Synthesis, antioxidant, anticoagulant, and fibrinolytic activities of new isatin derivative

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Background
Isatin as a product was first obtained from the oxidation of indigo dye by nitric acid and chromic acids by Otto Linné Erdman and Auguste Laurent in 1841.
Objective
This study presents the synthesis of some new isatin derivatives and examines their biological activities.
Materials and methods
2-Cyano-*N'*-(5-nitro-2-oxoindolin-3-ylidene)acetohydrazide (**2**) reacted with some reagents, namely, salicylaldehyde, phenyl isothiocyanate, ethyl chloroacetate, ethyl iodide, ethyl cyanoacetate, thioglycolic acid, phenyl isothiocyanate, malononitrile, and hydrazine hydrate to produce compounds **3, 4, 5, 6, 8, 9, 10, 11, and 12,** respectively. Moreover, compound **6** reacted with hydrazine hydrate to produce compound **7**. Antimicrobial activities of some newly synthesized compounds were studied.
Results
Antioxidant, anticoagulant, and fibrinolytic activities of the new synthesized compounds (**1–12**) were studied. Compound **10** exhibited highest fibrinolytic activity. On the contrary, compound **12** exhibited highest anticoagulant activities. Moreover, it was noticed that compound **9** exhibited highest antioxidant activity.
Conclusion
In summary, 14 novel isatin derivatives were synthesized and screened for their antioxidant, anticoagulant, and fibrinolytic activities. Some compounds displayed moderate-to-excellent activities such as antioxidant, anticoagulant, and fibrinolytic agents.

Keywords:
anticoagulant, antioxidant, nitro-isatin, pyridine, pyrimidine, thiazole

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Introduction

It has been reported that several isatin derivatives have antioxidant [1], anticoagulant [2], fibrinolytic [3], antibacterial, anticonvulsant, antifungal, anti-HIV, and anti-inflammatory activities [4,5]. Isatin as a product was first obtained from the oxidation of indigo dye by nitric acid and chromic acids by Otto Linné Erdman [6] and Auguste Laurent [7] in 1841. Isatin is a multifaceted heterocyclic compound found in some plants as a natural product, such as genus Isatis and in *Couroupita guianensis Aubl* [8,9] and has also been present in humans as a metabolic derivative of adrenaline [10]. With broad clinical and pharmacological applications, a wide variety of isatin derivatives can be synthesized from isatin [8,11,12]. Derivatives of isatin have recently drawn considerable attention of researchers worldwide owing to their wide applications as anti-HIV [4,5], anti-tubercular [13], anti plasmodial [14], anticonvulsant [15], and sedative and hypnotic [16] agents. Triazole as well as another class of azole group is a versatile pharmacophore, possessing diverse pharmacological properties [17,18] such as herbicidal [19], antitumor [20], antipsychotic [21], anticoagulant [22], antimicrobial [23], and antagonist [24]. Among the available substituted hydrazines, cyanoacetohydrazide is a convenient intermediate for the synthesis of a variety of heterocyclic compounds [25–36]. Schiff bases are found to be the most potent anticonvulsant agents [37], with a large spectrum in clinical application; they were studied by cyclic voltammetry, square wave, and differential pulse voltammetry over a wide pH range using a general certificate of education. The results of some studies of the electrochemical behavior of some isatin derivatives showed that the semicarbazone, nitro groups attached to the isatin ring and hydrazine give rise to separate and different

oxidation and reduction mechanisms [38]. The literature survey revealed that the introduction of electron-withdrawing groups, for example, nitro group at positions 5, 6, and 7, greatly increased activity from that of isatin, with substitution at the fifth position being most favorable. This is not surprising, as C-5 substitution has previously been associated with increased biological activity for a range of indole-based compounds [39,40]. Therefore, the aim of the present study was to examine the antimicrobial, antioxidant, and fibrinolytic and anticoagulation activities of synthesis of nitro-isatin and their derivatives by modification on structure.

Materials and methods

Chemistry

All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100. Elemental microanalyses were carried out in Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, using VarioElementar and were found within ±0.4% of the theoretical values. Infrared spectra were recorded on a FT/IR-4100 Jasco, Japan, Fourier transform infrared spectrometer at cm^{-1} scale using KBr disc technique at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. ^{1}H NMR and ^{13}C NMR spectra were determined by using a JEOL AS-500 NMR spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. Varian Gemini200-Oxford 300 MHz and Merury Plus-Oxford 400 MHz at Ministry of Defense, Chemical Warfare Department, The Main Chemical Warfare Laboratories, Cairo, Egypt. Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard. The mass spectra were measured with a GC MSQp1000EX Shimadzu, Cairo University, Cairo, Egypt, and with a Finnigan MAT SSQ-7000 mass spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. Follow-up of the reactions and checking the purity of the compounds were made by thin-layer chromatography on silica gel-precoated aluminum sheets (Type 60, F 254; Merck, Darmstadt, Germany) using chloroform/methanol (20 : 2, v/v), and the spots were detected by exposure to UV lamp at λ254 nanometer for few seconds and by iodine vapor. The chemical names given for the prepared compounds are according to the International Union of Pure and Applied Chemistry system.

