=O), 182.10 (C=S), 149.86 (2,6-C in pyridine ring), 38.70 (4C in pyridine ring), 18.72 (2,6-$CH_3$ in pyridine ring); MS (*m*/*z*, (relative abundance %)): 409.45 ($M^+$, 30.2), 350.39, 291.30, 261.27, 175.22, 147.12.

#### 4.1.9. 2,2′-[(2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-diyl) dicarbonyl]dihydrazine carbothioamide (**2b**)

$C_{17}H_{21}N_7O_2S_2$; C, 48.67; H, 5.50; N, 23.37; S, 15.29; Found: C, 48.64; H, 5.57; N, 23.31; S, 15.34%; IR (KBr, $cm^{-1}$): $\nu = 3372$ (NH), 3200 (NH–C=O), 3218 ($NH_2$), 3034 (Ar-H), 1718 (C=O), 1260 (C=S), 1091 (N–C–N), 808 (Ar-H); $^1$H NMR (DMSO-$d_6$): $\delta = 9.62$ (s, 2H, $NH_2$), 8.43 (s,1H, NH of pyridine ring), 8.09 (d, 1H, C3–CONH and C5–CONH), 7.39–7.22 (m, 5H, Ph-ring), 5.17 (s, 2H, C4–H), 2.37 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 2.12 (d, 1H, –NHCS); $^{13}$C NMR (DMSO-$d_6$): $\delta = 131.3, 128.5, 130.9, 141.8$ (4C in furyl ring), 106.8 (3,5-C in pyridine ring), 164.6 (C=O), 182.8 (C=S), 147.9 (2,6-C in pyridine ring), 34.6 (4C in pyridine ring), 18.9 (2,6-$CH_3$ in pyridine ring); MS (*m*/*z*, (relative abundance %)): 419.20 ($M^+$, 20.1), 301.34, 241.28, 185.2, 157.21, 81.11.

#### 4.1.10. 2,2′-{[4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diyl]dicarbonyl}dihydrazinecarbothioamide (**2c**)

$C_{17}H_{20}ClN_7O_2S_2$; C, 44.98; H, 4.40, N, 21.60, S, 14.13; Found: C, 44.92; H, 4.46; N, 21.64; S, 14.18%; IR (KBr, $cm^{-1}$): $\nu = 3325$ (NH), 3231 ($NH_2$), 3198 (NH–C=O), 3024 (Ar-H), 1707 (C=O), 1265 (C=S), 1097 (N–C–N), 827 (C–Cl); $^1$H NMR (DMSO-$d_6$ ): $\delta = 9.41$ (s, 2H, $NH_2$), 8.41 (bs,1H, NH of pyridine ring), 8.11 (d, 1H, C3–CONH and C5–CONH), 7.38–7.14 (m, 5H, Ph-ring), 5.10 (s, 2H, C4-H), 2.45 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 2.08 (d, 1H, NHCS); $^{13}$C NMR (DMSO-$d_6$ ): $\delta = 128.7, 108.3, 143.2, 152.8$ (4C in furyl ring), 105.3 (3,5, C in pyridine ring), 166.2 (C=O), 182.1 (C=S), 148.9 (2,6-C in pyridine ring), 39.3 (4C in pyridine ring), 18.2 (2,6-$CH_3$ in pyridine ring); MS (*m*/*z*, (relative abundance %)): 453.12 ($M^+$, 12.3), 335.78, 275.73, 219.70, 157.21, 81.11.

#### 4.1.11. 2,2′-{[4-(4-Hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diyl]dicarbonyl} dihydrazinecarbothioamide (**2d**)

