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New series of 6-substituted coumarin derivatives as effective factor Xa inhibitors: Synthesis, *in vivo* antithrombotic evaluation and molecular docking

Kamelia M. Amin, Nagwa M. Abdel Gawad, Doaa E. Abdel Rahman *, Mohamed K.M. El Ashry

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt

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ABSTRACT

Despite recent progress in antithrombotic therapy, there's still an unmet medical need for safe and orally available anticoagulants. Encouraged by the marked antithrombotic and anticoagulant activities of some coumarin derivatives, twenty-three new *N*-coumarinyl-4-amidinobenzamides **4a**–**f** and 6-heterocycle substituted coumarin derivatives **5**, **6a**,**b**, **10a**–**e**, **12a**–**e** and **14a**–**d** were synthesized and evaluated for their *in vivo* antithrombotic activity. The most active congeners were the unsubstituted amidine **4a** (36.5 s), coumarinyl oxadiazole **5** (42.3 s), bis coumarinyl oxadiazole **6b** (37.8 s) and coumarinyl pyrazole **10b** (38.5 s) that presented prothrombin time (PT) values comparable to the reference drug warfarin (42.3 s). Furthermore, docking studies were undertaken to gain insight into the possible binding mode of these compounds with the coagulation factor Xa (FXa) binding site.

1. Introduction

Hemostasis comprises the normal mechanisms that prevent blood loss from sites of vascular injury. Dysregulated hemostatic activity is believed to contribute to thrombotic diseases such as pulmonary embolism, myocardial infarction (MI) and stroke. Although considerable progress has been made to prevent or treat these diseases, thrombosis remains a major health problem in the developing countries and industrialized world, and the primary cause of morbidity and mortality [1].

Heparins, antithrombin dependent inhibitors, and vitamin K antagonists (principally warfarin) have been widely used in anticoagulant therapy. Despite their long history of use, their use is associated with problems such as difficulty in controlling their anticoagulant activity and their adverse effect on bleeding [2–4]. These limitations have provided the impetus for the development of newer antithrombotic agents with either superior efficacy or a better biosafety profile.

One attractive approach is the inhibition of FXa which is directly responsible for thrombin generation. FXa inhibitors can decrease the amplified generation of thrombin without decreasing the levels necessary to the primary hemostasis [5]. There is a major thrust on the development of orally bioavailable anti-FXa agents, which are slated to replace oral anticoagulants.

Coumarins are a large group of compounds that have been reported to possess a wide range of biological activities [6–13], including anticoagulant and antithrombotic properties [14–19]. Many coumarin derivatives especially 4-hydroxycoumarin displayed a significant anticoagulant action through antagonizing vitamin K action, e.g. warfarin (Fig. 1), phenprocoumon and acenocoumarol [3].

Furthermore, 6-substituted coumarin-3-carboxamides were reported as thrombin and FXa inhibitors [15]. These compounds were found to act as mechanism-based inhibitors through the nucleophilic attack by the activated hydroxyl group of Ser195 on the lactone moiety, forming the acyl-enzyme, leading to irreversible inactivation of the enzyme [15]. 3,6-Disubstitued analogues were also found to exhibit very potent thrombin inhibition [16]. Moreover a number of benzocoumarin amides [17] and pyrimidinocoumarins [19] have been recognized as potent antithrombotics and antiplatelet agents.

Literatures revealed that, many amidine containing compounds possess significant serine proteases FXa and thrombin inhibitory effects and anticoagulant activities [20–23]. It has also suggested that the amidine moiety acts as a replacement for the guanidine group that confers specificity for the active site S1 binding pocket of FXa [24]. Ximelagatran (Fig. 1) is an oral direct thrombin inhibitor containing an amidine moiety that used for prophylaxis against and treatment of thromboembolism [25]. Recently, a series of monobenzamidine-based FXa inhibitors has been shown to have a potent anticoagulant activity and high efficacy in a deep vein thrombosis (DVT) model [26].

* Corresponding author.
E-mail addresses: doaaezzat2004@yahoo.com, doaaezzat2004@cu.edu.eg (D.E. Abdel Rahman).

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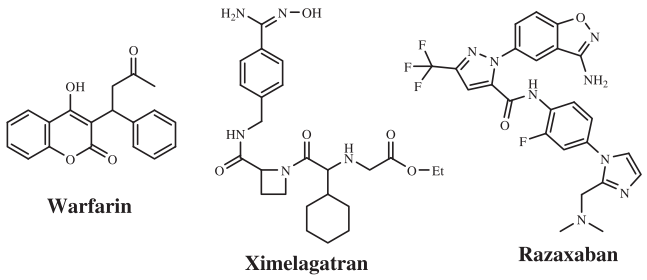


Fig. 1. Anticoagulant and FXa inhibitors.

It was observed that, heteroaromatic rings would be good neutral motifs for binding to the FXa S1 pocket as inhibitors with typical basic S1 binding elements such as amidines suffer often from poor bioavailability [27,28].

Pyrazole [14,29] and imidazole [28,29] moieties were present in several anticoagulant candidate, e.g. the FXa inhibitor clinical candidate razaxaban (Fig. 1). Furthermore, many reports showed that the pyridine [17] and pyrimidine [30] nuclei act as potential source of new antithrombotics.

Based on the above cited findings, we selected coumarin as the main scaffold for the design and synthesis of novel compounds as potent anticoagulants with safety profile.

Taking in consideration the presence of carboxamide groups in many FXa inhibitor agents [15,17,26,29], we aimed to design a set of novel compounds that have in addition to the main scaffold coumarin, carboxamide and benzamidine entities in one molecule as potential antithrombotic agents. Such conjugate structure might lead to a synergistic antithrombotic agents with similar efficacy or greater than other anticoagulant drugs and with high safety margin. Thus, a series of *N*-coumarinyl-4-amidinobenzamides **4a**–**f** was synthesized for *in vivo* antithrombotic screening.

In addition, with the aim of identifying new anticoagulant agents with improved pharmacokinetic and safety profile, it was of interest to design and synthesize 6-heterocycle substituted coumarins **5**, **6a**,**b**, **10a**–**e**, **12a**–**e** and **14a**–**d** as structure hybrids comprising basically a bioactive heterocyclic ring attached at the 6-position of the coumarin scaffold through various linkages. Such hybrid structure may lead to compounds with interesting biological profiles. All the newly synthesized compounds were tested for anticoagulant activity through determination of clotting time (CT) and prothrombin time (PT). In addition, attempt to elucidate a molecular target for activity was achieved via molecular docking of the prepared compounds in the active site of factor Xa using molecular operating Environment (MOE).

2. Experimental

2.1. Chemistry

Melting points were determined by open capillary tube method using Electrothermal 9100 melting point apparatus MFB-595-010M (Gallen Kamp, London, England) and were uncorrected. Microanalyses were carried out at The Regional Center for Mycology and Biotechnology, Al-Azhar University and the micro analytical center, Faculty of Science, Cairo University. Infrared Spectra were recorded as potassium bromide discs on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan) and Bruker FT-IR spectrophotometer and expressed in wave number υ_{max} (cm^{-1}). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer where ^1H spectra were run at 300 MHz and *JEOL-ECA500 NMR spectrometer where ^1H spectra were run at 500 MHz and ^{13}C spectra were run at 125 MHz in dimethylsulphoxide (DMSO-d_6). Chemical Shifts are quoted in δ as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard and *J* values are reported in Hz. Mass spectra were performed as EI at 70 eV on Fennigan MAT, SSQ 7000 mass spectrophotometer, Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX and Direct inlet unit of Shimadzu GC/MS-QP5050A. TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents was chloroform/methanol 9.5:0.5 and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

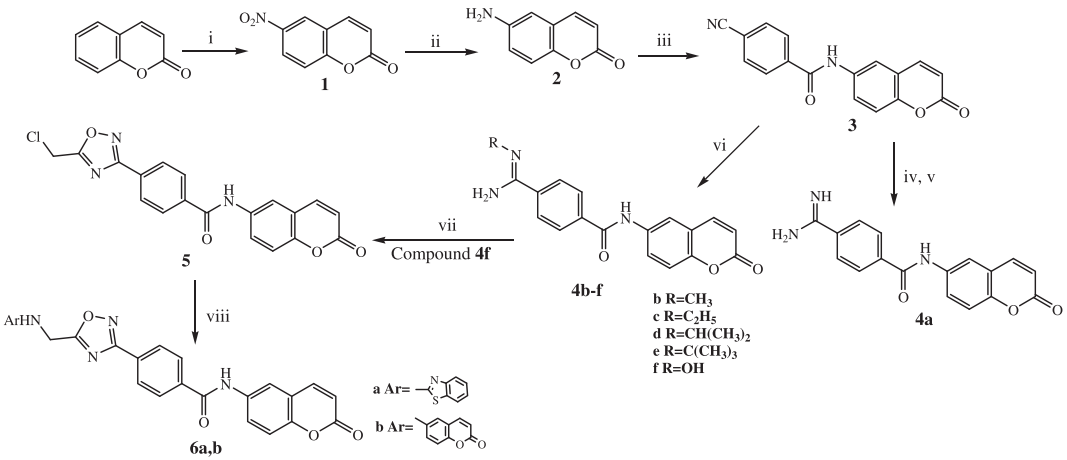
2.1.1. The synthesis of 6-nitrocoumarin **1** and 6-aminocoumarin **2** (Scheme 1) were prepared as reported in literature [31–33].

2.1.2. Synthesis of 4-cyano-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **3** (Scheme 1). To a cooled (−10 °C) solution of amine compound **2** (1.61 g, 10 mmol) and few drops triethylamine in dichloromethane (50 ml), 4-cyanobenzoyl chloride (1.65 g, 10 mmol) was added stepwise and the mixture stirred for 6 h. During stirring, the temperature was allowed to rise to room temperature. The product precipitated from the reaction mixture, filtered off and washed with a small portion of dichloromethane. It was crystallized from ethanol. Yield 70%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3287 (NH), 3088 (CH arom.), 2225 (CN), 1719, 1653 (2 CO) 1616, 1572, 1545 (NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 6.51 (d, 1H, *J* = 9.3 Hz, H-3 Ar), 7.43 (d, 1H, *J* = 9.0 Hz, H-8 Ar), 7.87 (d, 1H, *J* = 9.0 Hz, H-4 Ar), 8.03 (d, 1H, *J* = 8.1 Hz, H-7 Ar), 8.09 (s, 1H, H-5 Ar), 8.13 (d, 2H, *J* = 3.0 Hz, H-3′,5′ Ar), 8.21 (d, 2H, *J* = 2.4 Hz, H-2′,6′ Ar), 10.67 (s, 1H, NH, exchanged with D_2O). MS *m/z* (%): 290, M^+ (19.37%). Anal. Calcd. for $C_{17}H_{10}N_2O_3$ (290.27): C, 70.34; H, 3.47; N, 9.65. Found: C, 70.48; H, 3.60; N, 9.35.

