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Original article

Synthesis, antiarrhythmic and anticoagulant activities of novel thiazolo derivatives from methyl 2-(thiazol-2-ylcarbamoyl)acetate

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Abstract

A series of novel thiazolo derivatives **2**–**17** were synthesized by initial condensation of methyl 2-(thiazol-2-ylcarbamoyl)acetate **1** with phenyl isothiocyanate and further reactions using different organic reagents. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, EIMS spectral data and elemental analysis. Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50}. All the compounds were screened for their antiarrhythmic and anticoagulant activities and they showed high antiarrhythmic activity compared with procaine amide and lidocaine as positive controls. The detailed synthesis, spectroscopic data, LD_{50} and pharmacological activities of the synthesized compounds were reported.

Keywords: Thiazoles; Thiadiazoles; Bis-imides; Antiarrhythmic; Anticoagulant activities

1. Introduction

The thiazolyl group is of great importance in treating biological systems. Anti-inflammatory, analgesic and antipyretic activities are observed in some thiazolyl and benzothiazolyl derivatives [1–5]. Some synthetic thiazoles have exhibited a range of biological activities, such as antitumor, antifilarial, antibiotic, antibacterial, antifungal, and anti-inflammatory [6–9]. Recent studies have shown the synthesis of some new thiazole candidates as antimicrobial and anticancer agents [10–13]. In the previous work, acid hydrazides were very important compounds, for its high reactivity usefulness in heteroorganic synthesis, as key starting materials to form various classes of biologically and pharmacologically active candidates [14–20]. In addition, we reported that certain of our newly substituted heterocyclic compounds exhibited antiparkinsonian [21], antitumor [22–24], antimicrobial [25] and anti-inflammatory [26] activities. In view of these reports and in continuation of our previous works in heterocyclic chemistry, we have herein synthesized some new thiazole derivatives for their pharmacological screening.

2. Results and discussion

2.1. Chemistry

Methyl 2-(thiazol-2-ylcarbamoyl)acetate **1** was synthesized according to the reported procedure [27] and is used as a starting material. It was reacted with phenyl isothiocyanate in the presence of sodium hydride as a catalyst to afford the corresponding methyl mercaptoacrylic acid methyl ester derivative **2**, which was reacted with α-halocarbonyl compounds, namely, 2-chloroacetylacetone, chloroacetone, phenacyl bromide and ethyl bromoacetate in refluxing ethanol containing triethylamine as a catalyst to give *N*-phenylthiazolo derivatives **3**–**6**, respectively. It was condensed with hydrazonyl halides, namely, ethyl 2-[(4-chlorophenyl)hydrazono]-2-chloroacetate and 1-[(2-(4-sulfonamido)phenyl)-hydrazono]-1-chloropropan-2-one in the presence of triethylamine in refluxing ethanol to afford the corresponding thiadiazole derivatives **7** and **8**, respectively. Compound **8** was reacted with 1,2,4,5-tetrachlorophthalic

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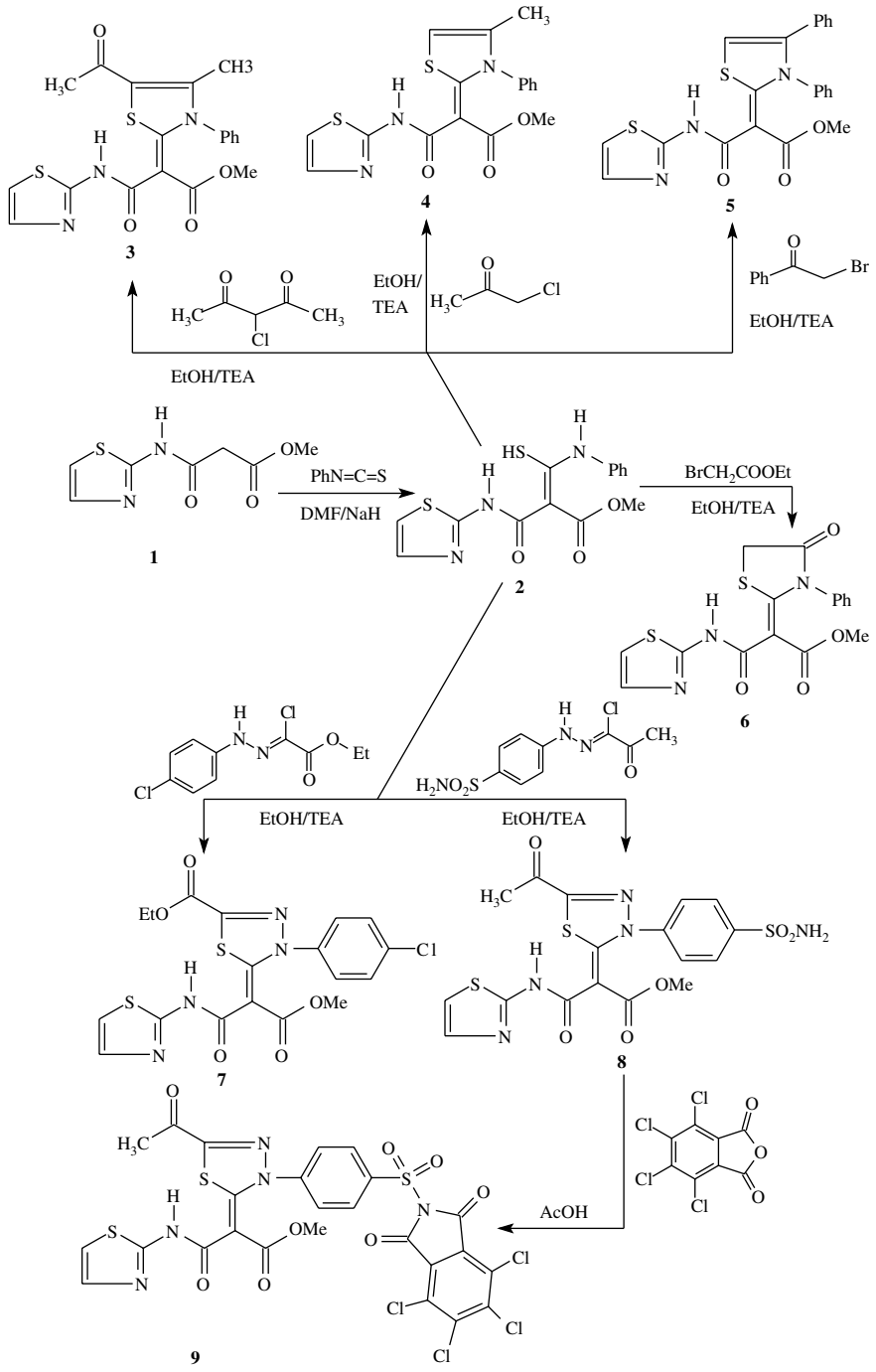
anhydride in refluxing glacial acetic acid to give the imide derivative **9** (Scheme 1). The structures of the synthesized compounds were assigned on the bases of its spectral data and elemental analysis (cf. Section 4 and Table 1).