The following compounds were synthesized:

2-Cyano-*N*'-*(5*-nitro-2-oxoindolin-3-ylidene) acetohydrazide (2): it was a mixture of nitro-isatin [41] (1.92 g, 0.01 mol) and cyanoacetohydrazide (0.99 g, 0.01 mol) in 1,4-dioxane (20 ml) warmed for 5 min. After slow evaporation, the solid which separated was collected by filtration and then recrystallized from 1,4-dioxane to give **2**. Yield: 85%; M.p. 230–232°C. IR spectrum (KBr, ν, cm^{-1}): 3200, 3189 (2NH), 2218 (C<td:glyph name=″tbnd6″/>N), 1714, 1694 (2C=O). ^{1}H NMR (DMSO-*d6*) δ: 3.1 (s, 2H, CH_2), 7.8–8.6 (m, 3H, Ar-H), 10.2, 10.5 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 27.1, 117.5, 122.9, 123.0, 124.6, 126.7, 133.9, 142.8, 147.1, 167.5, 171.8. MS: m/z=273 (27.5%) ($M^{\cdot+}$); Anal. Calcd. For $C_{11}H_7N_5O_4$ (273.2): C, 48.36; H, 2.58; N, 25.63%; found: C, 48.16; H, 2.48; N, 25.59%.

***N*'-(5-nitro-2-oxoindolin-3-ylidene)-2-oxochromane-3-carbohydrazide (3)**: it is a mixture of compound **2** (1.09 g, 0.004 mol) and salicylaldehyde (0.48 ml, 0.004 mol) in 1,4-dioxane (20 ml) and piperidine (0.5 ml) stirred for 3 h at room temperature. The reaction mixture was poured onto ice and acidified with dilute acetic acid. The precipitated was filtered off, and washed with cold water several times and then recrystallized from toluene to give **3.** Yield: 75%; M.p. more than 300°C. IR spectrum (KBr, ν, cm^{-1}): 3190, 3176 (2NH), 1710, 1698, 1685 (3C=O). ^{1}H NMR (DMSO-*d6*) δ: 3.3 (d, 2H, CH_2), 3.6 (t, 1H, CH), 7.4–8.3 (m, 7H, Ar-H), 10.3, 10.7 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 27.5, 55.9, 116.8, 118.2, 122.6, 122.9, 125.2, 126.3, 126.8, 129.8, 132.5, 133.8, 143.2, 146.5, 152.0, 168.5, 169.8, 174.9. MS: m/z=378 (8.6%) (M^{+}-2); Anal. Calcd. For $C_{18}H_{12}N_4O_6$ (380.3): C, 56.85; H, 3.18; N, 14.73%; found: C, 56.80; H, 3.10; N, 14.69%.

2-Cyano-3-(2-(5-nitro-2-oxoindolin-3-ylidene) hydrazinyl)-3-oxo-*N*-phenyl propanethioamide (4): compound **2** (2.73 g, 0.01 mol) was added to a stirred solution of potassium hydroxide (0.56 g, 0.01 mol) in *N, N*-dimethylformamide (20 ml). Phenyl isothiocyanate (1.35 g, 0.01 mol) was added to the resulting mixture after the mixture was stirred for 30 min. Stirring was continued for 12 h at room temperature. The reaction mixture was acidified with cold dilute HCl. The product that separated was filtered, washed with water, and recrystallized. Yield: 75%; M.p. 181–183°C. IR spectrum (KBr, ν, cm^{-1}): 3225, 3200, 3189 (3NH), 2215 (C&8801;N), 1690, 1683 (2C=O). ^{1}H NMR (DMSO-*d6*) δ: 3.4 (s, 1H,

CH), 7.1–8.4 (m, 8H, Ar-H), 10.5, 10.8, 11.1 (3s, 3H, 3NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 56.5, 115.3, 117.2, 121.8, 122.8, 126.4, 126.6, 126.9, 127.5, 128.6, 129.1, 134.5, 137.5, 143.2, 146.8, 167.8, 171.2, 193.6. MS: m/z=407 (1.3%) (M^+-1); Anal. Calcd. For $C_{18}H_{12}N_6O_4S$ (408.3): C, 52.94; H, 2.96; N, 20.58%; found: C, 52.90; H, 2.92; N, 20.50%.

2-Cyano-*N*'-(5-nitro-2-oxoindolin-3-ylidene)-2-(4-oxo-3-phenylthiazolidin-2-ylidene) acetohydrazide (5): to a stirred solution of potassium hydroxide (0.56 g, 0.01 mol) in *N, N*-dimethylformamide (20 ml), compound **2** (2.73 g, 0.01 mol) was added. After the mixture was stirred for 30 min phenyl isothiocyanate (1.35 g, 0.01 mol) was added to the resulting mixture. Stirring was continued at room temperature for 12 h and then ethyl chloroacetate (1.07 ml, 0.01 mol) was added and stirring was continued for additional 6 h. The reaction mixture was acidified with cold dilute acetic acid. The separated solid was filtered off, washed several times with cold water and recrystallized. Yield: 80%; M.p. 191–193°C. IR spectrum (KBr, ν, cm^{-1}): 3224, 3190 (2NH), 2217 (C&8801;N), 1715, 1695, 1689 (3C=O). ^1H NMR (DMSO-*d6*) δ: 4.1 (s, 2H, CH_2), 6.8–7.9 (m, 8H, Ar-H), 9.7, 10.5 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 32.5, 70.1, 114.5, 117.8, 122.6, 123.5, 125.6, 128.3, 128.5, 128.6, 128.8, 128.9, 133.6, 138.9, 143.7, 146.5, 167.3, 168.1, 168.6, 177.5. MS: m/z=447 (1.5%) (M^+-1); Anal. Calcd. For $C_{20}H_{12}N_6O_5S$ (448.4): C, 53.57; H, 2.70; N, 18.74%; found: C, 53.52; H, 2.68; N, 18.71%.