2.1.3. Synthesis of 4-[amino (imino) methyl]-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **4a** (Scheme 1). Into an ice-cooled solution of nitrile compound **3** (0.58 g, 2 mmol) in absolute ethanol (50 ml), gaseous HCl was bubbled until complete saturation was achieved (~30 min). The mixture was stirred for 24 h at room temperature and solvent was then evaporated under vacuum. The residue was dispersed in diethyl ether (20 ml), filtered and washed twice with diethyl ether (20 ml). The crude residue was dissolved in absolute ethanol (20 ml), ammonium acetate (0.46 g, 6 mmol) was added to the solution and stirred for another 24 h at room temperature. Diethyl ether was added dropwise into the reaction mixture until all solids were precipitated. The crude product was filtered, dried and crystallized from ethanol. Yield 37%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3258, 3240, 3151 (NH_2, 2 NH), 3043 (CH arom.), 1713, 1659 (2 CO), 1611, 1557 (C=N, NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 6.51 (d, 1H, *J* = 9.6 Hz, H-3 Ar), 7.43 (d, 1H, *J* = 9.0 Hz, H-8 Ar), 7.87 (d, 1H, *J* = 9.0 Hz, H-4 Ar), 8.04 (d, 1H, *J* = 8.1 Hz, H-7 Ar), 8.10–8.14 (m, 5H, H-5 Ar, H-2′,3′,5′,6′ Ar), 8.22 (s, 2H, NH_2 exchanged with D_2O), 10.67 (s, 2H, 2xNH exchanged with D_2O). MS *m/z* (%): 307, M^+ (1.76%). Anal. Calcd. for $C_{17}H_{13}N_3O_3$ (307.30): C, 66.44; H, 4.26; N, 13.67. Found: C, 66.32; H, 4.30; N, 13.88.

2.1.4. General procedure for synthesis of 4-[amino (substituted imino) methyl]-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **4b-f** (Scheme 1). To a solution of sodium metal (0.046 g, 2 mmol) in methanol (50 ml), substituted amine (2 mmol) and nitrile compound **3** (0.58 g, 2 mmol) were successively added and refluxed for 24 h. Solvent was evaporated and the residue was washed twice with petroleum ether. The product was crystallized from ethanol.

2.1.4.1. 4-[Amino (methylimino) methyl]-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **4b**. Yield 44%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3410, 3390 (NH_2, NH) 3100 (CH arom.) 2924, 2854 (CH aliph.), 1681, 1651 (2 CO), 1631, 1600, 1554 (C=N, NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.61 (s, 3H, CH_3), 6.50 (d, 1H,



Scheme 1. Reagents and conditions: (i) HNO_3, H_2SO_4, (ii) $SnCl_2$, HCl, (iii) 4-cyanobenzoylchloride, CH_2Cl_2, TEA, (iv) ethanol, HCl gas, (v) ammonium acetate, (vi) Na methoxide, substituted amines, (vii) chloroacetyl chloride, CH_2Cl_2, TEA, and (viii) aminoheterocycle, DMF, TEA.

J = 9.3 Hz, H-3 Ar), 7.43 (d, 1H, *J* = 9.0 Hz, H-8 Ar), 7.58–8.26 (m, 7H, H-4,5,7 Ar, H-2′,3′,5′,6′ Ar), 10.15 (s, 2H, NH_2 exchanged with D_2O), 10.75 (s, 1H, NH exchanged with D_2O) MS *m/z* (%): 319, M^+-2 (0.10%). Anal. Calcd. for $C_{18}H_{15}N_3O_3$ (321.33): C, 67.28; H, 4.71; N, 13.08. Found: C, 67.43; H, 4.92; N, 13.08.

2.1.4.2. 4-[Amino (ethylimino) methyl]-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **4c**. Yield 32%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3356, 3309 (NH_2, NH), 3080 (CH arom.), 2974, 2931 (CH aliph.), 1681, 1651 (2 CO), 1593, 1550, 1505 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.17 (t, 3H, *J* = 17.9 Hz, CH_3), 4.28 (q, 2H, *J* = 18.3 Hz, CH_2), 6.49 (d, 1H, *J* = 16.8 Hz, H-3 Ar), 6.95 (d, 1H, *J* = 7.7 Hz, H-8 Ar), 7.35–8.21 (m, 7H, H-4,5,7 Ar, H-2′,3′,5′,6′ Ar), 10.32 (s, 2H, NH_2), 10.85 (s, 1H, NH) ^{*13}C NMR (125 MHz, DMSO-d_6) δ ppm: 8.51 (CH_3), 51.85 (CH_2), 116.38 (C-3), 117.33 (C-5), 120.64 (C-7), 121.31 (C-8), 125.82 (C-10), 128.03 (C-2′,3′,5′,6′), 129.00 (C-6), 137.07 (C-1′,4′), 137.74 (C-4), 141.50 (C-9) 164.93 (C=O chromene), 167.91 (C=O, C=N). MS *m/z* (%): 335, M^+ (2.03%). Anal. Calcd. for $C_{19}H_{17}N_3O_3$ (335.36): C, 68.05; H, 5.11; N, 12.53. Found: C, 68.40; H, 5.24; N, 12.62.

2.1.4.3. 4-[Amino (isopropylimino) methyl]-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **4d**. Yield 45%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3367, 3294, 3174 (NH_2, NH), 3080 (CH arom.) 2924, 2850 (CH aliph.), 1716, 1658 (2 CO), 1620, 1570, 1543 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.65 (d, 6H, 2xCH_3), 2.95–3.20 (m, 1H, CH), 6.37 (d, 1H, *J* = 16.2 Hz, H-3 Ar), 6.87 (d, 1H, *J* = 8.4 Hz, H-8 Ar), 7.47–8.30 (m, 7H, H-4,5,7 Ar, H-2′,3′,5′,6′ Ar), 10.20 (s, 2H, NH_2 exchanged with D_2O), 10.40 (s, 1H, NH exchanged with D_2O). MS *m/z* (%): 349, M^+ (1.99%)). Anal. Calcd. for $C_{20}H_{19}N_3O_3$ (349.38): C, 68.75; H, 5.48; N, 12.03. Found: C, 68.93; H, 5.46; N, 12.10.

2.1.4.4. 4-[Amino (tert. butylimino) methyl]-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **4e**. Yield 43%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3363, 3302, 3174 (NH_2, NH), 3070 (CH arom.), 2940, 2850 (CH aliph.), 1720, 1655 (2 CO), 1647, 1620, 1590, 1535 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.17 (s, 9H, 3xCH_3), 6.50 (d, 1H, *J* = 9.6 Hz, H-3 Ar), 7.42 (d, 1H, *J* = 9.0 Hz, H-8 Ar), 7.50 (s, 2H, NH_2 exchanged with D_2O), 7.89 (d, 1H, *J* = 9.2 Hz, H-4 Ar), 7.98–8.23 (m, 6H, H-5,7 Ar, H-2′,3′,5′,6′ Ar), 10.60 (s, 1H, NH exchanged with D_2O). MS *m/z* (%): 363, M^+ (0.95%). Anal. Calcd. for $C_{21}H_{21}N_3O_3$ (363.41): C, 69.41; H, 5.82; N, 11.56. Found: C, 69.67; H, 5.95; N, 11.73.

2.1.4.5. 4-[Amino (hydroxyimino) methyl]-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **4f**. Yield 64%. mp 293–295 °C. IR υ_{max}/cm^{-1}: 3429 (OH), 3375, 3340 (NH_2, NH), 3100 (CH arom.), 1716, 1666 (2 CO), 1635, 1612, 1573, 1535 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 5.92 (s, 2H, NH_2 exchanged with D_2O), 6.50 (d, 1H, *J* = 9.6 Hz, H-3 Ar), 7.42 (d, 1H, *J* = 9.0 Hz, H-8 Ar), 7.88 (d, 1H, *J* = 9.2 Hz, H-4 Ar), 7.91 (s, 1H, H-5 Ar), 7.98 (d, 1H, *J* = 8.4 Hz, H-7 Ar), 8.12 (d, 2H, *J* = 3.0 Hz, H-3′,5′ Ar), 8.23 (d, 2H, *J* = 2.4 Hz, H-2′,6′ Ar), 9.83 (s, 1H, OH exchanged with D_2O), 10.46 (s, 1H, NH exchanged with D_2O). MS *m/z* (%): 323, M^+ (4.50%). Anal. Calcd. for $C_{17}H_{13}N_3O_4$ (323.30): C, 63.16; H, 4.05; N, 13.00. Found: C, 63.44; H, 4.13; N, 13.10.

2.1.5. Synthesis of 4-[5-(chloromethyl)-1,2,4-oxadiazol-3-yl]-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **5** (Scheme 1). To a solution of **4f** (0.64 g, 2 mmol) in dichloromethane (40 ml), chloroacetyl chloride (0.22 ml, 2 mmol) and few drops of triethylamine were added then refluxed for 48 h. The solution was concentrated, filtered and dried. The obtained product was crystallized from ethanol. Yield 55%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3336 (NH), 3082 (CH arom.), 2974 (CH aliph.), 1705, 1678 (2 CO), 1616, 1573, 1554 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 5.21 (s, 2H, CH_2), 6.50 (d, 1H, *J* = 9.6 Hz, H-3 Ar), 7.43 (d, 1H, *J* = 9.0 Hz, H-8 Ar), 7.90 (d, 1H, *J* = 9.0 Hz, H-4 Ar), 8.10 (d, 1H, *J* = 9.6 Hz, H-7 Ar), 8.13 (s, 1H, H-5 Ar), 8.18 (d, 2H, *J* = 3.0 Hz, H-3′,5′ Ar), 8.24 (d, 2H, *J* = 2.4 Hz, H-2′,6′ Ar), 10.65 (s, 1H, NH, exchanged with D_2O). MS *m/z* (%): 381, M^+ (24.27%) and 383, M^++2 (11.15%). Anal. Calcd. for $C_{19}H_{12}ClN_3O_4$ (381.77): C, 59.78; H, 3.17; N, 11.01. Found: C, 59.92; H, 3.17; N, 11.11.

2.1.6. General procedure for synthesis of *N*-(2-oxo-2*H*-chromen-6-yl)-4-[5-(substituted aminomethyl)-1,2,4-oxadiazol-3-yl]benzamide **6a**,**b** (Scheme 1). To a solution of **5** (0.76 g, 2 mmol) in dimethylformamide (20 ml), aminoheterocycle (2-aminobenzothiazole or 6-aminocoumarin) (2 mmol) and few drops of triethylamine were added and refluxed for 14 h. The reaction mixture was poured onto ice-water then filtered, dried and crystallized from ethanol.