Compound **1** was treated with hydrazine hydrate to give the corresponding hydrazide **10**, which was reacted with carbon disulfide and potassium hydroxide in ethanol to give potassium carbodithioate salt **11**. Compound **11** was reacted with phenacyl bromide in absolute ethanol under reflux to give the corresponding thiazolidine **12**, while it was condensed with hydrazonyl halides, namely, ethyl 2-[2-(4-bromophenyl) hydrazino]-2-chloroacetate and 1-[(2-*p*-sulfonamido-phenyl)-hydrazono]-1-chloropropan-2-one in refluxing ethanol to give the corresponding thiadiazoles **13** and **14**, respectively (Scheme 2). The structures of the synthesized compounds were assigned on the bases of its spectral data and elemental analysis (cf. Section 4 and Table 1).

Condensation of compound **10** with acid anhydrides, namely, 3,4,5,6-tetrachlorophthalic anhydride, 1,2,4,5-benzenetetracarboxylic acid dianhydride and 1,4,5,8-naphthalenetetracarboxylic acid dianhydride in refluxing glacial acetic acid afforded the corresponding imide derivative **15**, benzene-

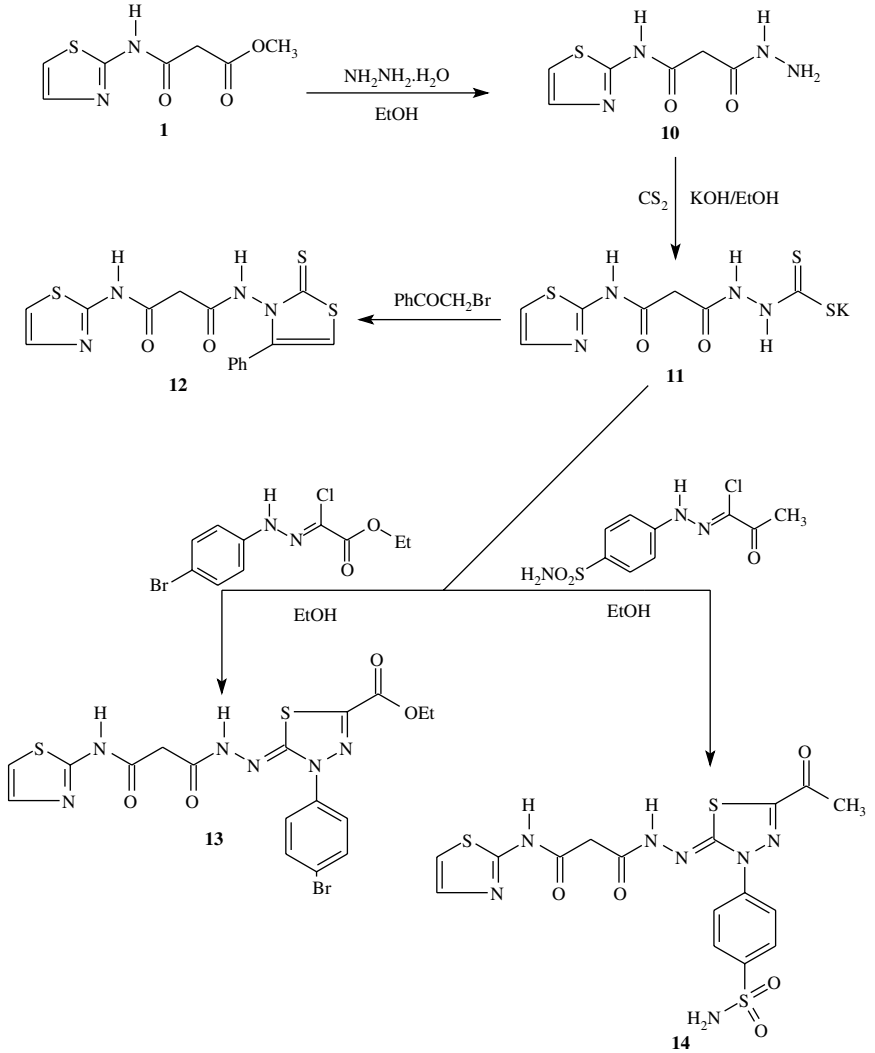


Scheme 1.

Table 1
Physical and analytical data for the synthesized compounds **2–17**

Compound	Molecular formula	MW	M.p. (°C)	Yield (%)	% Analysis of C, H, N, S found (calculated)			
					C	H	N	S
2	$C_{14}H_{13}N_3O_3S_2$	335	99–100	69	50.00 (50.14)	3.79 (3.91)	12.45 (12.53)	19.05 (19.12)
3	$C_{19}H_{17}N_3O_4S_2$	415	192–193	70	54.85 (54.93)	3.98 (4.12)	10.05 (10.11)	15.36 (15.43)
4	$C_{17}H_{15}N_3O_3S_2$	373	184–185	72	54.78 (54.68)	3.88 (4.05)	11.12 (11.25)	17.08 (17.17)
5	$C_{22}H_{17}N_3O_3S_2$	435	151–152	85	60.58 (60.67)	3.84 (3.93)	9.59 (9.65)	14.66 (14.72)
6	$C_{16}H_{13}N_3O_4S_2$	375	157–158	82	51.05 (51.19)	3.41 (3.49)	11.06 (11.19)	17.00 (17.08)
7	$C_{18}H_{15}ClN_4O_5S_2$	466	156–157	82	46.15 (46.30)	3.18 (3.24)	11.88 (12.00)	13.68 (13.73)
8	$C_{17}H_{15}N_5O_6S_3$	481	271–272	69	42.36 (42.41)	3.06 (3.14)	14.48 (14.54)	19.92 (19.97)
9	$C_{25}H_{13}Cl_4N_5O_8S_3$	749	>300	75	39.95 (40.07)	1.67 (1.75)	9.28 (9.35)	12.79 (12.83)
10	$C_6H_8N_4O_2S$	200	218–220	86	35.92 (35.99)	3.98 (4.03)	27.88 (27.98)	15.94 (16.01)
12	$C_{15}H_{12}N_4O_2S_3$	378	186–188	85	47.80 (47.86)	3.18 (3.21)	14.80 (14.88)	25.46 (25.55)
13	$C_{17}H_{15}BrN_6O_4S_2$	511	178–180	54	39.84 (39.93)	2.90 (2.96)	16.38 (16.43)	12.46 (12.54)
14	$C_{16}H_{15}N_7O_5S_3$	481	288–290	76	39.84 (39.91)	3.10 (3.14)	20.28 (20.36)	19.90 (19.97)
15	$C_{14}H_6Cl_4N_4O_4S$	468	>310	90	35.82 (35.92)	1.22 (1.29)	11.89 (11.97)	6.78 (6.85)
16	$C_{22}H_{14}N_8O_8S_2$	582	>310	95	45.28 (45.36)	2.36 (2.42)	19.16 (19.24)	10.95 (11.01)
17	$C_{26}H_{16}N_8O_8S_2$	632	>310	85	49.30 (49.37)	2.50 (2.55)	17.65 (17.71)	10.05 (10.14)

bis-substituted thiazole derivative **16** and naphthalene-bis-substituted thiazole derivative **17**, respectively (Scheme 3). The IR spectra of compounds **15–17** showed the absence of ν (NH_2) at 3308–3177 cm^{-1} of compound **10** and the presence of bands at 1742, 1739 and 1732 cm^{-1} corresponding to ν (C=O, imide ring). The structures of the synthesized compounds were assigned on the bases of its spectral data and elemental analysis (cf. Section 4 and Table 1).