2-Cyano-3-(ethylthio)-*N*'-(5-nitro-2-oxoindolin-3-ylidene)-3-(phenylamino) acrylohydrazide (6): compound **2** (2.73 g, 0.01 mol) was added to a stirred solution of potassium hydroxide (0.56 g, 0.01 mol) in *N, N*-dimethylformamide (20 ml). Phenyl isothiocyanate (1.35 g, 0.01 mol) was added after the mixture was stirred for 30 min. Stirring was continued for 12 h at room temperature and then ethyl iodide (0.62 ml, 0.01 mol) was added and stirring was continued for additional 6 h. The separated solid was filtered off, washed with cold water several times, and recrystallized. Yield: 86%; M.p. 170–172°C. IR spectrum (KBr, ν, cm^{-1}): 3228, 3200, 3189 (3NH), 2215 (C&8801;N), 1710, 1690 (2C=O). ^1H NMR (DMSO-*d6*) δ: 1.2 (t, 3H, CH_3), 3.2 (q, 2H, CH_2), 6.9–8.1 (m, 8H, Ar-H), 9.6, 10.1, 10.8 (3 s, 3H, 3NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 15.6, 25.8, 70.3, 114.5, 117.3, 121.5, 121.9, 123.3, 125.1, 125.2, 126.8, 128.7, 129.3, 133.6, 135.3, 142.6, 146.9, 167.3, 167.6, 177.9. MS: m/z=436 (1.1%) (M^+); Anal. Calcd. For $C_{20}H_{16}N_6O_4S$ (436.4): C, 55.04; H, 3.70; N, 19.26%; found: C, 54.99; H, 3.68; N, 19.24%.

5-Amino-*N*'-(5-nitro-2-oxoindolin-3-ylidene)-3-(phenylamino)-1*H*-pyrazole-4-carbohydrazide (7): it is a mixture of hydrazine hydrate 80% (0.005 mol) and **6** (0.87 g, 0.002 mol) in ethanol (20 ml), which was heated under reflux for 4 h. The solvent was evaporated in vacuo, and the solid product obtained was collected and recrystallized. Yield: 75%; M.p. 187–189°C. IR spectrum (KBr, ν, cm^{-1}): 3350, 3330 (NH_2), 3270, 3250, 3200, 3189 (4NH), 1699, 1685 (2C=O). ^1H NMR (DMSO-*d6*) δ: 6.8–8.3 (m, 8H, Ar-H), 9.6 (s, 2H, NH_2, D_2O exchangeable), 10.4, 10.7, 10.9, 11.1 (4 s, 4H, 4NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 85.6, 116.3, 116.5, 117.5, 122.3, 122.6, 123.5, 125.8, 128.4, 128.6, 133.6, 140.9, 142.3, 146.5, 151.2, 151.7, 162.8, 167.9. MS: m/z=406 (1.2%) (M^+); Anal. Calcd. For $C_{18}H_{14}N_8O_4$ (406.3): C, 53.20; H, 3.47; N, 27.58%; found: C, 53.18; H, 3.44; N, 27.53%.

Ethyl-5′-amino-5-nitro-2,7′-dioxo-4′,7′-dihydro-1′*H*-spiro[indoline-3,2′-pyrazolo-[1,5-a] pyrimidine]-3′-carboxylate (8): ethyl cyanoacetate (0.45 g, 0.004 mol) was added to a solution of compound **2** (1.09 g, 0.004 mol) in ethanol (20 ml) containing piperidine (0.5 ml). The reaction mixture was refluxed for 3 h, and then poured on ice and acidified with dilute acetic acid. The precipitated solid was filtered off, washed with cold water several times and recrystallized. Yield: 70%; M.p. more than 300°C. IR spectrum (KBr, ν, cm^{-1}): 3322, 3315 (NH_2), 3250, 3200, 3195 (3 NH), 1705, 1689 (3C=O). ^1H NMR (DMSO-*d6*) δ: 1.8 (t, 3H, CH_3), 3.3 (q, 2H, CH_2), 3.9 (s, 1H, CH), 6.4 (s, 2H, NH_2, D_2O exchangeable), 6.9–7.9 (m, 3H, Ar-H), 10.5, 10.9, 11.2 (3 s, 3H, 3NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 16.3, 60.5, 72.5, 78.9, 83.8, 110.2, 122.9, 126.7, 127.8, 140.5, 143.6, 146.8, 152.3, 159.6, 167.2, 168.6. MS: m/z=386 (8.3%) (M^+); Anal. Calcd. For $C_{16}H_{14}N_6O_6$ (386.3): C, 49.74; H, 3.65; N, 21.75%; found: C, 49.71; H, 3.62; N, 21.72%.