2.1.6.1. 4-{5-[(Benzo[d]thiazol-2-ylamino)methyl]-1,2,4-oxadiazol-3-yl}-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **6a**. Yield 65%. mp 285–286 °C. IR υ_{max}/cm^{-1}: 3414, 3290 (2 NH), 3089 (CH arom.), 2912 (CH aliph.), 1720, 1651 (2 CO), 1616, 1573, 1545 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.03 (s, 2H, CH_2), 6.80–8.55 (m, 13H, Ar H), 9.82 (s, 2H, 2xNH). ^{*13}C NMR (125 MHz, DMSO-d_6) δ ppm: 30.00 (CH_2), 114.52 (C-3), 117.08 (C-7,8,9″), 118.80 (C-5), 119.13 (C-6″), 119.64 (C-10,5″), 125.04 (C-7″,8″), 129.04 (C-3′,5′), 132.97 (C-2′,6′), 135.63 (C-6), 139.00 (C-1′,4′), 144.87 (C-4,9), 150.39 (C-4″), 160.50 (C=O chromene, C-3 oxadiazole), 164.63 (C=O, C-5 oxadiazole), 165.00 (C-2 benzothiazole). MS *m/z* (%): 498, M^++3 (0.94%). Anal. Calcd. for $C_{26}H_{17}N_5O_4S$ (495.51): C, 63.02; H, 3.46; N, 14.13. Found: C, 62.85; H, 3.56; N, 14.18.

2.1.6.2. *N*-(2-Oxo-2*H*-chromen-6-yl)-4-{5-[(2-oxo-2*H*-chromen-6-ylamino)methyl]-1,2,4-oxadiazol-3-yl}benzamide **6b**. Yield 45%. mp 262–265 °C. IR υ_{max}/cm^{-1}: 3425, 3336 (2 NH), 3078 (CH arom.), 2924, 2850 (CH aliph.), 1716, 1660 (2 CO), 1635, 1612, 1535 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.34 (s, 2H, CH_2), 6.50 (d, 2H, J = 9.3 Hz, H-3,3″ Ar), 7.42 (d, 2H, J = 8.7 Hz, H-8,8″ Ar), 7.84 (d, 2H, J = 8.7 Hz, H-4,4″ Ar), 7.89 (d, 2H, J = 8.7 Hz, H-7,7″ Ar), 7.96 (s, 2H, H-5,5″ Ar), 8.13 (d, 2H, J = 3.9 Hz, H-3′,5′ Ar), 8.24 (d, 2H, J = 2.4 Hz, H-2′,6′ Ar), 9.86 (s, 1H, NH, exchanged with D_2O) 10.49 (s, 1H, NH, exchanged with D_2O). MS *m/z* (%): 507, M^++1 (0.04%). Anal. Calcd. for $C_{28}H_{18}N_4O_6$ (506.47): C, 66.40; H, 3.58; N, 11.06. Found: C, 66.74; H, 3.76; N, 11.08.

2.1.7. The synthesis of ethyl *N*-(2-oxo-2*H*-chromen-6-yl)carbamate **7** and 4-(2-oxo-2*H*-chromen-6-yl)semicarbazide **8** (Scheme 2) were prepared as previously reported [34].

2.1.8. The synthesis of 1-(4-substituted phenyl)-3-(4-substituted phenyl)prop-2-en-1-one **9a–e** (Scheme 2) was adopted as described in literature [35–38].

2.1.9. General procedure for synthesis of *N*-(2-oxo-2*H*-chromen-6-yl)-3-(4-substituted phenyl)-5-(4-substituted phenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide **10a–e** (Scheme 2). To a solution of semicarbazide derivative **8** (1.09 g, 5 mmol) in ethanol (50 ml), the appropriate chalcone derivative **9a–e** (5 mmol) was added portionwise and the solution was refluxed for 24 h. The reaction mixture was concentrated and the solid obtained was filtered, dried and crystallized from ethanol.

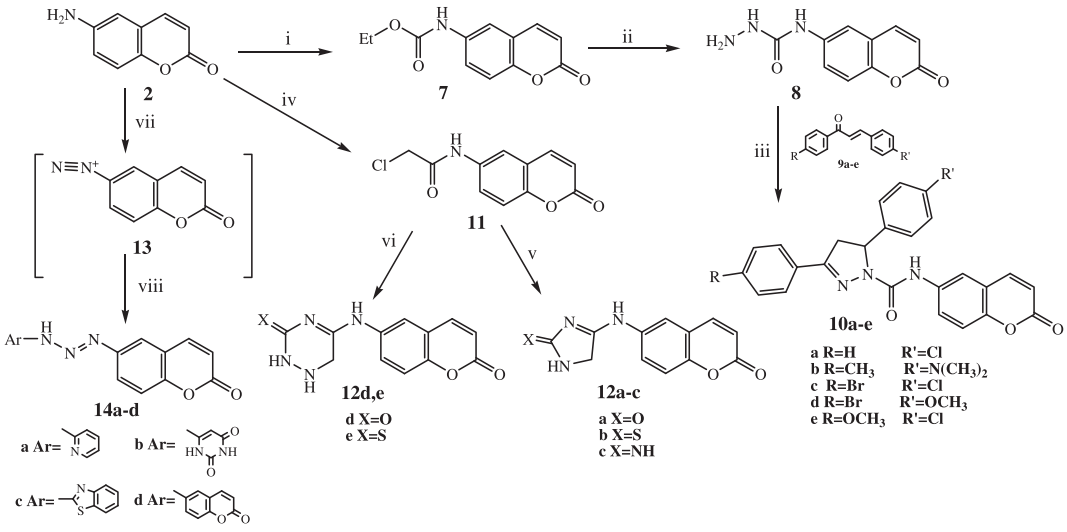
2.1.9.1. 5-(4-Chlorophenyl)-*N*-(2-oxo-2*H*-chromen-6-yl)-3-phenyl-4,5-dihydropyrazole-1-carboxamide **10a**. Yield 55%. mp 125–127 °C. IR υ_{max}/cm^{-1}: 3344 (NH), 3059, (CH arom.), 2924, 2854 (CH aliph.), 1710, 1681 (2 CO), 1650, 1589, 1560 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.73 (dd, 1H, J = 16.2 Hz, J = 10.8 Hz, CH_2), 3.96 (dd, 1H, J = 16.3 Hz, J = 10.8 Hz, CH_2), 4.81 (t, 1H, CH), 6.63–7.82 (m, 14H, Ar H), 10.95 (s, 1H, NH, exchanged with D_2O) ^{*13}C NMR (125 MHz, DMSO-d_6) δ ppm: 15.10 (CH_2 pyrazoline), 60.42 (CH pyrazoline), 100.38–149.30 (20 aromatic Cs), 154.31 (C=O, C-3 pyrazoline), 157.39 (C=O chromene). MS *m/z* (%): 444, M^++1 (22.83%). Anal. Calcd. for $C_{25}H_{18}ClN_3O_3$ (443.88): C, 67.65; H, 4.09; N, 9.47. Found: C, 68.04; H, 4.18; N, 9.72.

2.1.9.2. 5-(4-Dimethylaminophenyl)-*N*-(2-oxo-2*H*-chromen-6-yl)-3-(4-methylphenyl)-4,5-dihydropyrazole-1-carboxamide **10b**. Yield 77%. mp 120–122 °C. IR υ_{max}/cm^{-1}: 3332 (NH), 3089, (CH arom.), 2947, 2885 (CH aliph.), 1700, 1680 (2 CO), 1612, 1580, 1538, 1519 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.30 (s, 3H, CH_3), 2.75 (dd, 1H, J = 16.4 Hz, J = 10.5 Hz, CH_2), 2.85 (s, 6H, $N(CH_3)_2$), 3.30 (dd, 1H, J = 16.2 Hz, J = 10.5 Hz, CH_2), 4.71 (t, 1H, CH), 6.68 (d, 3H, J = 5.7 Hz, H-3,3″,5″ Ar), 7.16 (d, 3H, J = 6.6 Hz, H-8,2″,6″ Ar), 7.19 (d, 3H, J = 6.0 Hz, H-4,3′,5′ Ar), 7.25 (s, 1H, NH exchanged with D_2O), 7.49 (s, 1H, H-5 Ar), 7.51 (d, 3H, J = 6.6 Hz, H-7,2′,6′ Ar). MS *m/z* (%): 466, M^+ (0.69%). Anal. Calcd. for $C_{28}H_{26}N_4O_3$ (466.53): C, 72.09; H, 5.62; N, 12.01. Found: C, 72.35; H, 5.69; N, 12.18.

2.1.9.3. 3-(4-Bromophenyl)-5-(4-chlorophenyl)-*N*-(2-oxo-2*H*-chromen-6-yl)-4,5-dihydropyrazole-1-carboxamide **10c**. Yield 45%. mp 182–183 °C. IR υ_{max}/cm^{-1}: 3329 (NH), 3050 (CH arom.), 2950, 2860 (CH aliph.), 1710, 1678 (2 CO), 1585, 1559, 1528 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.82 (dd, 1H, J = 15.5 Hz, J = 10.5 Hz, CH_2), 3.44 (dd, 1H, J = 16.4 Hz, J = 10.8 Hz, CH_2), 4.86 (t, 1H, CH), 7.25–8.20 (m, 13H, Ar H), 7.71 (s, 1H, NH exchanged with D_2O). MS *m/z* (%): 526, M^++4 (8.70%). Anal. Calcd. for $C_{25}H_{17}BrClN_3O_3$ (522.78): C, 57.44; H, 3.28; N, 8.04. Found: C, 57.82; H, 3.41; N, 8.43.

2.1.9.4. 3-(4-Bromophenyl)-5-(4-methoxyphenyl)-*N*-(2-oxo-2*H*-chromen-6-yl)-4,5-dihydropyrazole-1-carboxamide **10d**. Yield 53%. mp 122–124 °C. IR υ_{max}/cm^{-1}: 3336 (NH), 3078 (CH arom.), 2958, 2939, 2873 (CH aliph.), 1707, 1665 (2 CO), 1608, 1585, 1550, 1512 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.80 (dd, 1H, J = 15.9 Hz, J = 10.8 Hz, CH_2), 3.37 (dd, 1H, J = 16.2 Hz, J = 10.8 Hz, CH_2), 3.73 (s, 3H, OCH_3), 4.80 (t, 1H, CH), 6.87–6.91 (m, 4H, H-3,8,3″,5″ Ar), 7.26–7.29 (m, 4H, H-4,7,2″,6″ Ar), 7.54 (d, 5H, J = 2.4 Hz, H-5,2′,3′,5′ 6′ Ar), 7.59 (s, 1H, NH, exchanged with D_2O). MS *m/z* (%): 518, M^+ (17.54%). Anal. Calcd. for $C_{26}H_{20}BrN_3O_4$ (518.36): C, 60.24; H, 3.89; N, 8.11. Found: C, 60.41; H, 4.15; N, 8.33.