Scheme 2.

2.2. Pharmacological screening

2.2.1. Antiarrhythmic activity

Procaine amide (5 mg/kg i.v.) and lidocaine (5 mg/kg i.v.) lead to an increase in LD_{100} by 65%, which corresponds to an LD_{100} of approximately 9 μg/100 mg (Fig. 1).

From Table 2, one can see that all the tested compounds showed potent antiarrhythmic activities, and compounds **4**, **8**, **11** and **12** are the most potent than procaine amide and lidocaine.

2.2.1.1. Structure–activity relationship (SAR) of antiarrhythmic activities

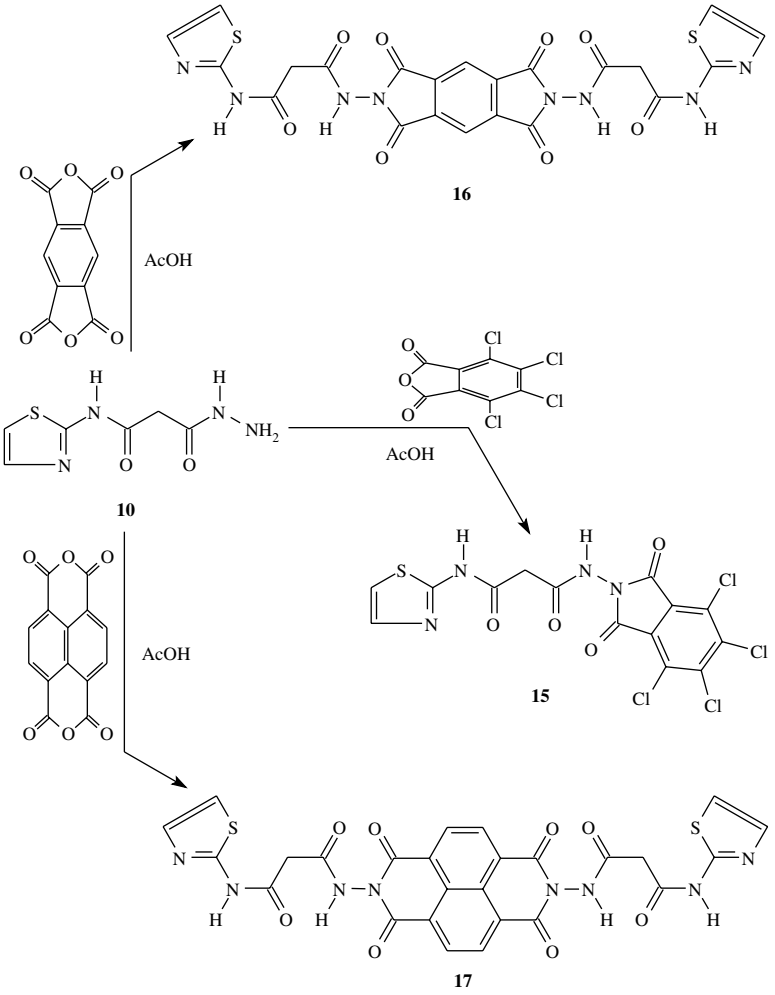
- Thiazole nucleus is essential for activity.
- Increasing the number of sulfur atoms sharply increases the antiarrhythmic activity.

2.2.2. Effects of the tested compounds on coagulation assessed in vitro

A concentration-dependent increase in activated partial thromboplastin time (aPTT) was observed after the addition of the tested compounds to either rabbit or human plasma. In a buffer assay system, compounds **16** and **17** stimulated the inhibition of thrombin by heparin cofactor II (HCII), but was approximately 20 and 40 times, respectively, more active by weight than unfractionated heparin (Fig. 2).

In contrast, all other tested compounds did not stimulate the inhibition of thrombin by antithrombin III (ATIII) over the concentration range of 0.2–2000 μg/mL. In rabbit plasma incubated with increasing concentrations of the tested compounds and trace amounts of ^{125}I-thrombin, covalent ^{125}I-thrombin–heparin cofactor II (HCII) complexes were detected at concentrations $\geq$10 μg/mL, but no ^{125}I-thrombin–antithrombin III (ATIII) complexes were detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography.

2.2.2.1. Effects of the tested compounds on coagulation assessed in vivo. A 1 mg/kg intravenous bolus of compounds **16** and **17** in anesthetized rabbits appeared to represent a threshold dosage by increasing the aPTT 4- and 7-fold, respectively (Fig. 3), with the increase showing some variation but persisting for at least 120 min. Arterial blood pressure



Scheme 3.

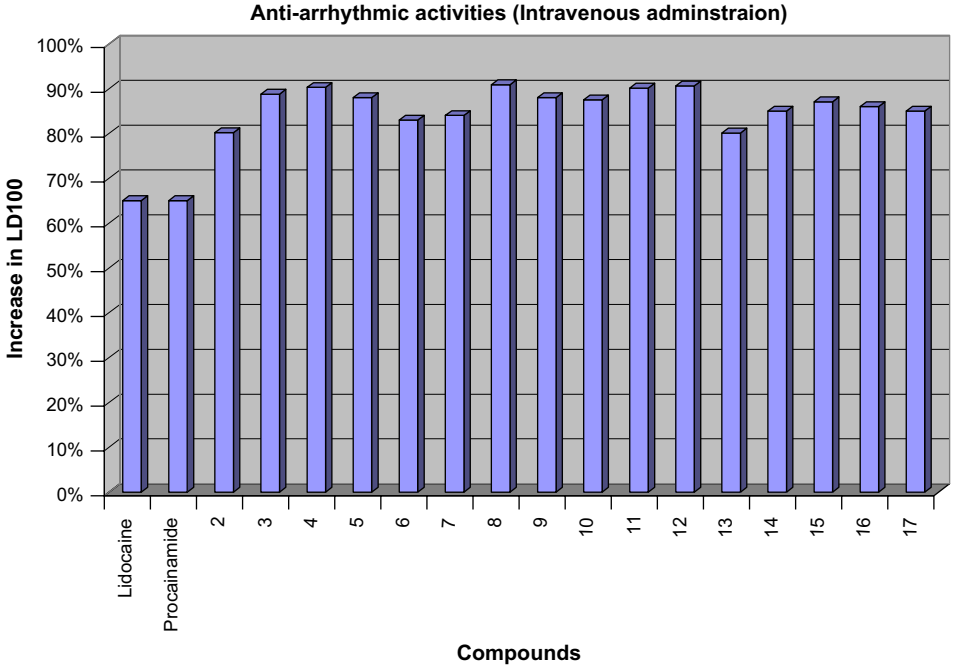


Fig. 1. Antiarrhythmic activities of the newly synthesized compounds.

was unchanged throughout the 2 h observation interval in rabbits with the 1 mg/kg dosage (68.12 mmHg at baseline and 67.4 mmHg after 2 h, $n = 4$).

Oral administration of 1 mg/kg of compounds **16** and **17** in other rabbits that were allowed to regain consciousness after the removal of the orogastric tube resulted in a nearly 2- and 3-fold, respectively, increase in aPTT by 4 h, which was significant compared with baseline values by 8 h. After 12 h, aPTT levels had returned toward baseline levels (Fig. 4).