***N*'-(5-nitro-2-oxoindolin-3-ylidene)-2-(4-oxo-4,5-dihydrothiazol-2-yl)aceto hydrazide (9)**: it is a mixture of compound **2** (1.09 g, 0.004 mol) and thioglycolic acid (0.3 ml, 0.004 mol) in dry pyridine (20 ml) that was refluxed for 3 h. The reaction mixture was poured on ice cold acetic acid after cooling. The solid separated was filtered off, washed with cold water several times, and recrystallized from toluene to give **9**. The remaining solid which was insoluble in toluene

was recrystallized from 1,4-dioxane to give **9**. Yield: 80%; M.p. more than 300°C. IR spectrum (KBr, ν, cm^{-1}): 3208, 3189 (2 NH), 1700, 1670, 1655 (3C=O). ^{1}H NMR (DMSO-*d6*) δ: 3.1 (s, 2H, CH_2), 4.1 (s, 2H, CH_2), 7.1–8.4 (m, 3H, Ar-H), 10.4, 10.6 (2 s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 38.5, 43.2, 119.1, 122.5, 123.6, 125.3, 134.5, 142.7, 146.8, 162.3, 168.2, 171.3, 175.8. MS: m/z=347 (1.2%) (M^+); Anal. Calcd. For $C_{13}H_9N_5O_5S$ (347.3): C, 44.96; H, 2.61; N, 20.17%; found: C, 44.92; H, 2.59; N, 20.15%.

4-Imino-*N*′-(5-nitro-2-oxoindolin-3-ylidene)-3-phenyl-2-thioxothiazolidine-5-carbohydrazide (10): to a solution of compound **2** (2.73 g, 0.01 mol) in ethanol (15 ml) containing triethylamine (0.5 ml), elemental sulfur (0.32 g, 0.01 mol) and phenyl isothiocyanate (1.35 g, 0.01 mol) were added. The reaction mixture heated with continuous stirring for 2 h at 60°C. The reaction mixture was acidified with cold dilute acetic acid after cooling. The precipitated product was filtered off, washed with cold water several times and recrystallized. Yield: 75%; M.p. 170–172°C IR spectrum (KBr, ν, cm^{-1}): 3223, 3210, 3189 (3 NH), 1715, 1695 (2C=O). ^{1}H NMR (DMSO-*d6*) δ: 3.3 (s, 1H, CH), 7.9-8.1 (m, 8H, Ar-H), 10.2, 11.0, 11.3 (3s, 3H, 3NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 58.6, 117.2, 122.3, 123.6, 125.1, 127.6, 129.1, 129.3, 133.0, 132.3, 132.5, 133.6, 143.6, 146.3, 165.9, 167.3, 171.2, 192.4. MS: m/z=440 (0.95%) ($M^{\cdot+}$); Anal. Calcd. For $C_{18}H_{12}N_6O_4S_2$ (440.4): C, 49.09; H, 2.75; N, 19.08%; found: C, 49.01; H, 2.71; N, 19.01%.

7′-Ethoxy-5-nitro-2,5′-dioxo-1′,5′-dihydro-3′*H*-spiro[indoline-3,2′-[1,2,4]triazolo-[1,5-a]pyridine]-6′,8′-dicarbonitrile (11): a mixture of 2-(3,4-dimethoxybenzylidene) malononitrile (0.94 g, 0.004 mol) and **2** (1.09 g, 0.004 mol) in 1,4-dioxane (25 ml) in the presence of triethylamine (0.5 ml) was refluxed for 3 h. Then, it was concentrated and acidified with cold dilute acetic acid. The solid separated out was filtered off, washed with cold water several times and recrystallized. Yield: 80%; M.p. 241–243°C. IR spectrum (KBr, ν, cm^{-1}): 3222, 3215, 3187 (3 NH), 2215, 2225 (2C&8801;N), 1701, 1690 (2C=O). ^{1}H NMR (DMSO-*d6*) δ: 1.2 (t, 3H, CH_3), 4.2 (q, 2H, CH_2), 7.1-7.9 (m, 3H, Ar-H), 10.4, 10.6, 10.9 (3 s, 3H, 3NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 15.8, 65.4, 65.8, 68.3, 104.6, 108.2, 115.2, 115.6, 122.7, 125.6, 130.7, 143.9, 147.8, 158.2, 160.4, 167.8, 184.3. MS: m/z=392 (1.2%) (M^+-1); Anal. Calcd. For $C_{17}H_{11}N_7O_5$ (393.3): C, 51.91; H, 2.82; N, 24.93%; found: C, 51.89; H, 2.78; N, 24.90%.

***N*′-(6-nitro-3-oxo-2,3-dihydrocinnolin-4(1*H*)-ylidene)hydrazinecarbohydrazide (12)**: a mixture of hydrazine hydrate 80% (0.01 mol) and **2** (1.09 g, 0.004 mol) and in 1,4-dioxane (20 ml) was refluxed for 2 h. The solid precipitated after cooling was collected by filtration and recrystallized. Yield: 85%; M.p. more than 300°C. IR spectrum (KBr, ν, cm^{-1}): 3370, 3333 (NH_2), 3255, 3222, 3200, 3189 (4 NH), 1701, 1695 (2C=O). ^{1}H NMR (DMSO-*d6*) δ: 4.3 (s, 2H, NH_2, D_2O exchangeable), 7.0–8.0 (m, 3H, Ar-H), 10.1, 10.7, 10.8, 11.2 (4s, 4H, 4NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 111.7, 114.3, 121.5, 125.3, 135.6, 144.3, 146.7, 153.2, 157.4. MS: m/z=278 (4.4%) (M^+-1); Anal. Calcd. For $C_9H_9N_7O_4$ (279.2): C, 38.72; H, 3.25; N, 35.12%; found: C, 38.70; H, 3.21; N, 35.10%.

Pharmacology

Antioxidant activity (DPPH assay)

From modified synthesized isatin compounds, stock solutions will be prepared by dissolving 10 mg of samples in 1 ml DMSO is an organo sulfur compound with the formula $(CH_3)_2SO$, and then diluted to several dilutions. From each concentration, a triplicate of 10 µl will be prepared and then 90 µl of DPPH was added on Eliza plate, and after that plate will be stored in dark cover with aluminum foil for 30 min and then measured at 520 nm on ELISA reader.