2.1.9.5. 5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-*N*-(2-oxo-2*H*-chromen-6-yl)-4,5-dihydropyrazole-1-carboxamide **10e**. Yield 49%. mp 118–119 °C. IR υ_{max}/cm^{-1}: 3414 (NH), 3070 (CH arom.), 2931, 2839 (CH aliph.), 1720, 1666 (2 CO), 1600, 1570, 1550 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.80 (dd, 1H, J = 16.2 Hz, J = 10.5 Hz, CH_2), 3.15 (dd, 1H, J = 16.2 Hz, J = 10.5 Hz, CH_2), 3.81 (s, 3H, OCH_3), 4.80 (t, 1H, CH), 5.50 (s, 1H, NH, exchanged with D_2O), 6.92–8.19 (m, 13H, Ar H). MS *m/z* (%): 473, M^+ (22.99%) and 475, M^++2 (17.52%). Anal. Calcd. for



Scheme 2. Reagents and conditions: (i) ethyl chloroformate, glacial acetic acid, anhydrous Na acetate, (ii) hydrazine hydrate, ethanol, (iii) substituted chalcones **9a–e**, ethanol, (iv) chloroacetyl chloride, dry benzene, (v) urea or thiourea or guanidine, acetone, K_2CO_3, (vi) semicarbazide or thiosemicarbazide, acetone, K_2CO_3, (vii) $NaNO_2$/HCl, and (viii) aminoheterocycle, sodium acetate.

$C_{26}H_{20}ClN_3O_4$ (473.91): C, 65.89; H, 4.25; N, 8.87. Found: C, 65.99; H, 4.56; N, 9.13.

2.1.10. The synthesis of 2-chloro-*N*-(2-oxo-2*H*-chromen-6-yl)acetamide **11** (Scheme 2) was prepared as described previously in literature [39].

2.1.11. General procedure for synthesis of *N*- substituted heterocyclic-6-amino-2*H*-chromen-2-one **12a–e** (Scheme 2). To solution of chloroacetamido derivative **11** (0.576 g, 2 mmol) in dry acetone (50 ml) containing anhydrous potassium carbonate (0.276 g, 2 mmol), amine derivative (urea, thiourea, guanidine, semicarbazide or thiosemicarbazide) (2 mmol) was added. The solution was refluxed for 24 h then concentrated, filtered and dried. The obtained solid was crystallized from ethanol.

2.1.11.1. 4-(2-Oxo-2*H*-chromen-6-ylamino)-1*H*-imidazol-2(5*H*)-one **12a**. Yield 44%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3444 (2 NH), 3080 (CH arom.), 2910, 2840 (CH aliph.), 1732, 1658 (2 CO), 1624, 1570 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.55 (s, 2H, CH_2), 6.45–8.09 (m, 5H, Ar H), 10.00 (s, 1H, NH), 10.40 (s, 1H, NH). MS *m/z* (%): 243, M^+ (5.09%). Anal. Calcd. for $C_{12}H_9N_3O_3$ (243.22): C, 59.26; H, 3.73; N, 17.28. Found: C, 59.30; H, 3.79; N, 17.53.

2.1.11.2. 6-(2-Thioxo-2,5-dihydro-1*H*-imidazol-4-ylamino)-2*H*-chromen-2-one **12b**. Yield 48%. mp 240–242 °C. IR υ_{max}/cm^{-1}: 3271, 3224 (2NH), 3093 (CH arom.), 1689 (CO), 1654, 1620, 1573 (C=N, NH, C=C), 1265 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.52 (s, 2H, CH_2), 6.44 (d, 1H, *J* = 9.3 Hz, H-3 Ar), 7.32 (d, 1H, *J* = 9.3 Hz, H-7 Ar), 7.62 (d, 1H, *J* = 8.7 Hz, H-8 Ar), 7.93 (s, 1H, H-5 Ar), 8.01 (d, 1H, *J* = 9.3 Hz, H-4 Ar), 10.40 (s, 2H, 2xNH exchanged with D_2O). MS *m/z* (%): 259, M^+ (0.50%). Anal. Calcd. for $C_{12}H_9N_3O_2S$ (259.28): C, 55.59; H, 3.50; N, 16.21. Found: C, 55.84; H, 3.59; N, 16.38.

2.1.11.3. 6-(2-Imino-2,5-dihydro-1*H*-imidazol-4-ylamino)-2*H*-chromen-2-one **12c**. Yield 53%. mp 198–199 °C. IR υ_{max}/cm^{-1}: 3298, 3255, 3167 (3NH), 3089 (CH arom.), 2997, 2962 (CH aliph.), 1701 (CO), 1620, 1573 (C=N, NH, C=C). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.29 (s, 2H, CH_2), 6.49 (d, 1H, *J* = 9.3 Hz, H-3 Ar), 7.00 (s, 1H, NH exchanged with D_2O), 7.39 (d, 1H, *J* = 9.0 Hz, H-7 Ar), 7.67 (d, 1H, *J* = 9.0 Hz, H-8 Ar), 8.05 (s, 1H, H-5 Ar), 8.09 (d, 1H, *J* = 9.6 Hz, H-4 Ar), 10.64 (s, 2H, 2xNH exchanged with D_2O). ^{*13}C NMR (500 MHz, DMSO-d_6) δ ppm: 44.00 (C-5 imidazoline), 117.12 (C-5), 117.24 (C-3), 118.59 (C-7), 119.23 (C-8), 123.97 (C-10), 135.34 (C-9), 144.75 (C-6), 150.19 (C-4), 160.46 (C=O chromene), 165.36 (C-2,4 imidazoline). MS *m/z* (%): 242, M^+ (0.67%). Anal. Calcd. for $C_{12}H_{10}N_4O_2$ (242.23): C, 59.50; H, 4.16; N, 23.13. Found: C, 59.86; H, 4.18; N, 22.87.

2.1.11.4. 5-(2-Oxo-2*H*-chromen-6-ylamino)-1,2-dihydro-1,2,4-triazin-3(6*H*)-one **12d**. Yield 58%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3444, 3398, 3336 (3NH), 3078 (CH arom.), 2958, 2858 (CH aliph.), 1732, 1658 (2 CO), 1620, 1570 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 4.57 (s, 2H, CH_2), 6.40 (s, 3H, 3xNH exchanged with D_2O), 6.51 (d, 1H, *J* = 9.9 Hz, H-3 Ar), 7.45 (d, 1H, *J* = 9.0 Hz, H-7 Ar), 7.70 (d, 1H, *J* = 9.3 Hz, H-8 Ar), 7.79 (s, 1H, H-5 Ar), 8.05 (d, 1H, *J* = 9.3 Hz, H-4 Ar). MS *m/z* (%): 259, M^++1 (0.83%). Anal. Calcd. for $C_{12}H_{10}N_4O_3$ (258.23): C, 55.81; H, 3.90; N, 21.70. Found: C, 56.18; H, 4.10; N, 22.06.

2.1.11.5. 6-(3-Thioxo-1,2,3,6-tetrahydro-1,2,4-triazin-5-ylamino)-2*H*-chromen-2-one **12e**. Yield 42%. mp 230–232 °C. IR υ_{max}/cm^{-1}: 3444, 3290 3228 (3NH), 3101 (CH arom.), 1701 (CO), 1616, 1573 (C=N, NH, C=C), 1265 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.52 (s, 2H, CH_2), 6.45 (d, 1H, *J* = 9.0 Hz, H-3 Ar), 7.33 (d, 1H, *J* = 9.3 Hz, H-7 Ar), 7.63 (d, 1H, *J* = 8.7 Hz, H-8 Ar), 7.99 (s, 1H, H-5 Ar), 8.01 (d, 1H, *J* = 9.6 Hz, H-4 Ar), 10.35 (s, 3H, 3xNH exchanged with D_2O). MS *m/z* (%): 274, M^+ (0.31%). Anal. Calcd. for $C_{12}H_{10}N_4O_2S$ (274.30): C, 52.54; H, 3.67; N, 20.43. Found: C, 52.90; H, 3.58; N, 20.62.

2.1.12. General procedure for synthesis of 6-(3-substituted triaz-1-enyl)-2*H*-chromen-2-one **14a–d** (Scheme 2). To a solution of the amine compound **2** (0.6 g, 3.75 mmol) in concentrated hydrochloric acid (0.65 ml) and water (2.5 ml), solution of sodium nitrite (0.26 g, 3.75 mmol) in water (2 ml) was added portionwise and stirred for 15 min. at 0 °C. Solution of aminoheterocycle (3.75 mmol) in concentrated hydrochloric acid (0.35 ml) and water (2 ml) was then added and the suspension was stirred for further 15 min. Sodium acetate (1.05 g, 12 mmol) dissolved in water (2 ml) was added and stirred for 1 h at room temperature then left over night. The product was filtered, dried and crystallized from chloroform.

2.1.12.1. 6-[3-(Pyridin-2-yl)triaz-1-enyl]-2*H*-chromen-2-one **14a**. Yield 75%. mp 245–247 °C. IR υ_{max}/cm^{-1}: 3433 (NH), 3080 (CH arom.), 1724 (CO), 1597, 1531 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 6.45–8.10 (m, 9H, Ar H), 8.80 (s, 1H, NH, exchanged with D_2O). MS *m/z* (%): 267, M^++1 (2.73%). Anal. Calcd. for $C_{14}H_{10}N_4O_2$ (266.25): C, 63.15; H, 3.79; N, 21.04. Found: C, 63.41; H, 3.92; N, 21.41.

2.1.12.2. 6-[3-(2-Oxo-2*H*-chromen-6-yl)triaz-2-enyl]-1,2,3,4-tetrahydropyrimidin-2,4-dione **14b**. Yield 78%. mp > 300 °C. IR υ_{max}/cm^{-1}: 3380, 3320, 3170 (3 NH), 3051 (CH arom.), 2920 (CH) 1724, 1676 (3 CO), 1624, 1550 (NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 6.49–8.12 (m, 6H, CH and Ar H), 10.09 (s, 1H, NH, exchanged with D_2O), 10.47 (s, 1H, NH exchanged with D_2O), 10.96 (s, 1H, NH, exchanged with D_2O). MS *m/z* (%): 299, M^+ (2.75%). Anal. Calcd. for $C_{13}H_9N_5O_4$ (299.24): C, 52.18; H, 3.03; N, 23.40. Found: C, 52.48; H, 3.34; N, 23.62.

2.1.12.3. 6-[3-(Benzo[d]thiazol-2-yl)triaz-1-enyl]-2*H*-chromen-2-one **14c**. Yield 76%. mp 185–187 °C. IR υ_{max}/cm^{-1}: 3394 (NH), 3100 (CH arom.), 1705 (CO), 1635, 1562 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 6.51–7.76 (m, 9H, aromatic H), 12.80 (s, 1H, NH, exchanged with D_2O). ^{*13}C NMR (125 MHz, DMSO-d_6) δ ppm: 117.17–153.30 (14 aromatic Cs), 160.49 (C=O chromene), 167.11 (C-2 benzothiazole). MS *m/z* (%): 322, M^+ (51.79%). Anal. Calcd. for $C_{16}H_{10}N_4O_2S$ (322.34): C, 59.62; H, 3.13; N, 17.38. Found: C, 59.99; H, 3.24; N, 17.58.