2.2.2.2. Effect of the tested compounds on thrombosis. The incidence of complete arterial occlusion in the control group was 100% (7 of 7) compared to 42.85% (3 of 7) and 24.57% (2 of 7) in rabbits that received 3 mg/kg of compounds **16** and **17** orally, respectively. However, the average time to occlusion was nearly increased by 10- and 12-fold in compounds **16** and **17** treated animals compared with controls (Fig. 5).

Surgical bleeding time was increased from 96.25 sat. baseline to 315.86 by 6 h (3.28) and to 416.8 by 6 h (4.33) in rabbits treated with compounds **16** and **17**, respectively.

2.2.2.3. Structure–activity relationship (SAR) of anticoagulant and antithrombic activities. Dianhydrides, namely, 1,2,4,5-benzenetetracarboxylic acid dianhydride and 1,4,5,8-naphthalenetetracarboxylic acid dianhydride moieties are essential for anticoagulant and antithrombic activities.

Table 2
Antiarrhythmic activities of the newly synthesized compounds **2–17**

Compound (5 mg/kg)	Percentage increase in LD_{100}
2	80.20
3	88.80
4	90.30
5	88.00
6	83.00
7	84.00
8	90.90
9	88.00
10	87.50
11	90.15
12	90.60
13	80.10
14	85.00
15	87.10
16	86.00
17	85.00

2.2.3. Determination of acute toxicity (LD_{50})

LD_{50} was determined using rats. They were injected with different increasing doses of the synthesized compounds (Table 3). The dose that killed 50% of the animal was calculated according to Austen and Brocklehurst [28].

3. Conclusion

A series of novel thiazolo derivatives were synthesized by initial condensation of methyl 2-(thiazol-2-ylcarbamoyl)acetate with phenyl isothiocyanate and further reactions using different organic reagents. All the synthesized compounds were screened for their antiarrhythmic and anticoagulant activities and they showed high antiarrhythmic activity compared with procaine amide and lidocaine as positive controls. Thiazole nucleus is essential for activity and increasing the number of sulfur atoms sharply increases the antiarrhythmic activity. Also, dianhydrides, namely, 1,2,4,5-benzenetetracarboxylic acid dianhydride and 1,4,5,8-naphthalenetetracarboxylic acid dianhydride moieties are essential for anticoagulant and antithrombic activities.

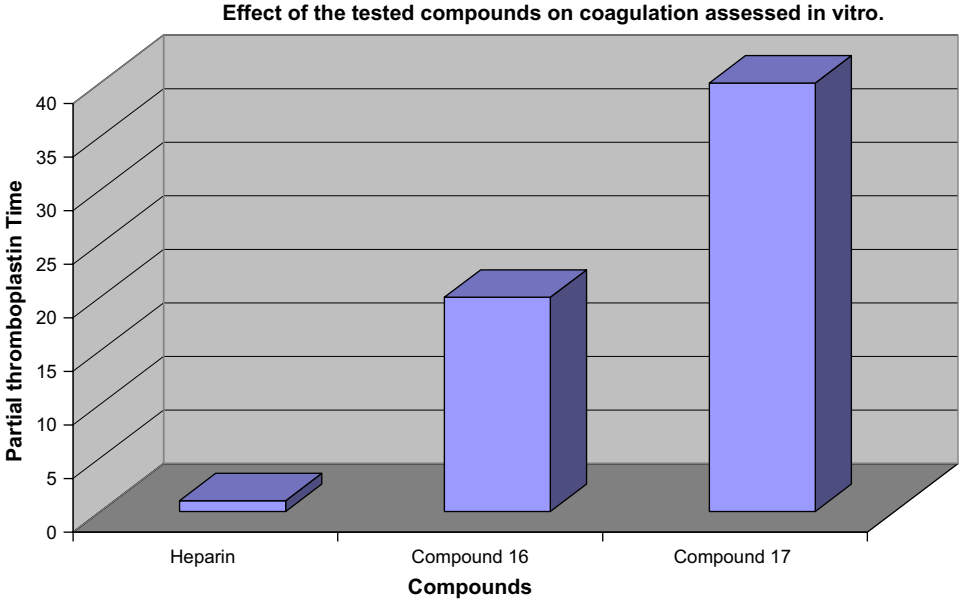


Fig. 2. Effect of the tested compounds on coagulation assessed in vitro.

4. Experimental

4.1. Chemistry

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus and are uncorrected. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Centre, Cairo, Egypt. Infrared (IR) spectra were recorded on Carl Zeise spectrophotometer model "UR 10" spectrophotometer using the KBr disc technique. ^{1}H NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6) and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. The mass spectra (EIMS) were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60 F_{254}, Merck, Darmstadt, Germany) using $CHCl_3$/EtOH, 9:1 (v/v) as eluent and the spots were detected by exposure to UV lamp at λ_{254} (nm) for few seconds. The starting material, methyl 2-(thiazol-2-ylcarbamoyl)acetate **1**, was prepared according to the reported procedures [27].

*4.1.1. 3-Mercapto-3-phenylamino-2-(thiazol-2-ylcarbamoyl)acrylic acid methyl ester (**2**)*

A mixture of sodium hydride (0.36 g, 15 mmol) and methyl 2-(thiazol-2-ylcarbamoyl)acetate **1** (2 g, 10 mmol) in dimethylformamide (30 mL) was stirred at room temperature for 20 min, then phenyl isothiocyanate (1.4 g, 10 mmol) was added with stirring. The reaction mixture was stirred for 6 h

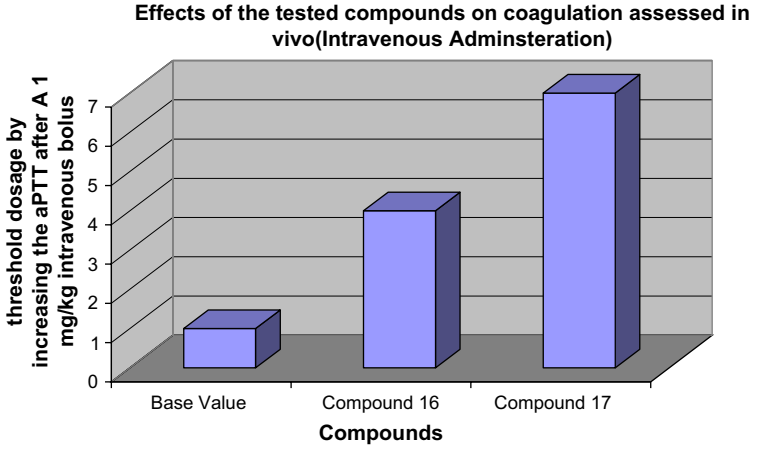


Fig. 3. Effect of the tested compounds on coagulation assessed in vivo (intravenously).