Anticoagulation activity

The anticoagulation activities of the different synthesized compounds adopting the method of USA, Pharmacopoeia [42], for the assay of sodium heparin were evaluated as follows:

Reagents

The following reagents were used: standard heparin sodium preparation, human plasma and calcium chloride solution 1% (w/v), and saline solution 0.9% (w/v).

Procedure

Hard-glass test tubes (31×100 mm) were cleaned overnight by immersion in chromic acid. Either 0.8 ml of sample solution (0.01%), 0.8 ml of standard heparin sodium solution (1.4 U.S.P unit/0.8 ml), or 0.8 ml saline solution as a control to each tube was added. Moreover, 1 ml plasma and 0.2 ml calcium chloride solution were added to each of the prepared tubes. The tubes were incubated in a water bath at 37°C. Each tube was stoppered, and the time was immediately recorded. The average of three readings is the time required for clotting was then determined.

Fibrinolytic activity

By exposing a plasma clot to the effect of an aqueous solution (at suitable concentration) of the investigated, the fibrinolytic activity was determined. Under the same conditions mentioned previously for determination of anticoagulation activity, preparation of the plasma clot was achieved [42].

Procedure

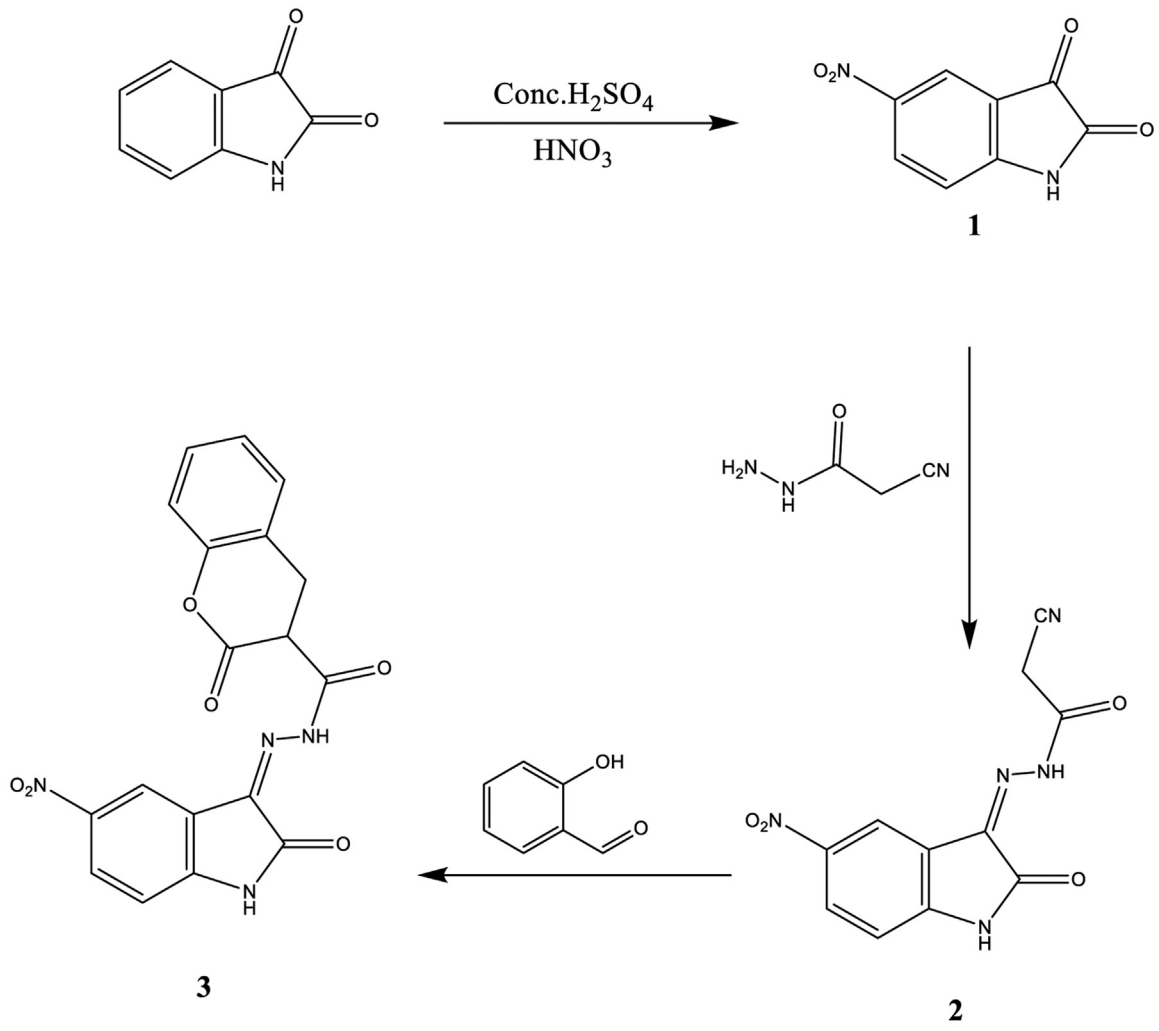
By immersion overnight in chromic acid, sets of three-hard-glass test tubes (31×100 mm) were cleaned. 1 ml plasma and 0.2 ml calcium chloride solution (1% w/v) were added to each tube 0.8 ml saline solution (0.89% w/v). After mixing, the tubes were incubated in a water bath at 37°C, and 1 ml of either the saline solution, Pentosan polysulfate (Hemoclar) preparation (2 mg/tube), or the tested sample (1 mg/tube), was added individually when clotting was complete. The lyses percentages of the plasma clots at 37°C were recorded with each sample and compared with the standard Pentosan polysulfate (Hemoclar).