2.1.12.4. 6-[3-(2-Oxo-2*H*-chromen-6-yl)triaz-1-enyl]-2*H*-chromen-2-one **14d**. Yield 62%. mp 260–261 °C. IR υ_{max}/cm^{-1}: 3417 (NH), 3070 (CH arom.), 1724 (2 CO), 1608, 1570 (NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 6.50 (d, 2H, *J* = 9.2 Hz, H-3,3′ Ar), 7.41 (d, 2H, *J* = 8.4 Hz, H-7,7′ Ar), 7.65 (s, 2H, H-5,5′ Ar), 7.73 (d, 2H, *J* = 8.7 Hz, H-8,8′ Ar), 8.08 (d, 2H, *J* = 9.2 Hz, H-4,4′ Ar), 12.74 (s, 1H, NH, exchanged with D_2O). MS *m/z* (%): 333, M^+ (0.49%). Anal. Calcd. for $C_{18}H_{11}N_3O_4$ (333.30): C, 64.86; H, 3.33; N, 12.61. Found: C, 65.16; H, 3.41; N, 12.80.

2.2. Anticoagulant activity

All the prepared compounds in addition to the key intermediate 6-aminocoumarin **2** were evaluated for antithrombotic activity (clotting time, CT and prothrombin time, PT) in mice.

The *in vivo* study was performed at the Pharmacology Department, at the National Research Institute, Giza, Egypt, after approval from the Ethics committee of the center and in accordance with the recommendations of the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985).

The experiment was carried out on 156 western albino male mice weighing from 25 to 30 g. The mice were divided into 26 groups of 6 mice each. Mice within group 1 were kept as control and received propylene glycol in distilled water only, whereas those of group 2–25 were given the tested compounds and mice in the last group 26 were given warfarin as the standard control. The tested compounds and warfarin that were dissoluted or suspended in propylene glycol were administered orally with daily dose of 0.4 mmol/kg for 3 successive days. Blood samples were

taken after 24 h from the last injection then CT and PT were determined [40–42].

2.2.1. Clotting time (CT) determination

A drop of blood from each mouse was drawn on a clean dry glass slide. One end of the capillary tube was dipped into blood drop gently without pressure. The capillary was allowed to be filled with blood by lowering the end of fitted capillary. Around three quarter of the capillary length was undipped in blood without sucking. Using stopwatch, a small piece of capillary was broken. Breaking was repeated at regular time intervals, till fibrin thread appeared at the broken end of capillary tube. The clotting time of blood was determined by recording time interval between dipping the blood and the first appearance of fibrin thread at the broken ends of capillary tube using a stop watch [41].

2.2.2. Prothrombin time (PT) determination

Blood was drawn into a commercial vacuum tube containing 3.8% sodium citrate solution (ratio of 9 part blood to 1 part sodium citrate is critical).

Blood was mixed well by inversion and centrifugation for 15 min. soon after blood collection to separate the blood cells. Unless samples are to be processed immediately, the plasma was transferred into a plastic tube. Plasma that is clearly haemolysed or contains >10,000 platelets per cubic milliliter or red cells is not suitable for coagulation testing.

The PT reagent (Biorexfars®) was pre-incubated at 37 °C for at least 10 min. The reagent was maintained as suspension by magnetic stirring or mixing by inversion immediately to homogenize the content prior to use. 100 μl of test or control plasma was pipetted into a test cuvette and incubated at 37 °C for 1 min., 200 μl of the pre-incubated PT reagent was added and time of clotting was recorded in seconds [41].

2.3. Molecular docking

Docking studies of sixteen novel coumarin analogues were performed by Molecular Operating Environment (MOE) 2008.10 release of chemical computing group, Canada [43]. The program operated under "Widnows Xp" operating system installed on an Intel Pentium IV PC with a 2.8 MHz processor and 512 RAM. All minimization were performed with MOE until a RMSD gradient of 0.05 kcal mol^{-1} $Å^{-1}$ with MMFF 94 force field and the partial charges were automatically calculated. The score function, dock function (s, kcal/mol), developed by MOE program was used for the evaluation of the binding affinity of the ligand.

2.3.1. Preparation of the target factor Xa

The X-ray crystal structure of the enzyme with amidine ligand RPR200095, 4-({4-[(6-chloro-1-benzothien-2-yl)sulfonyl]-2-oxopiperazin-1-yl}methyl)benzene carboxyimidamide, (PDB code: 1NFY) [44] was obtained from the protein data bank in PDB format. The enzyme was prepared for docking studies: (i) acting on only one chain of amino acids containing one molecule of RPR200095, (ii) 3D protonation for the amino acid side chain and RPR200095, (iii) isolation of the active site, fixation to be dealt with as a rigid structure and recognition of the amino acids, (iv) creation of dummies around the active site, (v) studying the interactions of the ligand (RPR200095) with the amino acids of the active site.

2.3.2. Preparation of compounds for docking

The 3D structures of the synthesized compounds were built using MOE and subjected to the following procedure: (i) 3D protonation of the structures, (ii) Running conformational analysis using systemic search, (iii) Selecting the least energetic conformer, (iv) Applying the same docking protocol used with RPR200095.

2.3.3. Docking running

Prior to the docking of the coumarin derivatives, redcoking of the native ligand bound in the factor Xa (FXa) active site was performed to validate the docking protocol. The generated most stable conformer of each compound was virtually docked into the predefined active site of FXa. The developed dock models were energetically minimized and used to predict the interaction of the ligand with the amino acids in the active site of the enzyme.

3. Results and discussion

3.1. Chemistry

The targeted compounds **4a–f**, **5**, **6a**,**b**, **10a**–**e**, **12a**–**e** and **14a**–**d** were synthesized as depicted in Schemes 1 and 2.

The key intermediate compound 6-aminocoumarin **2** was prepared in two steps from coumarin according to the reported procedures (Scheme 1) [31–33]. The amine compound **2** was acylated with 4-cyanobezoyl chloride under cold conditions and in presence of triethylamine to capture the resulting HCl and gave 4-cyano-*N*-(2-oxo-2*H*-chromen-6-yl)benzamide **3** (Scheme 1). The proposed structure was confirmed by spectral and analytical data. IR spectrum showed two stretching bands at 3287 and 2225 cm^{-1} assigned to NH and C≡N groups, respectively, and two bands at 1719 cm^{-1} and 1653 cm^{-1} attributed to the C=O groups. ^1H NMR spectrum displayed a D_2O exchangeable singlet signal corresponding to NH proton at δ = 10.67 ppm and additional two doublet signals at 8.13 and 8.21 assigned to added aromatic protons H-3′,5′ Ar and H-2′,6′ Ar, respectively. Furthermore, MS of the compound revealed a molecular ion peak M^+ at *m*/*z* 290.

Nitrile **3** was subsequently subjected to Pinner reaction, treatment with dry gaseous HCl in absolute ethanol and reaction of the resulting imidate salt with ammonium acetate yielded amidine **4a** (Scheme 1) adopting the same procedure of Anderluh et al. [45]. Pinner conversion was achieved also using 4 N HCl in dioxane but use of HCl gas provided higher yield than using 4 N HCl in dioxane although the later is operationally simple. This was attributed to isolation of imidate salt that also known as Pinner salt, in good to moderate yields and was stable at room temperature. The imidate salts undergoes simple reaction with amine compound providing amidine [46]. The structure of the amidine derivative **4a** was deduced by spectral and analytical data. IR spectrum showed the disappearance of C≡N stretching band and appearance of NH_2 and 2NH stretching bands at 3258, 3240 and 3151 cm^{-1}. The ^1H NMR spectrum proved the presence of two singlet signals at δ = 8.22 and 10.67 ppm corresponding to these D_2O exchangeable protons of NH_2 and NH groups, respectively. The MS of the compound exhibited a molecular ion peak M^+ at *m*/*z* 307.

Refluxing nitrile compound **3** with substituted amines in presence of sodium methoxide yielded substituted amidines **4b**–**f** (Scheme 1). The structures of the target compounds **4b**–**f** were confirmed by spectral and analytical data. Their IR spectra showed the disappearance of C≡N stretching band and the presence of OH band at 3429 cm^{-1} for compound **4f**, aliphatic CH bands at 2974–2850 for compounds **4b**–**e**, in addition to the presence of stretching bands at 3410–3151 cm^{-1} for the new NH_2 and the former amide NH groups. Furthermore, ^1H NMR spectra showed D_2O exchangeable signals of NH_2 and NH protons at δ = 5.92–10.85 ppm, additional signals for aliphatic protons at δ = 1.17–4.28 ppm for compounds **4b**–**e**, and a singlet signal at δ = 9.83 corresponding for OH in compound **4f** that exchanged with D_2O. ^{13}C NMR spectrum for compound **4c** showed signals at 8.51, 51.85, 164.93 and 167.91 corresponding to CH_3, CH_2, C=O chromene, C=O amide and C=N, respectively, in addition to signals corresponding to 14

Table 1
Antithrombotic activity (CT and PT) of the tested compounds and warfarin at an oral daily dose of 0.4 mmol/kg for successive 3 days.

Compound	CT (s)	PT (s)
Control	9.8 ± 0.60[b]	13.3 ± 1.02[b]
2	12.7 ± 1.63[b]	13.5 ± 0.76[b]
4a	18.3 ± 0.88[a,b]	36.5 ± 1.98[a,b]
4b	18.0 ± 1.48[a,b]	20.7 ± 1.99[a,b]
4c	17.3 ± 1.33[a,b]	14.5 ± 0.76[b]
4d	14.2 ± 1.42[b]	15.3 ± 0.97[b]
4e	14.5 ± 0.76[b]	17.3 ± 1.05[b]
4f	17.5 ± 0.89[a,b]	26.0 ± 2.10[a,b]
5	17.7 ± 0.88[a,b]	42.3 ± 2.32[a]
6a	20.7 ± 0.80[a]	13.8 ± 0.98[b]
6b	13.7 ± 0.88[b]	37.8 ± 1.42[a]
10a	13.7 ± 0.56[b]	11.0 ± 0.52[b]
10b	21.0 ± 1.79[a]	38.5 ± 0.99[a]
10c	23.5 ± 0.96[a]	17.3 ± 1.02[b]
10d	24.8 ± 1.01[a]	18.5 ± 0.56[b]
10e	22.2 ± 0.79[a]	14.0 ± 1.29[b]
12a	17.3 ± 0.49[a,b]	15.7 ± 1.45[b]
12b	22.3 ± 0.80[a]	13.3 ± 0.88[b]
12c	14.2 ± 0.70[b]	12.5 ± 0.76[b]
12d	18.7 ± 0.71[a,b]	15.2 ± 0.95[b]
12e	20.8 ± 1.01[a]	16.0 ± 0.86[b]
14a	24.0 ± 1.77[a]	29.5 ± 2.22[a,b]
14b	16.7 ± 0.88[a,b]	19.3 ± 1.43[a,b]
14c	19.5 ± 0.76[a]	11.8 ± 0.66[b]
14d	28.0 ± 1.65[a,b]	16.2 ± 1.35[b]
Warfarin	23.2 ± 1.25[a]	42.3 ± 1.61[a]

[a] $P < 0.05$: Statistically significant from control (Dunnett's test).
[b] $P < 0.05$: Statistically significant from warfarin (Dunnett's test).

aromatic carbons. MS spectra of the title compounds showed the appearance of their respective molecular ion peaks.