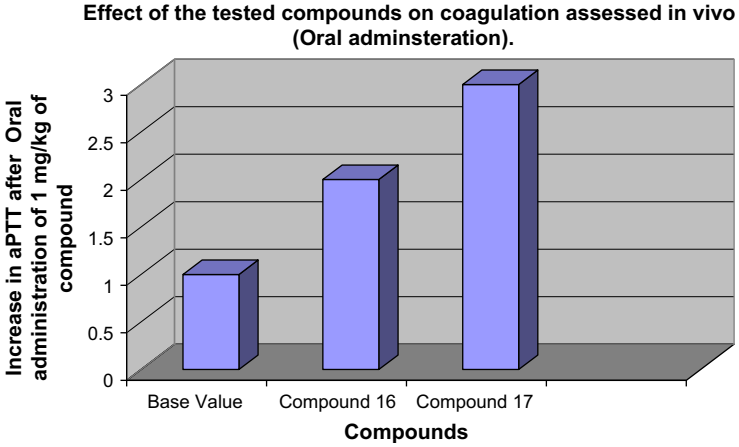


Fig. 4. Effect of the tested compounds on coagulation assessed in vivo (oral administration).

at room temperature and poured onto crushed ice containing hydrochloric acid (pH ~ 7). The obtained solid was filtered off, washed with water, dried and crystallized from ethanol to afford compound **2**. IR (KBr, ν, cm^{-1}): 1679 (C=O, amide), 1740 (C=O, ester), 3152 (NH, amide), 3314 (NH); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 1.23 (s, 1H, SH), 4.21 (s, 3H, OCH_3), 7.09 (d, 1H, $J_{4,5} = 7.80$ Hz, thiazole-H), 7.13 (d, 1H, $J_{5,4} = 7.82$ Hz, thiazole-H), 7.30–7.84 (m, 5H, Ar-H), 11.91 (s, 1H, NH, exchangeable with D_2O), 12.49 (s, 1H, NH, exchangeable with D_2O); EIMS: *m/z* (%) 335 (M^+, 26) and at 243 (100, base peak).

*4.1.2. Synthesis of thiazol-2-ylidene derivatives (**3–6**)*

To a mixture of compound **2** (0.67 g, 2 mmol) and appropriate α-halocarbonyl compounds, namely, 2-chloroacetylacetone, chloroacetone, phenacyl bromide and ethyl bromoacetate (2 mmol) in absolute ethanol (20 mL), triethylamine (0.3 mL) was added portionwise. The reaction mixture was refluxed for 1.5 h then left to cool to room temperature. The formed solid was filtered off, washed with ethanol, and crystallized from DMF/EtOH to afford the corresponding *N*-phenyl thiazole derivatives **3–6**, respectively.

*4.1.2.1. 2-(5-Acetyl-4-methyl-3-phenyl-3H-thiazol-2-ylidene)-N-thiazol-2-yl-malonamic acid methyl ester (**3**).* IR (KBr, ν, cm^{-1}): 1620 (C=O, amide), 1726 (CO, ester), 3069 (NH); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 1.26 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 4.28 (s, 3H, OCH_3), 7.05 (d, 1H, $J_{4,5} = 7.84$ Hz, thiazole-H), 7.09 (d, 1H, $J_{5,4} = 7.81$ Hz, thiazole-H), 7.25–7.35 (m, 5H, Ar-H), 10.81 (s, 1H, NH, exchangeable with D_2O); EIMS: *m/z* (%) 415 (M^+, 12) and at 186 (100, base peak).

*4.1.2.2. 2-(4-Methyl-3-phenyl-3H-thiazol-2-ylidene)-N-thiazol-2-yl-malonamic acid methyl ester (**4**).* IR (KBr, ν, cm^{-1}): 1598 (C=O, amide), 1678 (C=O, ester), 3167 (NH); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 1.25 (s, 3H, CH_3), 4.37 (s, 3H, OCH_3), 6.11 (s, 1H, thiazole-H), 7.08 (d, 1H, $J_{4,5} = 7.79$ Hz, thiazole-H), 7.22 (d, 1H, $J_{5,4} = 7.80$ Hz, thiazole-H), 7.24–7.49 (m, 5H, Ar-H), 11.31 (s, 1H, NH,

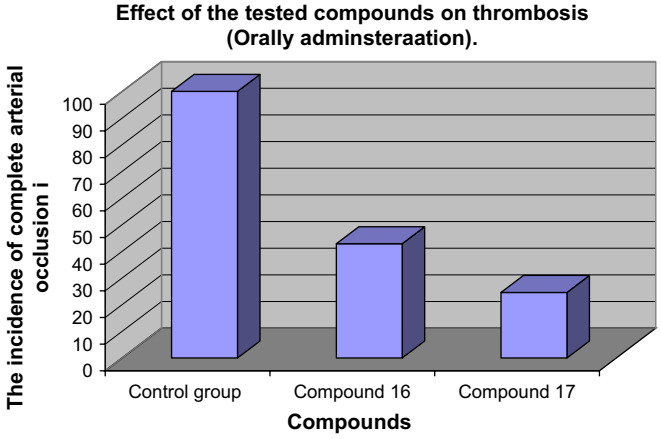


Fig. 5. Effect of the tested compounds on thrombosis.

Table 3
Acute toxicity (LD_{50}) of the newly synthesized compounds **2**–**17**

Compound	LD_{50} (mg/kg)
2	543 ± 0.57
3	674 ± 0.68
4	434 ± 0.46
5	724 ± 0.78
6	444 ± 0.49
7	435 ± 0.47
8	456 ± 0.46
9	564 ± 0.58
10	736 ± 0.75
11	723 ± 0.76
12	292 ± 0.39
13	342 ± 0.39
14	546 ± 0.59
15	443 ± 0.48
16	336 ± 0.38
17	445 ± 0.49

exchangeable with D_2O); EIMS: *m/z* (%) 373 (M^+, 100, base peak), corresponding to the molecular formula $C_{17}H_{15}N_3O_3S_2$.

*4.1.2.3. 2-(3,4-Diphenyl-3H-thiazol-2-ylidene)-N-thiazol-2-yl-malonamic acid methyl ester (**5**).* IR (KBr, ν, cm^{-1}): 1597 (C═O, amide), 1636 (C═O, ester), 3196 (NH); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 4.29 (s, 3H, OCH_3), 6.02 (s, 1H, thiazole-H), 7.12 (d, 1H, $J_{4,5} = 7.53$ Hz, thiazole-H), 7.20 (d, 1H, $J_{5,4} = 7.55$ Hz, thiazole-H), 7.31–7.73 (m, 10H, Ar-H), 11.49 (s, 1H, NH, exchangeable with D_2O); EIMS: *m/z* (%) 435 (M^+, 16) and at 404 (100, base peak).

*4.1.2.4. 2-(4-Oxo-3-phenyl-thiazolidin-2-ylidene)-N-thiazol-2-yl-malonamic acid methyl ester (**6**).* IR (KBr, ν, cm^{-1}): 1577 (C═O, amide), 1621 (C═O, ring), 1728 (C═O, ester), 3068 (NH); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 4.18, 4.29 (dd, 2H, thiazolidinone-H), 4.38 (s, 3H, OCH_3), 7.02 (d, 1H, $J_{4,5} = 8.7$ Hz, thiazole-H), 7.09 (d, 1H, $J_{5,4} = 8.65$ Hz, thiazole-H), 7.25–7.95 (m, 5H, Ar-H), 9.29 (s, 1H, NH, exchangeable with D_2O); EIMS: *m/z* (%) 375 (M^+, 6) and at 273 (100, base peak).