Results and discussion

Chemistry

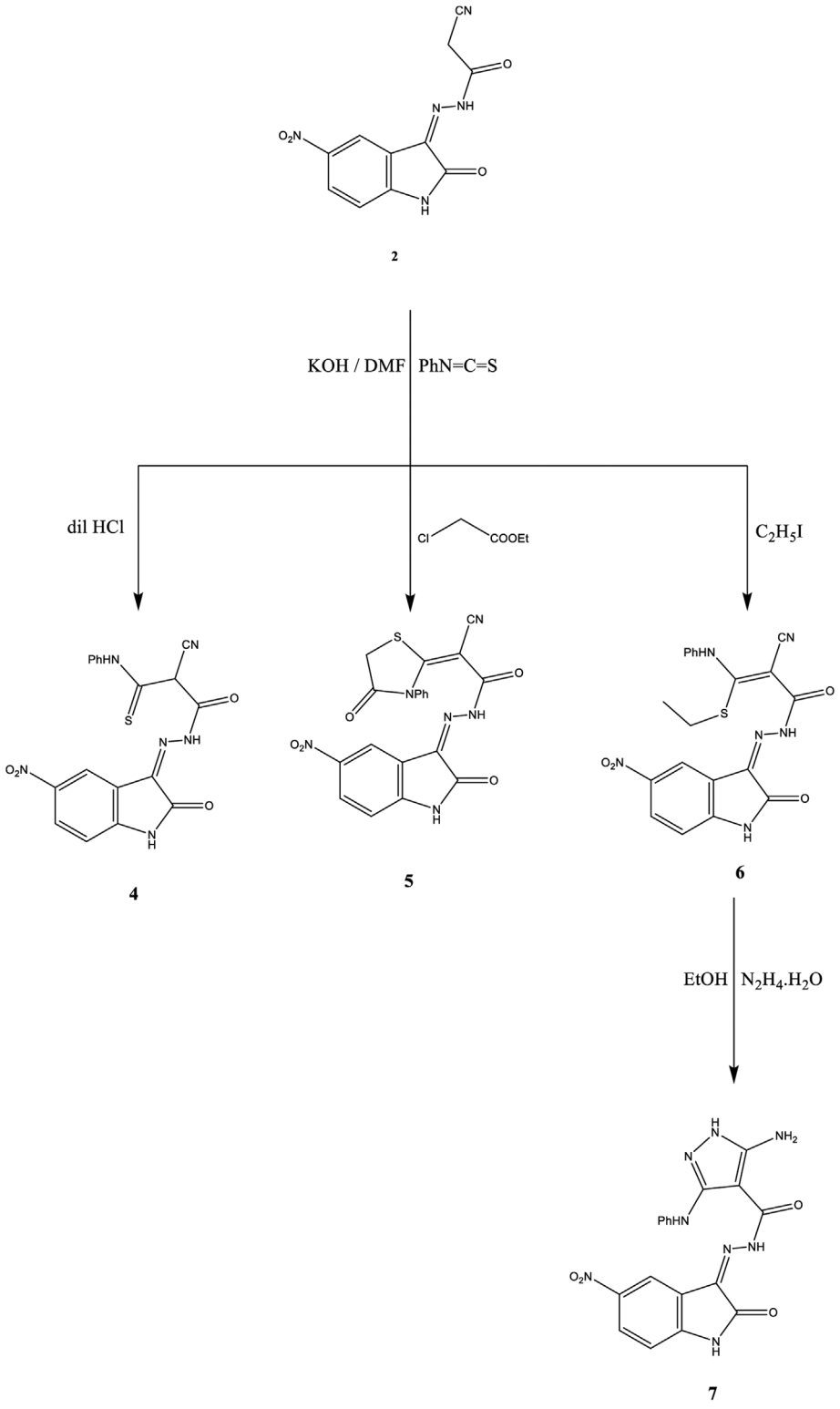
Compound 2-cyano-*N*'-(5-nitro-2-oxoindolin-3-ylidene)acetohydrazide (**2**) was synthesized by the reaction of nitro-isatin with cyanoacetohydrazide in 1,4-dioxane which then reacted with salicylaldehyde to give *N*'-(5-nitro-2-oxoindolin-3-ylidene)-2-oxochromane-3-carbohydrazide **3** (Scheme 1). IR spectra of **2** showed absorption bands at 1694, 1714 cm^{-1} due to the respective (2C=O) and absorption band at 2218 cm^{-1} due to the presence of (C&8801; N). ^{13}C NMR spectrum of compound **2** exhibited confirmatory signal of CH_2 at δ 27.1. The IR spectra of **3** showed absorption bands at 1685, 1698, and 1710 cm^{-1} due to the respective (3C=O) and disappearance of C&8801;N group band. Moreover, compound **2** reacted with phenyl isothiocyanate to synthesize 2-cyano-3-(2-(5-nitro-2-oxoindolin-3-ylidene)hydrazinyl)-3-oxo-*N*-phenyl-propane thioamide (**4**). Furthermore, compound **2** was allowed to react with ethyl choloroacetate

Scheme 1



Synthesis of nitro-isatin derivatives 1–3.