1,2,4-Oxadiazoles were prepared in general in two steps by the O-acylation of an amidoxime with an activated carboxylic acid derivative, typically an active acyl chloride, followed by cyclization and cyclodehydration by heating [47–50]. Accordingly, cyclization of amidoxime derivative **4f** to the corresponding 1,2,4-oxadiazole derivative was achieved through acylation with chloroacetyl chloride in dichloromethane and subsequently refluxing to be cyclized and afforded the corresponding oxadiazole derivative **5** (Scheme 1). The formation of compound **5** was supported from its spectral and microanalytical data. IR spectrum confirmed the presence of only one NH band at 3336 cm^{-1} and the disappearance of NH_2, OH bands that involved in the cyclization sequence. The ^1H NMR spectrum revealed the absence of NH_2 and OH signals and the appearance of singlet signal assigned to aliphatic CH_2 at $\delta = 5.21$ ppm. Additionally, the mass spectrum revealed the two isotopic molecular ion peak at 381 and 383 for M^+ and M^++2, respectively.

Refluxing 5-chloromethyl-1,2,4-oxadiazole derivative **5** with appropriate arylamine in dimethylformamide in presence of triethylamine yielded target compounds **6a**,**b** in good yield (Scheme 1). Triethylamine was used to provide basic medium as N-alkylation of aromatic amine is usually carried by the treatment of the amine with an alkylhalide in the presence of a base [51].

Analysis of compounds **6a** and **6b** confirmed their proposed structures. IR spectra showed the presence of two bands corresponding to 2 NH at 3425–3290 cm^{-1}. The ^1H NMR spectrum of compound **6a** displayed a singlet signal at $\delta = 9.82$ ppm assigned to 2NH and a singlet signal corresponding to aliphatic CH_2 at $\delta = 4.03$ ppm, in addition to the expected extra aromatic protons. ^{13}C NMR spectrum of the same compound **6a** exhibited signals at $\delta = 30.00$ ppm corresponding to CH_2, 160.50 ppm for C=O chromene and C-3 oxadiazole, 164.63 ppm for C=O and C-5 oxadiazole and 165.00 ppm for C-2 benzothiazole in addition to extra signals corresponding to 20 aromatic carbons.

The synthetic steps adopted for the preparation of *N*-(2-oxo-2*H*-chromen-6-yl)-3-(4-substituted phenyl)-5-(4-substituted phenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide **10a**–**e**, 6-(2-substituted-2,5-dihydro-1*H*-imidazol-4-yl)amino-2*H*-chromen-2-one **12a**–**c**, 6-(3-substituted-1,2,3,6-tetrahydro-1,2,4-triazin-5-yl)amino-2*H*-chromen-2-one **12d**,**e**, and 6-substituted triazenyl-2*H*-chromen-2-one **14a**–**d** were illustrated in Scheme 2. Known intermediates such as ethyl *N*-(2-oxo-2*H*-chromen-6-yl) carbamate **7**, 4-(2-oxo-2*H*-chromen-6-yl)semicarbazide **8** [34] and 1-(4-substituted phenyl)-3-(4-substituted phenyl)prop-2-en-1-one **9a**–**e** [35–38] were prepared according to the reported procedures.

Condensation of appropriate chalcones **9a**–**e** with 4-coumarinylsemicarbazide **8** in ethanol afforded pyrazoline-1-carboxamide derivatives **10a**–**e** with high yield and purity (Scheme 2). The structures of the compounds **10a**–**e** were confirmed by spectral and analytical data. Their IR spectra showed the presence of only one NH stretching band at 3414–3329 cm^{-1}. ^1H NMR spectra exhibited two doublet of doublet signals at $\delta = 2.73$–2.82 and 3.15–3.96 ppm corresponding to the two protons of pyrazoline CH_2 due to vicinal and geminal coupling. They showed a prominent ABX system (J_{Ha-Hx} 15.5–16.4 Hz, J_{Ha-Hb}10.5–10.8 Hz) and triplet signal at $\delta = 4.71$–4.86 ppm attributed to pyrazoline CH. The ^{13}C NMR spectrum for compound **10a** showed signals at

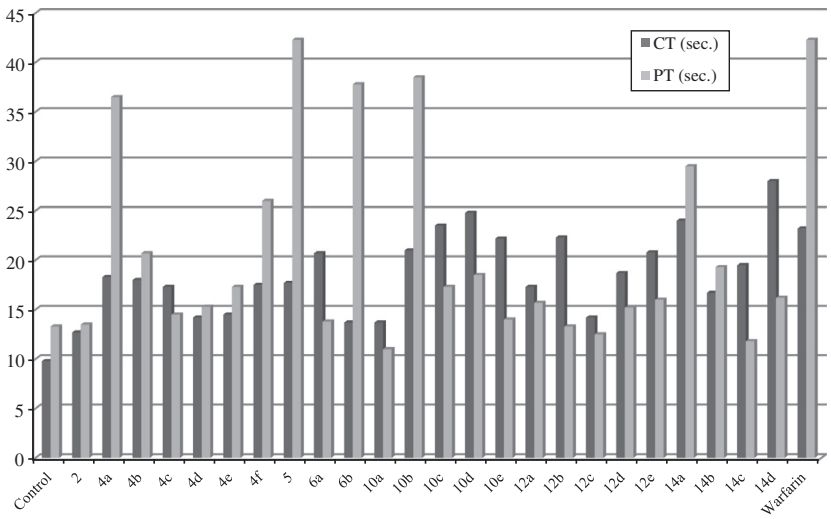


Fig. 2. The bar diagram showing antithrombotic activity (CT and PT) of the tested compounds and their comparison to control, 6-aminocoumarin **2** and warfarin.

δ = 15.10 ppm for CH_2 pyrazoline, 60.42 for CH pyrazoline, 154.31 for C=O and C-3 pyrazoline, 157.39 for C=O chromene in addition to extra signals corresponding to 20 aromatic carbons. Mass spectra showed their molecular ion peaks.

2-Chloro-*N*-(2-oxo-2*H*-chromen-6-yl)acetamide **11** was prepared according to the reported procedure [39].

6-(2-Substituted-2,5-dihydro-1*H*-imidazol-4-yl)amino-2*H*-chromen-2-one **12a–c** were prepared by refluxing 2-chloroacetamide derivative **11** with urea or thiourea or guanidine in acetone in presence of potassium carbonate to obtain imidazol-2-one, imidazol-2-thione, imidazol-2-imine, respectively (Scheme 2). The assignment of the products **12a–c** was based on spectral and analytical data. IR spectra showed the presence of additional NH(s) band(s) at 3444–3167 cm^{-1}. The ^1H NMR spectra displayed additional singlet signal(s) at δ = 7.00–10.64 ppm and a singlet signal at δ = 3.52–4.55 ppm for NH(s) and CH_2 protons, respectively. The ^{13}C NMR spectrum for compound **12c** supported the carbon skeleton of its structure as it exhibited signals at 44.00 for C-5 imidazoline, 160.46 ppm for C=O chromene and 165.36 for C-2,4 imidazoline in addition to signals corresponding to 8 aromatic

Table 2
Docking results.

Compound	Energy score S (kcal/mol)	Amino acid interactions	Interacting groups	H-bond length (Å)
RPR200095	−30.0421	Gly 218	CO piperazine	2.92
		Trp 215 (arene cation)	NH, NH_2 amidine	
		Ile 175 (through a water molecule)	NH amidine	1.96
		Phe 174 (arene arene, arene cation)	Phenyl, NH_2 amidine	
		Thr 98 (through water molecule)	NH amidine	1.96
4a	−18.1529	Arg 222 (arene cation)	Phenyl of chromene	
		Gly 216	NH amide	1.95
		Ile 175 (through a water molecule)	NH_2 amidine	1.92
		Phe 174 (arene arene)	Phenyl	
		Arg 143	CO chromene	2.86
		Thr 98 (through a water molecule)	NH_2 amidine	1.92
		Glu 97	NH amidine	2.37
4b	−16.6205	Ile 175 (through a water molecule)	NH_2 amidine	1.60
		Phe 174 (arene arene)	Phenyl	
		Thr 98 (through a water molecule)	NH_2 amidine	1.60
4d	−15.7368	Ile 175 (through two water molecules)	NH_2 amidine	1.56, 2.37
		Phe 174 (arene arene)	Phenyl	
		Tyr 99	CO amide	2.85
		Thr 98 (through a water molecule)	NH_2 amidine	1.56
4e	−14.9181	Ile 175 (through two water molecules)	NH_2 amidine	1.82, 2.37
		Phe 174 (arene arene)	Phenyl	
		Thr 98 (through a water molecule)	NH_2 amidine	1.82
4f	−15.7201	Gly 216	NH amide	1.90
		Ile 175 (through water molecule)	NH_2 amidine	2.07
		Arg 143	CO chromene	2.77
		Thr 98 (through a water molecule)	NH_2 amidine	2.07
		Glu 97	OH oxime	1.51
5	−0.8626	Gly 216	NH amide	2.20
		Phe 174 (arene arene)	Oxadiazole	
		Arg 143	CO chromene	2.72
6b	−14.1764	Gly 216	NH amide	1.92
		Phe 174 (arene arene)	Phenyl, Oxadiazole	
		Glu 97	NH amino methyl	1.92, 2.14
10b	−28.2342	Arg 222 (arene cation)	Phenyl	
		Ile 175 (through a water molecule)	CO chromene	2.77
		Thr 98 (through a water molecule)	CO chromene	2.77
10c	−20.3689	Ser 195	CO chromene	2.82
		Phe 174 (arene arene)	Phenyl	
10d	−23.6996	Ser 195	CO chromene	2.65
		Phe 174 (arene arene)	Phenyl	
12a	−15.8009	His 57 (through a water molecule)	CO imidazole	2.52
		Gly 193 (through a water molecule)	CO imidazole	2.83
12d	−16.6637	Ile 175 (through a water molecule)	CO chromene	3.26
		Tyr 99	CO triazine	3.02
		Thr 98 (through a water molecule)	CO chromene	3.26
12e	−17.9016	Gly 216	NH, =N triazine	1.47, 3.13
		Tyr 99	NH triazine	2.76
14a	−16.1391	Gly 216	NH triazene	1.58
		Phe 174 (arene arene),	Pyridine	
14b	−15.5532	Gly 216	NH pyrimidine, CO pyrimidine	2.03, 2.88
		Gln 192	CO pyrimidine	2.67
		Ile 175 (through a water molecule)	CO chromene	3.03
		Thr 98 (through a water molecule)	CO chromene	3.03
14d	−19.1792	Ile 175 (through a water molecule)	CO chromene	2.82
		Thr 98 (through a water molecule)	CO chromene	2.82