*4.1.3. Synthesis of thiadiazole derivatives **7** and **8***

A mixture of compound **2** (0.67 g, 2 mmol) and appropriate hydrazonyl chlorides, namely, ethyl 2-[(4-chlorophenyl)hydrazono]-2-chloroacetate and 1-[(2-(4-sulfonamido)phenyl)-hydrazono]-1-chloropropan-2-one (2 mmol) in absolute ethanol (20 mL) in the presence of triethylamine (0.3 mL) was refluxed for 1 h. After cooling, the formed solid was filtered off, washed with ethanol, dried and crystallized from DMF/EtOH to afford the corresponding thiadiazoles **7** and **8**, respectively.

*4.1.3.1. 4-(4-Chlorophenyl)-5-[methoxycarbonyl-(thiazol-2-yl-carbamoyl)methylene]-4,5-dihydro-[1,3,4]thiadiazole-2-carboxylic acid ethyl ester (**7**).* IR (KBr, ν, cm^{-1}): 1679 (C═O, amide), 1713, 1725 (C═O, ester), 3168 (NH); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 1.33 (t, 3H, CH_3), 3.08 (s, 3H, OCH_3), 4.22 (q, 2H, CH_2), 6.69 (d, 1H, $J_{4,5} = 8.11$ Hz, thiazole-H), 7.17 (d, 1H, $J_{5,4} = 8.15$ Hz, thiazole-H), 7.42 (d, 2H, $J = 7.65$ Hz, Ar-H), 7.62 (d, 2H, $J = 7.58$ Hz, Ar-H), 10.95 (s, 1H, NH, exchangeable with D_2O); EIMS: *m/z* (%) 468 ($M^+ + 2$, 36) and at 386 (100, base peak).

*4.1.3.2. 2-[5-Acetyl-3-(4-sulfamoyl-phenyl)-3H-[1,3,4]thiadiazol-2-ylidene]-N-thiazol-2-yl-malonamic acid methyl ester (**8**).* IR (KBr, ν, cm^{-1}): 1326 (S═O), 1595 (C═O, amide), 1727 (C═O, ester), 1717 (C═O), 3352–3234 (NH_2, NH); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 3.12 (s, 3H, CH_3), 4.31 (s, 3H, OCH_3), 7.20 (d, 1H, $J_{4,5} = 8.10$ Hz, thiazole-H), 7.44 (d, 1H, $J_{5,4} = 8.12$ Hz, thiazole-H), 7.61 (d, 2H, $J = 7.83$ Hz, Ar-H), 7.77 (d, 2H, $J = 7.84$ Hz, Ar-H), 10.22 (s, 1H, NH, exchangeable with D_2O), 11.22 (s, 2H, NH_2, exchangeable with D_2O); EIMS: *m/z* (%) 481 (M^+, 18) and at 315 (100, base peak).

*4.1.4. 2-{5-Acetyl-3-[4-(4,5,6,7-tetrachloro-1,3-dioxo-1,3-dihydro-isoindole-2-sulfonyl)phenyl]-3H-[1,3,4]thiadiazol-2-ylidene}-N-thiazol-2-yl-malonamic acid methyl ester (**9**)*

A mixture of compound **8** (0.48 g, 1 mmol) and 3,4,5,6-tetrachlorophthalic anhydride (0.29 g, 1 mmol) in glacial acetic acid (50 mL) was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure; the obtained solid was filtered off and crystallized from AcOH/H_2O to yield the imide derivative **9**. IR (KBr, ν, cm^{-1}): 1327 (S═O), 1661 (C═O, amide), 1733 (C═O, ester), 1710 (C═O), 3233 (NH); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 2.55 (s, 3H, CH_3), 4.35 (s, 3H, OCH_3), 6.95 (d, 1H, $J_{4,5} = 8.21$ Hz, thiazole-H), 7.10 (d, 1H, $J_{5,4} = 8.18$ Hz, thiazole-H), 7.65 (d, 2H, $J = 7.85$ Hz, Ar-H), 7.85 (d, 2H, $J = 7.84$ Hz, Ar-H), 11.23 (s, 1H, NH, exchangeable with D_2O); EIMS: *m/z* (%) 749 (M^+, 12) and at 241 (100, base peak).

*4.1.5. 2-Hydrazinocarbonyl-N-thiazol-2-yl-acetamide (**10**)*

A mixture of ester **1** (2 g, 10 mmol) and hydrazine hydrate (3.5 mL, 80 mmol) in absolute ethanol (50 mL) was refluxed for 1 h. The precipitated solid was filtered off, dried and crystallized from ethanol to afford the corresponding hydrazide **10**. IR (KBr, ν, cm^{-1}): 3330–3280 (NH, NH_2), 1682, 1675 (2C═O), 1654 (C═N); ^1H NMR (DMSO-d_6, 270 MHz) δ (ppm): 3.82 (s, 2H, CH_2), 4.85 (br s, 2H, NH_2, exchangeable with D_2O), 7.24 (d, 1H, $J_{4,5} = 7.31$ Hz, thiazole-H), 7.28 (d, 1H, $J_{5,4} = 7.30$ Hz, thiazole-H), 11.35 (s, 1H, NH, exchangeable with D_2O), 12.15 (s, 1H, NH, exchangeable with D_2O); EIMS: *m/z* (%) 200 (M^+, 100, base peak).

*4.1.6. Hydrazinecarbodithionic acid potassium salt (**11**)*

To a mixture of hydrazide **10** (2 g, 10 mmol) in ethanol (50 mL) and ethanolic potassium hydroxide (0.84 g, 15 mmol, 50 mL), carbon disulfide (5 mL) was added. The reaction mixture was heated under reflux for 3 h, the solid formed was filtered off and dried to afford potassium salt of compound **11** in 85% yield. IR (KBr, ν, cm^{-1}): 3380–3100 (3 NH), 1655 (C═O).

*4.1.7. N-(4-Phenyl-2-thioxothiazol-3-yl)-N′-thiazol-2-yl-malonamide (**12**)*

A mixture of potassium salt of compound **11** (0.63 g, 2 mmol) and phenacyl bromide (0.4 g, 2 mmol) in ethanol (20 mL) was heated under reflux for 3 h. The formed solid was collected by filtration, washed with ethanol, dried and crystallized from methanol to give the corresponding thiazole derivative **12**. IR (KBr, ν, cm^{-1}): 1043 (C=S), 1688 (C=O, amide), 3188 (NH); ^{1}H NMR (DMSO-d_6, 270 MHz) δ (ppm): 3.34 (s, 2H, CH_2), 6.98 (d, 1H, $J_{4,5} = 7.78$ Hz, thiazole-H), 7.08 (d, 1H, $J_{4,5} = 7.76$ Hz, thiazole-H), 7.26–7.44 (m, 5H, Ar-H), 8.18 (s, 1H, CH), 10.12 (s, 1H, NH, exchangeable with D_2O), 11.28 (s, 1H, NH, exchangeable with D_2O); EIMS: *m/z* (%) 378 (M^+, 10) and at 341 (100, base peak).