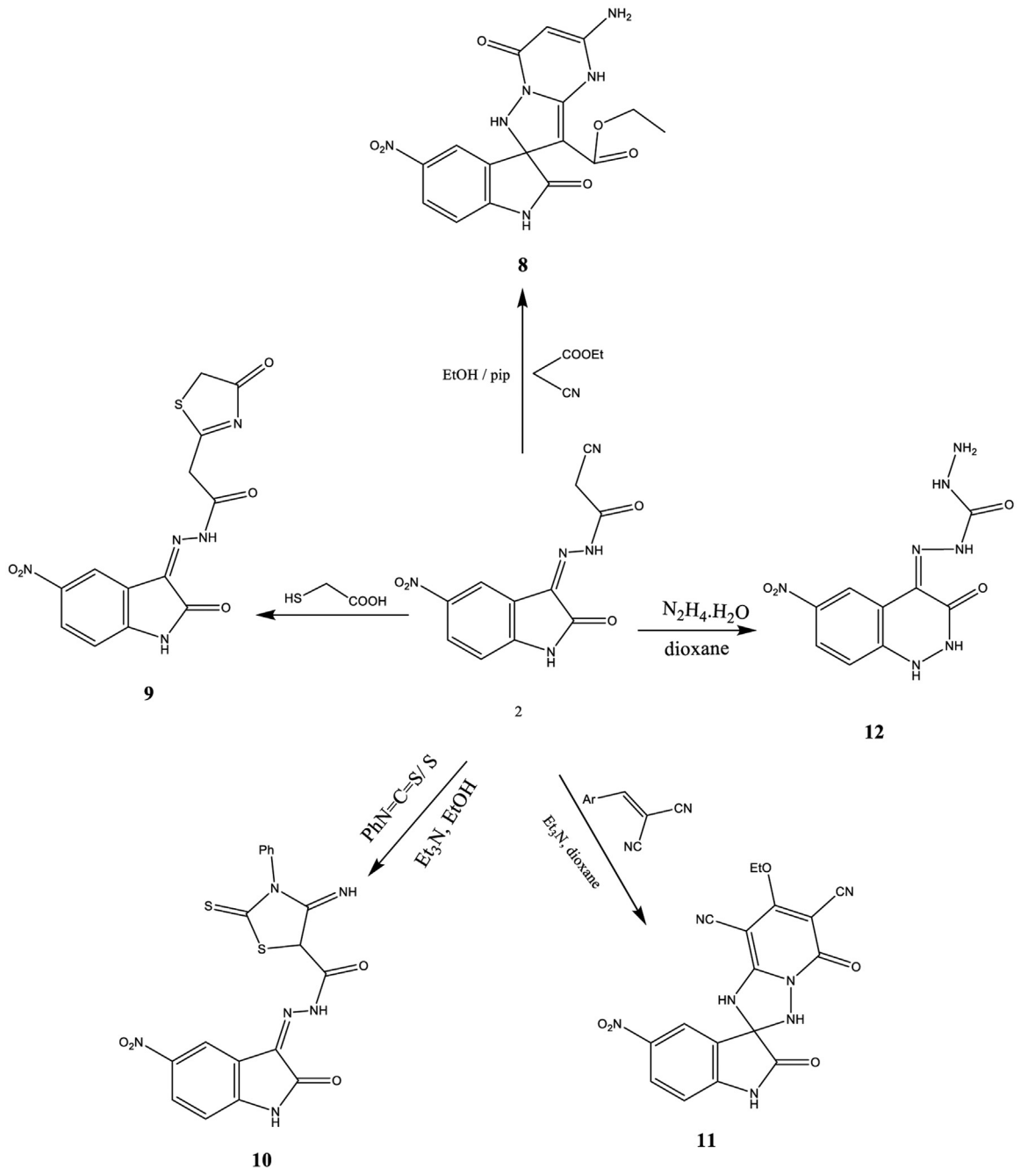
Scheme 2



Synthesis of nitro-isatin derivatives 4–7.

to give 2-cyano-*N*'-(-5-nitro-2-oxoindolin-3-ylidene)-2-(4-oxo-3-phenylthiazolidin-2-ylidene)aceto hydrazide (**5**). Compound 2-cyano-3-(ethylthio)-*N*'-)-5-nitro-2-oxoindolin-3-ylidene)-3-(phenyl amino) acrylohydrazide (**6**) was yielded by the reaction of compound **2** with ethyl iodide, which then reacted with hydrazine hydrate to give 5-amino-*N*'-(5-nitro-2-oxoindolin-3-ylidene)-3-(phenylamino)-*1H*-pyrazole-4-carbohydrazide (**7**) (Scheme 2). ^{13}C NMR spectrum of compound **6** exhibited confirmatory signal of CH_3 at δ 15.6. ^{1}H NMR (DMSO-d_6) spectra of compound **7** revealed signal at δ 6.8–8.3

Scheme 3



Synthesis of nitro-isatin derivatives 8–12.