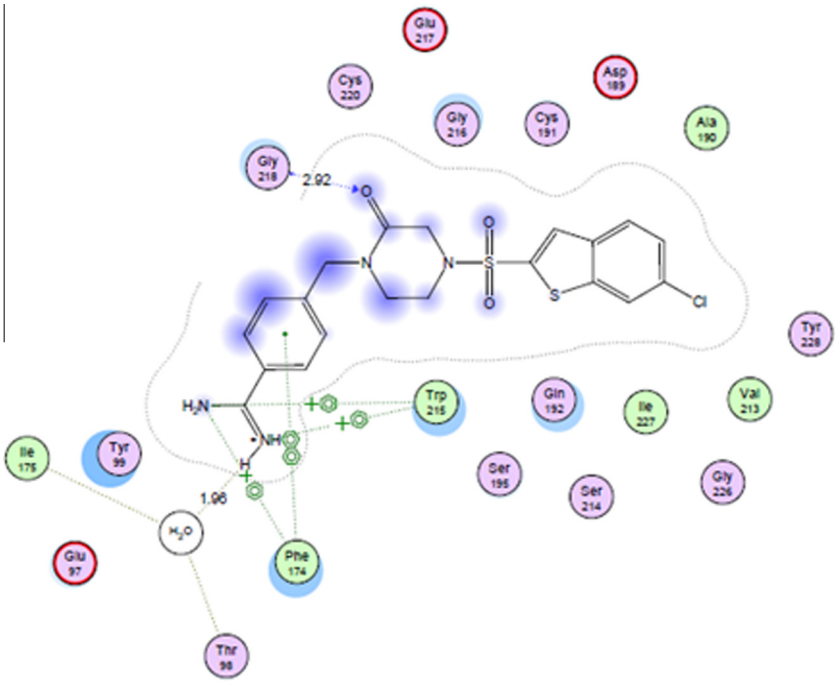


Fig. 3. 2D interactions of RPR200095 on the active site of FXa.

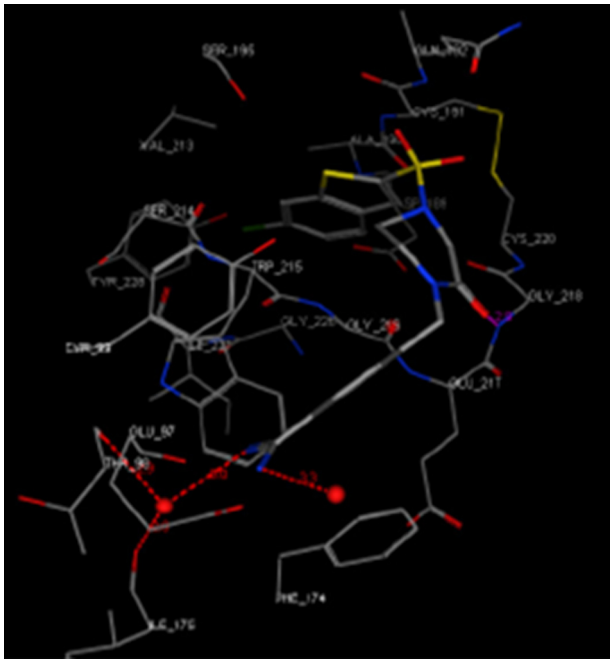


Fig. 4. 3D interactions of RPR200095 on the active site of FXa.

carbons. Additionally, mass spectra of **12a-c** demonstrated their molecular ion peaks.

6-(3-Substituted -1,2,3,6-tetrahydro-1,2,4-triazin-5-yl)amino-2*H*-chromen-2-one **12d**,**e** were prepared in a similar manner by reacting 2-chloroacetamide derivative with semicarbazide or thiosemicarbazide (Scheme 2). The structures of the synthesized compounds were deduced by spectral and microanalytical data. IR spectra showed the presence of additional NH stretching bands at 3444–3228 cm^{-1}. The ^1H NMR spectrum showed three singlet signals at δ = 6.40–10.35 ppm for NH protons and a singlet for CH_2 of triazine ring at δ = 3.52–4.57 ppm.

Diazotization of the key amine compound **2** with sodium nitrite in presence of concentrated hydrochloric acid provided the diazonium salt which upon treatment with the appropriate heterocyclic amines, mainly pyridine, pyrimidine, benzothiazole, and coumarin amines, furnished the expected triazenyl compounds **14a**–**d** upon raising pH to 7 with the aid of sodium acetate (Scheme 2) [52]. The structures of **14a**–**d** were confirmed by IR spectra that showed the presence of NH bands at 3433–3170 cm^{-1} and ^1H NMR spectra that proved the presence of D_2O exchangeable singlet signal assigned to one NH group for compounds **14a**,**c**,**d** at δ = 8.80–12.80 ppm and to three NH groups at δ = 10.09, 10.47 and 10.96 ppm in case of compound **14b**. ^{13}C NMR spectrum for compound **14c** showed bands at δ = 160.49 and 167.11 ppm corresponding to C=O chromene and C-2 benzothiazole, respectively in addition to signals for 14 aromatic carbons. The MS spectra revealed appearance of molecular ion peaks of the target derivatives.

3.2. Anticoagulant activity

All the prepared compounds in addition to the key intermediate 6-aminocoumarine **2** were evaluated for antithrombotic activity (CT and PT) in mice.

The whole blood clotting time is a rough measure for all intrinsic clotting factors in the absence of tissue factors. Whole blood, when removed from the vascular system and exposed to a foreign surface, will form a solid clot. Within limits, the time required for the formation of the solid clot is a measure of the coagulation system [53].

One stage prothrombin time test specifically evaluates the presence of factors II, V, VII and X [54]. A drop in the concentration of any of these factors will cause the blood to take longer time to clot. During therapy with oral anticoagulant, the activity of vitamin K-dependent clotting factors (II, VII, IX, X, Protein C and Protein S) is impaired and PT is increased. During oral anticoagulation therapy, the activity of vitamin K-dependent clotting factors (II, VII, IX, Protein C and Protein S) is reduced and PT is increased [55,56]. Therefore, PT is the method of choice for monitoring oral anticoagulant therapy [42].

Results of the experiments were expressed as mean (M) ± standard error (SE) in seconds. The significance of difference between groups was determined using one-way analysis of variance (ANOVA) (Table 1, and Fig. 2).

All amidino derivatives showed significant prolongation in CT except compounds **4d** and **4e**. Concerning PT results, unsubstituted amidine derivative **4a** proved to be the most potent anticoagulant of this series with high PT value (36.5 s) comparable to that of warfarin (PT, 42.3 s). Substitution on the amidine nitrogen of **4a** with hydroxy and methyl group afforded **4f** and **4b** derivatives with good activity (PT values, 26.0 and 20.7 s, respectively). The reduction in the activity was more pronounced in compounds with N-ethyl **4c** and branched alkyl **4d**,**e**, probably due to the increased steric bulkeness of the large *N*- alkyl substituents.

Cyclization of amidoxime moiety in **4f** resulted in the oxadiazole derivative **5**, the most potent amongst all tested compounds, displaying excellent PT value (42.3 s.) similar to that of warfarin. Elongation of compound **5** by incorporation of heterocycle moieties was found to affect the antithrombotic activity. While the coumarinyl congener **6b** showed also high activity with PT value (37.8 s), the benzothiazole counterpart **6a** was inactive (PT, 13.8 s). Interestingly, on contrary to PT results, the reduction in the CT value was more pronounced in compound **6b**.

For pyrazole carboxamide derivatives **10a**–**e**, the presence of electron attracting substituent (chloro, bromo, methoxy) on both phenyl moieties was unfavorable, as anticougulant activities of **10c**–**e** were observed to be dropped significantly (PT:14.0-18.5 s) whereas electron donating groups (methyl, dimethylamino) in congener **10b** correlated to its high activity (PT: 38.5 s). However, replacement of bromo and methoxy substituent in compounds **10c** and **10e**, respectively, with hydrogen significantly abolished activity of the resulting compound **10a** (PT 11.0 s).

Furthermore, imidazoles **12a**–**c** and their bioisoster triazines **12d**,**e** linked to the coumarin scaffold through NH spacer, produced significant increase in CT except iminoimidazolo derivative **12c**. On contrary, all imidazolino and triazino compounds did not show increase in PT compared to control group.

Finally, all heterocyclic triazenyl derivatives **14a**–**d** showed an increase in CT especially pyridino **14a** (24.0 s) and coumarin **14d** (28.0 s) derivatives. Notably, a decrease in PT values to a much lower extent had already been observed in going from pyridine **14a** (29.5 s) to pyrimidine **14b** (19.3 s) and coumarin **14d** (16.2 s) moieties. The benzothiazole congener **14c** showed inactive PT value (11.8 s) compared to control group.

3.3. Molecular docking

The trypsin like serine protease factor Xa, converting prothrombin to thrombin, is located at the convergence point of the intrinsic and extrinsic pathway and plays a pivotal role in the blood coagulation cascade. This process involves signal amplification as one molecule of factor Xa activates many molecules of prothrombin to thrombin [57–60]. Therefore, inhibition of factor Xa is thought to be an effective treatment for a variety of thrombotic events with less bleeding risk than direct thrombin inhibition.

Four binding pockets were identified within the active site, labelled S1 to S4. The S1 and S4 binding pockets are the important ones that are exploited by FXa inhibitors. The S1 pocket is a narrow cleft defined by Asp 189, Ala 190 and Gln 192 and favors positively charged moieties such as amine, guanidine and benzamidine [44]. The other main binding pocket, the S4 pocket, is shaped by the side chains of Tyr 99, Phe 174 and Trp 215 [61,62].

The binding affinity of the ligand was evaluated with energy score (S, kcal/mol). The compound which revealed the highest binding affinity, minimum dock score, is the one forming the most stable ligand-enzyme complex. Length of the hydrogen bond and arene cation interaction were also used to assess the binding models. The results of docking studies; dock score, involved FXa active

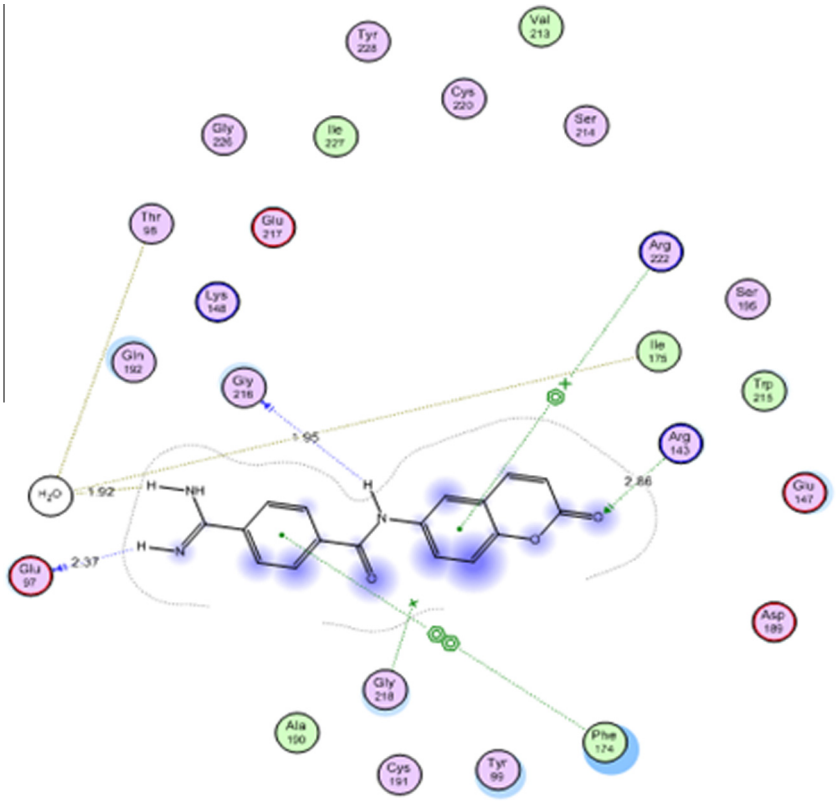


Fig. 5. 2D interactions of **4a** on the active site of FXa.