*4.1.8. 4-(4-Bromophenyl)-5-{[2-(thiazol-2-ylcarbamoyl)-acetyl]-hydrazono}-4,5-dihydro[1,3,4]-thiadiazole-2-carboxylic acid ethyl ester (**13**)*

To a solution of potassium salt of compound **11** (0.63 g, 2 mmol) in ethanol (20 mL), ethyl 2-[2-(4-bromo-phenyl)hydrazono]-2-chloroacetate (0.61 g, 2 mmol) was added. The reaction mixture was heated under reflux for 4 h; after cooling, the precipitated solid was collected by filtration, washed with ethanol and crystallized from ethanol to afford compound **13**. IR (KBr, ν, cm^{-1}): 1634 (C=O, amide), 1678 (C=O), 3168 (NH); ^{1}H NMR (DMSO-d_6, 270 MHz) δ (ppm): 1.20 (t, 3H, CH_3), 3.37 (s, 2H, CH_2), 4.18 (q, 2H, CH_2), 3.37 (s, 2H, CH_2), 7.18 (d, 1H, $J_{4,5} = 7.76$ Hz, thiazole-H), 7.25 (d, 1H, $J_{4,5} = 7.75$ Hz, thiazole-H), 7.38 (d, 2H, $J = 7.78$ Hz, Ar-H), 7.54 (d, 2H, $J = 7.79$ Hz, Ar-H), 10.94, 11.31 (2s, 2H, 2NH, exchangeable with D_2O); EIMS: *m/z* (%) 511 (M^+, 14) and at 386 (100, base peak).

*4.1.9. 2-[5-Acetyl-3-(4-sulfamoyl-phenyl)-3H-[1,3,4]thiadiazol-2-ylidenehydrazinocarbonyl]-N-thiazol-2-yl-acetamide (**14**)*

A mixture of potassium salt of compound **11** (0.63 g, 2 mmol) and 1-[(2-*p*-sulfonamido-phenyl)-hydrazono]-1-chloropropan-2-one (0.55 g, 2 mmol) in ethanol (20 mL) was refluxed for 1 h. The formed solid was filtered off, dried and crystallized from methanol to afford compound **14**. IR (KBr, ν, cm^{-1}): 1330 (S=O), 1667 (C=O, amide), 1685 (C=O), 3232, 3208 (NH_2); ^{1}H NMR (DMSO-d_6, 270 MHz) δ (ppm): 2.61 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 7.10 (d, 1H, $J_{4,5} = 8.21$ Hz, thiazole-H), 7.21 (d, 1H, $J_{4,5} = 8.18$ Hz, thiazole-H), 7.62 (d, 2H, $J = 7.84$ Hz, Ar-H), 7.86 (d, 2H, $J = 7.82$ Hz, Ar-H), 10.90 (s, 1H, NH, exchangeable with D_2O), 11.18 (s, 1H, NH), 12.29 (s, 2H, NH_2, exchangeable with D_2O); EIMS: *m/z* (%) 481 (M^+, 4) and at 236 (100, base peak).

*4.1.10. N-(4,5,6,7-Tetrachloro-1,3-dioxo-1,3-dihydro-isoindol-2-yl)-N′-thiazol-2-yl-malonamide (**15**)*

Compound **15** was synthesized by using the same procedure of synthesis of derivative **9** but using compound **10** as a starting material. IR (KBr, ν, cm^{-1}): 1671 (C=O), 1742 (C=O, ring), 3230 (NH); ^{1}H NMR (DMSO-d_6, 270 MHz) δ (ppm): 3.84 (s, 2H, CH_2), 7.22 (d, 1H, $J_{4,5} = 7.54$ Hz, thiazole-H), 7.26 (d, 1H, $J_{5,4} = 7.55$ Hz, thiazole-H), 10.38, 11.15 (2s, 2H, 2 NH, exchangeable with D_2O); EIMS: *m/z* (%) 468 (M^+, 8) and at 299 (100, base peak).

*4.1.11. Synthesis of bis-imide derivatives **16** and **17***

A mixture of compound **10** (0.4 g, 2 mmol) and dianhydrides, namely, 1,2,4,5-benzenetetracarboxylic acid dianhydride and 1,4,5,8-naphthalenetetracarboxylic acid dianhydride (1 mmol) in glacial acetic acid (50 mL) was heated under reflux for 6 h. The obtained solid was filtered off and crystallized to yield the corresponding bis-imide derivatives **16** and **17**.

*4.1.11.1. N-{1,3,5,7-Tetraoxo-6-[2-(thiazol-2-ylcarbamoyl)acetylamino]-3,5,6,7-tetrahydro-1H-pyrrolo[3,4-f]isoindol-2-yl}-N′-thiazol-2-yl-malonamide (**16**).* IR (KBr, ν, cm^{-1}), 1678 (C=O, amide), 1739 (C=O, ring), 3163 (NH); ^{1}H NMR (DMSO-d_6, 270 MHz) δ (ppm): 3.78 (s, 2H, CH_2), 7.08 (d, 1H, $J_{4,5} = 7.78$ Hz, thiazole-H), 7.16 (d, 1H, $J_{5,4} = 7.80$ Hz, thiazole-H), 7.86 (s, 2H, Ar-H), 10.90, 11.15 (2s, 4NH, exchangeable with D_2O); EIMS: *m/z* (%) 582 (M^+, 22) and at 386 (100, base peak).

*4.1.11.2. N-{1,3,6,8-Tetraoxo-7-[2-(thiazol-2-ylcarbamoyl)-acetylamino]-3,6,7,8-tetrahydro-1H-benzo[lmn][3,8]phenanthrolin-2-yl}-N′-thiazol-2-yl-malonamide (**17**).* IR (KBr, ν, cm^{-1}): 1686 (C=O, amide), 1732 (C=O, ring), 3279 (NH); ^{1}H NMR (DMSO-d_6, 270 MHz) δ (ppm): 3.78 (s, 2H, CH_2), 7.08 (d, 1H, $J_{4,5} = 7.76$ Hz, thiazole-H), 7.16 (d, 1H, $J_{5,4} = 7.75$ Hz, thiazole-H), 7.46–7.88 (m, 4H, Ar-H), 10.94, 11.10 (2s, 4NH, exchangeable with D_2O); EIMS: *m/z* (%) 632 (M^+, 14) and at 186 (100, base peak).

4.2. Pharmacological activity

4.2.1. Antiarrhythmic activity [29–34]

4.2.1.1. Purpose and rational. The plant alkaloid aconitine persistently activates sodium channel. Infusion of aconitine into the anesthetized rat causes ventricular arrhythmias. Drugs that are considered to have antiarrhythmic properties can be tested in aconitine-intoxicated rats.

4.2.1.2. Procedure. Male Ivanovas rats weighing 300–350 g are used. The animals are anesthetized by intraperitoneal injection of 1.25 g/kg urethane: 5 mg/kg aconitine dissolved in 0.1 N HNO_3 is administered by continuous infusion into the saphenous vein (0.1 mL/min) and the electrocardiogram (ECG) in lead II is recorded every 30 s. The test compound is injected i.v. at a screening dose of 3 mg/kg, 5 min before the start of the aconitine infusion; 24 animals are used per compound.

4.2.1.3. Evaluation. The antiarrhythmic effect of a test compound is measured by the amount of aconitine/100 g of animal. (Duration of infusion) which induces.

- Ventricular extra systoles.
- Ventricular tachycardia.
- Ventricular fibrillation.

Higher doses of aconitine in the treated group as compared to an untreated control group are an indication of antiarrhythmic activity.