$C_{17}H_{21}N_7O_2S_2$; C, 46.88; H, 4.86; N, 22.51; S, 14.72; Found: C, 46.81; H, 22.57; N, 4.81; S, 14.77%; IR (KBr, $cm^{-1}$): $\nu = 3342$ (NH), 3220 ($NH_2$), 3028 (Ar-H), 3192 (NH–C=O), 1242 (C=S), 1472 (C–OH), 1717 (C=O), 1091 (N–C–N); $^1$H NMR (DMSO-$d_6$): $\delta = 9.71$ (s, 2H, $NH_2$), 9.41 (s, 1H, OH), 8.64 (bs, 1H, NH of pyridine ring), 8.01 (d, 1H, C3–CONH and C5–CONH), 7.33–7.27 (m, 5H, Ph-ring), 5.11 (s, 2H, C4–H), 2.25 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 2.02 (d, 1H, –NHCS); $^{13}$C NMR (DMSO-$d_6$): $\delta = 155.8, 137.1, 130.3, 114.2$ (4C in furyl ring), 102.9 (C in pyridine ring), 164.9 (C=O), 184.6 (3,5, C=S), 148.1(2,6-C in pyridine ring), 43.8 (4C in pyridine ring), 19.2 (2,6-$CH_3$ in pyridine ring); MS (*m*/*z*, (relative abundance %)): 434.52 ($M^+$, 27.2), 257.28, 201.26, 173.21, 157.21, 81.11.

#### 4.1.12. 2,2′-{[4-(4-nitrophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diyl]dicarbonyl}dihydrazinecarbothioamide (**2e**)

$C_{17}H_{20}N_8O_4S_2$; C, 43.96; H, 4.34; N, 24.12; S, 13.81; Found: C, 43.91; H, 4.38; N, 24.17; S, 13.87%; IR (KBr, $cm^{-1}$): $\nu = 3310$ (NH), 3241 ($NH_2$), 3218 (NH–C=O), 3041 (Ar-H), 1272 (C=S), 1710 (C=O), 1091 (N–C–N), 1530 (C–$NO_2$); $^1$H NMR (DMSO-$d_6$ ): $\delta = 9.77$ (s, 2H, $NH_2$), 8.60 (bs, 1H, NH of pyridine ring), 8.15 (d, 1H, C3–CONH and C5–CONH), 7.42–7.18 (m, 5H, Ph-ring), 5.17 (s, 2H, C4-H), 2.31 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 2.08 (d, 1H, –NHCS); $^{13}$C NMR (DMSO-$d_6$ ): $\delta = 143.2, 123.7, 126.7$ (4C in furyl ring), 102.9 (3,5-C in pyridine ring), 164.9 (C=O), 181.9 (C=S), 149.9 (2,6-C in pyridine ring), 44.5 (4C in pyridine ring), 19.7 (2,6-$CH_3$ in pyridine ring); MS (*m*/*z*, (relative abundance %)): 464.52 ($M^+$, 12.78), 346.34, 286.20, 258.23, 230.21, 202.20, 81.11.

#### 4.1.13. 2,2′-{[4-(4-methoxyhenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diyl]dicarbonyl} dihydrazinecarbothioamide (**2f**)

$C_{18}H_{23}N_7O_3S_2$; C, 48.09; H, 5.16; N, 21.81; S, 14.27; Found: C, 48.05; H, 5.19; N, 21.88; S, 14.21%; IR (KBr, $cm^{-1}$): $\nu = 3323$ (NH), 3251 (NH–C=O), 3231 ($NH_2$), 3034 (Ar-H), 1717 (C=O), 1251 (C=S), 1091 (N–C–N), 808 (Ar-H); $^1$H NMR (DMSO-$d_6$): $\delta = 9.82$ (s, 2H, $NH_2$), 8.57 (bs, 1H, NH of pyridine ring), 8.05 (d, 1H, C3–CONH and C5–CONH), 7.33–7.27 (m, 5H, Ph-ring), 5.21 (s, 2H, C4–H), 3.81 (s, 3H, –O$\underline{CH_3}$), 2.10 (d, 1H, –NHCS) 2.25 (s, 6H, C2–$CH_3$ and C6–$CH_3$); $^{13}$C NMR (DMSO-$d_6$ ): $\delta = 111.8, 108.3, 143.2, 152.8$ (4C in furyl ring), 105.3 (3,5, C in pyridine ring), 166.2 (3,5, C=O), 181.7 (3,5, C=S), 147.7 (2,6-C in pyridine ring), 44.7 (4C in pyridine ring), 18.8 (2,6-$CH_3$ in pyridine ring), 55.9 (–$OCH_3$); MS (*m*/*z*, (relative abundance %)): 449.21 ($M^+$, 29.12), 331.36, 271.31, 243.25, 215.29, 185.26, 157.21.