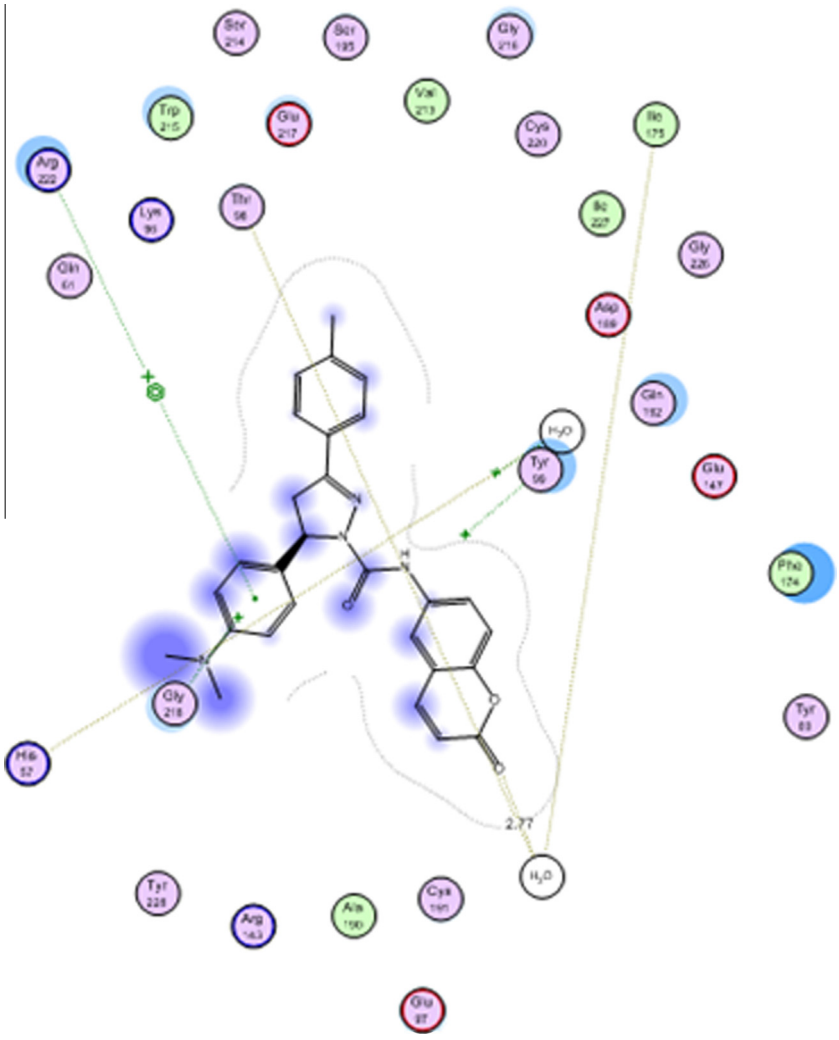


Fig. 6. 2D interactions of **10b** on the active site of FXa.

site amino acid interacting ligand moieties and hydrogen bond length for each compound and reference inhibitor are listed in Table 2 and Figs. 3–7.

Analysis of the docking results revealed that:

(i) The inhibitor (RPR200095)-FXa complex was precisely reproduced by the docking procedure as demonstrated by low root mean square deviation, rmsd (0.3279) and dock score (−30.0421 kcal/mol, Table 2), i.e. the docking protocol was valid. As shown in Fig. 3 and 4, the inhibitor RPR200095 nearly fits in the active site forming various hydrogen bonding interactions with the active site residues: CO of piperazine with Gly 218 (2.92 Å), NH of amidine with Ile 175 (1.96 Å) and Thr 98 (1.96 Å) through water molecule. Also, inhibitor forms arene cation interaction of NH and NH_2 of amidine with Trp 215 and Phe 174 and arene arene interaction of phenyl ring with Phe 174.

(ii) The docking scores for compounds **4a**,**b**,**d**–**f**, **5**, **6b**, **10b**–**d**, **12a**,**d**,**e** and **14a**,**b**,**d** were all in the range −28.2342 to −0.8626 kcal/mol. A significant correlation between dock scores and anticoagulant activity, PT value, of the compounds was not routinely observed.

For benzamidine derivatives **4a**,**b**,**d**–**f** (dock score, −18.1529 to −14.9181 kcal/mol), a high negative dock score was estimated to

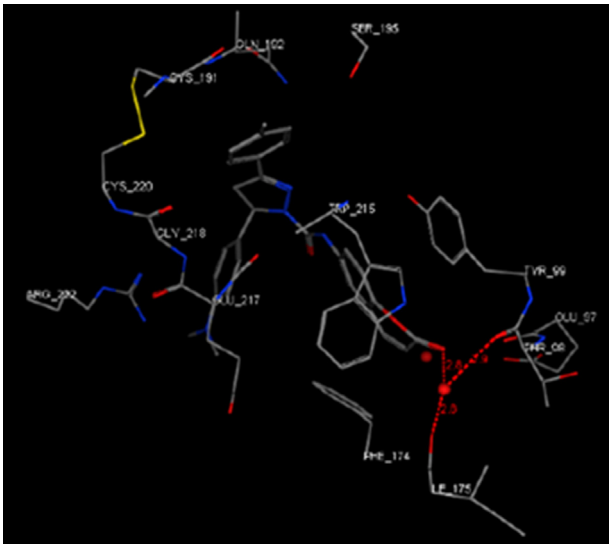


Fig. 7. 3D interactions of **10b** on the active site of FXa.

unsubstituted amidine derivative **4a**, the fourth most active compound (PT: 36.5 s), whereas the *N*-hydroxy derivative **4f** with relatively lower PT value (PT: 26.5 s), was found to have albeit less

negative dock score (−15.7368 kcal/mol). However, the docking scores of *N*-alkyl congeners **4b** (−16.6205 kcal/mol), **4d** (−15.7368 kcal/mol) and **4e** (−14.9181 kcal/mol) were correlated to their moderate anticoagulant activity (PT: 20.7, 15.3 and 17.3 s, respectively).

For the heterocycle coumarins series, surprisingly, the oxadiazole derivative **5** which is the first most active analogue (PT: 42.3 s) has the lowest dock score (−0.8626 kcal/mol). The same observation was noticed for the bicoumarinyl oxadiazole analogue **6b** as it showed a low docking score (−14.1764 kcal/mol) irrespective of its high PT value (PT: 37.8 s).

In contrast, the second most active analogue, the pyrazole **10b** (PT: 38.5 s) has the highest score (−28.2342 kcal/mol) and the congeners **10c**,**d** were found to have significant lower docking scores (−20.3689 and −23.6996 kcal/mol, respectively) which may reflect their much poorer PT values (17.3 and 18.5 s, respectively).

Furthermore, the docking scores for the imidazole **12a** (−15.8009 kcal/mol) and the triazine analogues **12d**,**e** (−16.6637 and −17.9016 kcal/mol, respectively), were correlated to their moderate activity (PT: 15.7 s) and (PT: 15.2 and 16.0 s, respectively).

Among the triazenyl derivatives **14a**,**b**,**d**, the pyridine congener **14a** with PT value (29.5 s) has a docking score (−16.1391 kcal/mol) that was lower than that obtained by the less active coumarinyl derivative **14d** (PT: 16.2 s. and dock score −19.1792 kcal/mol).

(iii) Inspection of the binding mode also demonstrated that all compounds show from one to five hydrogen bonds and/or arene arene or arene cation interactions with the enzyme active site residue. Arg 222, Gly 216, Ser 195, Gly 193, Gln 192, Ile 175, Phe 174, Arg 143, Tyr 99, Thr 98 and Glu 97 are the amino acids residue involved in these interactions. In common with RPR200095 FXa inhibitors, most of the docked compounds interacted with at least one of the S4 binding pocket amino acid residues Ile 175, Phe 174 and Thr 98.

For example, the fourth most active compound **4a** (energy score: −18.1529 kcal/mol) mediated five strong hydrogen bonds with Gly 216 (1.95 Å), Ile 175 through a water molecule (1.92 Å), Arg 143 (2.86 Å), Thr 98 through a water molecule (1.92 Å) and Glu 97 (2.37 Å) through NH amide, NH_2 amidine, CO chromene and NH amidine respectively, arene cation interaction between benzene ring of coumarin and Arg 222 and arene arene interaction between phenyl ring and Phe 174 (Fig. 5).

Furthermore, the pyrazole carboxamide **10b** (−28.2342 kcal/mol.), the first one in score ranking and the second most active compound showed two strong hydrogen bonds through a water molecule Ile 175 (2.77 Å) and Thr 98 (2.77 Å) through CO chromene and arene cation interaction between phenyl and Arg 222 (Fig. 6 and 7).

4. Conclusion

A total of twenty-three newly prepared coumarin derivatives of two series: *N*-coumarinyl-4-amidinobenzamides (**4a**–**f**) and 6-heterocycle substituted coumarin derivatives (**5**, **6a**,**b**, **10a**–**e**, **12a**–**e**, **14a**–**d)** were tested in mice to determine if they have any anticoagulant effects by investigating the CT and PT values. The obtained results revealed many compounds with high CT and PT values comparable to their precursor aminocoumarin **2** and the control group, while a few of them presented PT values similar to that of the reference drug warfarin.

Amongst the compounds, the oxadiazole **5** was the most active expressing the same PT value of warfarin (42.3 s) followed by pyrazole **10b** (PT: 38.5 s), coumarinyl oxadiazole **6b** (PT: 37.8 s.) and the unnsubstituted amidine **4a** (PT: 36.5 s.). Thus, the coumarin scaffold with those substituents in presence of carboxamide moiety would form suitable matrix for the development of more enhanced candidates with outstanding oral antithrombotic activity and low side effects.

Although the correlation between dock score and measured PT by the compounds was not routinely observed, most of the docked compounds shared some binding interactions with FXa similar to those of the native ligand inhibitor. This suggests that these compounds might possibly act as FXa inhibitors, and this may contribute at least in part to their anticoagulant activity.

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