Statistical significance between the groups is assessed by the Student's *t*-test.

4.2.2. Anticoagulant activity

4.2.2.1. In vitro experiments [35]. The tested compounds were tested for anticoagulant activity in both human and rabbit plasma. Blood was withdrawn from the antecubital vein of consenting, healthy human volunteers and from the central ear artery of unanesthetized rabbits. The blood was placed in tubes containing 3.8% sodium citrate (one part citrate to nine parts blood) and centrifuged at $1000 \times g$ for 10 min. A stock solution (1.5 mg/mL) of the tested compounds was prepared and diluted serially with pancreas and blood sugar (PBS). A 10-mL aliquot of diluted tested compounds or 10 μL of PBS as a control was added to 100 μL of plasma for assay of aPTT as described below. aPTT for each dilution was measured in triplicate and the results averaged. Concentration versus aPTT experiments were repeated for both rabbit and human plasma obtained from three different individuals.

To investigate the basis for the anticoagulant activity of the tested compounds, we tested its ability to stimulate inhibition of thrombin by HCII or ATIII. Human α-thrombin (10 nM, Haematologic Technologies, Essex Junction, VT), HCII (29 nM) or ATIII (51 nM) purified from human plasma as described previously and the tested compounds or heparin (Sigma Chemical Co., St. Louis, MO) at the final concentrations indicated were incubated in 0.1 mL of 0.02 M Tris–HCl containing 0.15 M NaCl and 1 mg/mL polyethylene glycol at pH 7.4 (TS buffer). Thrombin was added last to initiate the reaction.

After 1 min, residual thrombin activity was determined by the addition of 0.5 mL tosyl-Gly-Pro-Arg-*p*-nitroanilide (Boehringer Mannheim, Indianapolis, IN) followed by the measurement of the rate of change in absorbance at 405 nm.

To determine whether the tested compounds stimulated inhibition of thrombin by HCII or ATIII in plasma ex vivo, aliquots (2.5 μL) of citrated rabbit plasma were incubated in 50 μL of TS buffer for 1 min with several concentrations of the tested compounds and a trace amount of ^{125}I labeled thrombin (0.3 pmol; 13,000 cpm) prepared as described previously [35]. ^{125}I-thrombin–HCII and ^{125}I-thrombin–ATIII complexes were then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and detected by autoradiography as described [36].

4.2.2.2. Animal preparations. All procedures involving animals were in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Centre, Cairo, Egypt). New Zealand white rabbits of either sex weighing 3.0–3.8 kg were fasted for 12–24 h and anesthetized with an intramuscular injection of 50 mg/kg of ketamine and 18 mg/kg of xylazine, supplemented as needed. Body temperature was maintained by placing the animals on a heating pad. A 22-gauge catheter (Surflo, Terumo Medical Co., Elkton, MD) was inserted into a femoral vein or a pediatric feeding tube (Davol, Bard Co., Cranston, RI) was inserted through the mouth and into the stomach for delivery of the tested compounds or PBS vehicle. A catheter was inserted into a femoral artery or a jugular vein for the withdrawal of blood samples. Blood pressure was monitored continuously in those animals with an arterial catheter.

4.2.2.3. In vivo experiments [36]. To determine the effect of the tested compounds on coagulation after i.v. administration, the selected dosage dissolved in 5 mL of PBS was infused as a bolus into the femoral vein. Serial arterial blood samples were withdrawn before and 1, 5, 10, 30, 60 and 120 min after drug infusion for analysis of aPTT.

To determine the effect of the tested compounds on coagulation after oral administration, a baseline blood sample was collected from the jugular vein and the selected dosage dissolved in 5 mL of PBS was injected as a bolus into the stomach. The feeding tube was immediately withdrawn to avoid regurgitation. However, if regurgitation occurred, the experiment was stopped and the rabbit euthanized. Blood samples were collected before and 1, 2, 4, 8, 12 and 24 h after the administration of the tested compounds for analysis of aPTT. Rabbits regained consciousness after 1–2 h, and were returned to their cages after 4 h and permitted access to food and water.

To assess the effect of the tested compounds administered orally on in vivo thrombosis, preparations described previously for arterial and venous thrombosis were implemented simultaneously in the same animals [37]. Briefly, after a 24-h fast and induction of anesthesia, an oral dosage of the tested compounds shown to increase aPTT 2-fold or PBS vehicle as a control was administered. For induction of arterial thrombosis, a common carotid artery was exposed and instrumented with a proximal Doppler flow probe and a distal transluminal needle electrode consisting of the tip of a 23-gauge needle crimped on the end of 30-gauge Teflon-insulated silver-coated copper wire (A-M Systems, Everett, WA). To avoid induction of cerebral infarction from embolism with thrombus, an arteriovenous shunt consisting of a 5-cm length of silanized polypropylene tubing (1.57 mm I.D., 2.41 mm O.D.) was inserted between the carotid artery and the jugular vein. Mean flow velocity was decreased to 50% of the baseline level by tightening the ligature used to anchor the electrode on either side of the vessel. Five hours after oral administration of the tested compounds, identified as the interval needed to achieve a 2-fold increase in aPTT, electrical injury to the carotid artery was initiated by application of 250 mA of anodal current to the indwelling electrode for 2 h.

For induction of venous thrombosis, a 5-cm silanized polypropylene tube (1.57 mm I.D., 2.41 mm O.D) was advanced via a jugular vein into the superior vena cava. A preweighed 7 cm length of 34-gauge copper wire, looped at one end and with eight, 3 cm strands of cotton thread attached was then pushed through the catheter into the vena cava exposing the threads to the circulation. The catheter was flushed and clamped closed with a hemostat. Initiation of thrombosis

was considered to begin at the time the wire with threads was advanced into the vessel, which coincided with the onset of electrical current in the carotid artery.

Surgical bleeding time was measured and arterial blood samples were withdrawn before and 5, 6 and 7 h after oral administration of the tested compounds or PBS vehicle for assay of aPTT. After the 7 h sample, the resulting venous thrombi were removed, blotted dry and weighed. The effect of the tested compounds on arterial thrombosis was assessed by measuring the frequency of cyclic flow variations resulting from transient accumulation and dislodgment of platelets at the site of electrical injury. The incidence and time of onset of complete arterial occlusion, if it occurred, were also recorded. *aPTT and surgical bleeding time*: aPTT was measured with use of a Coag-A-Mate XM automated coagulation timer (Organon Teknika, Durham, NC). A sample of 100 μL of citrated plasma was warmed for 60 s to 37 °C and then 100 μL of prewarmed activator reagent (Automated APTT, Organon Teknika) was added and allowed to incubate for 300 s. Prewarmed 20 mM calcium chloride (100 mL) was then added and aPTT was recorded as the time for clot formation. Making an incision in the edge of the ear with a scalpel blade and immersing the ear in warmed saline assessed surgical bleeding time. The time required for bleeding to stop was taken as the bleeding time.

4.2.2.4. Statistical analysis. Results are expressed as the mean + S.D. Interval changes in aPTT were compared between the tested compounds-treated and controls by multiple analysis of variance. An unpaired Student's *t*-test was used to compare the incidence and time of onset of arterial thrombosis and the weight of venous thrombus between the tested compounds-treated and the control groups. A value of P 0.05 was considered significant.

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