*4.1.14. 2,2′-{[4-(4-dimethylnitrophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diyl]dicarbonyl}dihydrazinecarbothioamide (**2g**)*

$C_{19}H_{26}N_8O_2S_2$; C, 49.33; H, 5.67; N, 24.22; S, 13.86; Found: C, 49.37; H, 5.69; N, 24.27; S, 13.82%; IR (KBr, $cm^{-1}$): $\nu$ = 3321 (NH), 3211 ($NH_2$), 3118 (NH–C=O), 3021 (Ar-H), 1712 (C=O), 1248 (C=S), 1091 (N–C–N), 808 (Ar-H); $^1$H NMR (DMSO-$d_6$): $\delta$ = 9.66 (s, 2H, $NH_2$), 8.52 (s, 1H, NH of pyridine ring), 8.03 (d, 1H, C3–CONH and C5–CONH), 6.62–7.07 (m, 4H, Ph-ring), 5.13 (s, 2H, C4–H), 3.06 (s, 1H, $-N(CH_3)_2)_2$, 19 (s, 6H, C2–$CH_3$ and C6–$CH_3$), 2.07 (d, 1H, –NHCS); $^{13}$C NMR (DMSO-$d_6$): $\delta$ = 112.8, 134.8, 128.3, 148.2, 152.8 (4C in furyl ring), 106.3 (3,5-C in pyridine ring), 165.2 (C=O), 181.1 (C=S), 147.9 (2,6-C in pyridine ring), 39.3 (4C in pyridine ring), 40.8 ($N(CH_3)_2$), 46.5 (4C in pyridine ring), 18.2 (2,6-$CH_3$ in pyridine ring), MS (*m*/*z*, (relative abundance %)): 462.22 ($M^+$, 16.24), 432.56, 344.41, 284.35, 256.29, 213.23, 199.24, 185.26.

### *4.2. Human blood plasma preparation*

Human blood was collected from healthy individual donors in conical tubes with 2.5% sodium citrate solution. The plasma was separated from the blood cells by centrifuging at 5400 rpm at 4 °C for 20 min. The blood plasma was stored at −70 °C until use.

### *4.3. Anticoagulant activity experiment*

The anticoagulant study was carried out according to the method in the literature [26,27]. The anticoagulant activity of the compounds were determined by activated partial thromboplastin time (APTT) and prothrombin time (PT) coagulation assays (Fisher scientific company, USA). Briefly, citrated normal human plasma (90 μl) was mixed with 10 μl of the synthesized compounds and incubated for 1 min at 37 °C. Then the APTT assay reagent (100 μl) was added to the mixture and incubated for 5 min at 37 °C. Thereafter, 0.05 mm CaCl2 (100 μl) was added and clotting times were recorded using a coagulometer. The prothrombin time assay (PT) was only performed on the purified synthesized compounds to determine their type of coagulation inhibition pathway. For the PT assay, citrated normal human plasma (90 μl) was mixed with 10 μl of synthesized compound solutions and incubated for 10 min at 37 °C. Then pre-incubated PT assay reagent (200 μl) was added and the clotting times were recorded. The activity was compared with heparin, a commercial anticoagulant. To analyse the relative clotting potency, the compounds and heparin were dissolved in water and normal saline was used as control. The relative potencies were calculated as APTT index (Sample APTT/Control APTT ratio) or PT Index (Sample PT/Control PT ratio) using the following equation [28].

$$\text{APTT index} = \text{APTTs}/\text{PTTc}$$

$$\text{PT index} = \text{PTs}/\text{PTC}$$

Where, APTTs and PTs were APTT and PT at determined sample concentrations, respectively, while APTTc and PTc were those of the control assays.

## 5. Conclusion

We have synthesized a new series of 1,4-dihydropyridine derivatives (**1a**–**g**) and (**2a**–**g**). The synthesized compounds (**2a**–**g**) were screened for anticoagulant activity. Among these compounds, **2a** was found to have highly response anticoagulation action (time 720.35 s) at a concentration of 30 μg/mL compared with the other compounds.

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