(m, 8H, Ar-H), 9.6 (s, 2H, NH_2, D_2O exchangeable), 10.4, 10.7, 10.9, 11.1 (4 s, 4H, 4NH, D_2O exchangeable). Moreover, we synthesized ethyl-5′-amino-5-nitro-2,7′-dioxo-4′,7′-dihydro-*1′H*-spiro[indoline-3,2′-pyrazolo [1,5-a]pyrimidine]-3′-carboxylate (**8**) from the reaction of compound **2** with ethyl cyanoacetate. Compound *N*′-(5-nitro-2-oxoindolin-3-ylidene)-2-(4-oxo-4,5-dihydrothiazol-2-yl)acetohydrazide (**9**) was synthesized by the reaction of thioglycolic acid with compound **2**. Moreover, compound **2** reacted with elemental sulfur to yield 4-imino-*N*′-(5-nitro-2-oxoindolin-3-ylidene)-3-phenyl-2-thioxothiazolidine-5-carbohydrazide (**10**). Malononitrile reacted with compound **2** to give 7′-Ethoxy-5-nitro-2,5′-dioxo-1′,5′-dihydro-*3′H*-spiro[indoline-3,2′-[1,2,4]triazolo [1,5-a]pyridine]-6′,8′-dicarbonitrile (**11**). Furthermore, *N*′-(6-nitro-3-oxo-2,3-dihydrocinnolin-4*(1H)*-ylidene) hydrazinecarbohydrazide (**12**) was synthesized from the reaction of compound **2** with hydrazine hydrate (Scheme 3). IR spectra of **9** showed absorption bands at 1655, 1670, and 1700 cm^{-1} due to the presence of (3C=O) and disappearance of C&8801;N group band. Moreover, ^1H NMR (DMSO-d_6) spectra of compound **12** revealed signal at δ 4.3 ppm representing NH_2 and

D_2O exchangeable, and signals at δ 10.1, 10.7, 10.8, 11.2 ppm representing 4NH groups.

Pharmacology

Fibrinolytic and anticoagulant activities of isatin derivatives

Fibrinolytic activity in isatin derivatives was analyzed and the novel isatin derivatives exhibited the promising results in pharmaceutics, and also anticoagulant activity in isatin derivatives. From the results shown in Table 1, compound **10** exhibited highest fibrinolytic activity (the percentage of lysis of plasma clot was 80%), whereas compounds **2** and **4** exhibited higher fibrinolytic activity as compared with those of 'rest compounds' (50% lysis of plasma clot) and standard 'Pentosan polysulfate (Hemoclar)' preparation (75% lysis) (Fig. 1). However, compound **12** exhibited the highest anticoagulant activity for 29 min (Fig. 2). Then compounds **1** and **2** considered also exhibited highest anticoagulant activity compared with the rest compounds, despite being still lower than that of standard 'Heparin' preparation (Fig. 2). The significant variation in pharmacological activities of the isatin compounds happened may be owing to the position of branching and chemical structure features.

Table 1 Anticoagulant and fibrinolytic activities of novel compounds

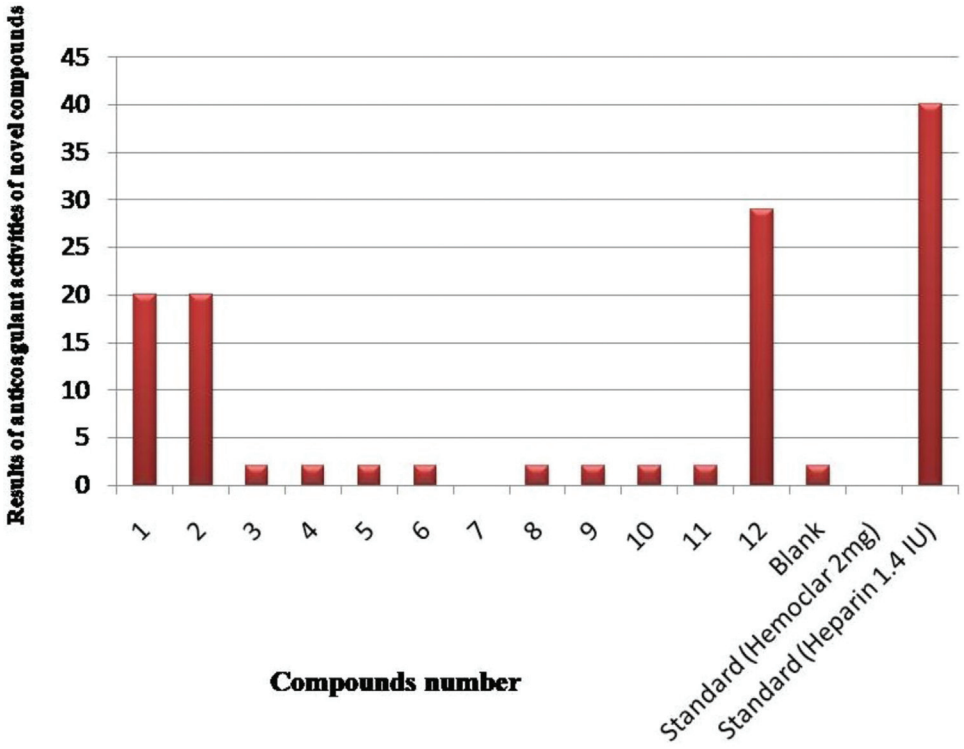
Compounds no.	Anticoagulant	Fibrinolytic
1	20 min	+2
2	20 min	+4
3	2 min	+3
4	2 min	+4
5	2 min	+2
6	2 min	+3
7	NA	NA
8	2 min	+2
9	2 min	+2
10	2 min	+6
11	2 min	+2
12	29 min	+2
Blank	2	–
Standard (Hemoclar 2 mg)	–	75
Standard (Heparin 1.4 IU)	? 30	–

Activity color key c**olor:** Higher activity. Moderate activity. Low activity.

Antioxidant activity of isatin derivatives

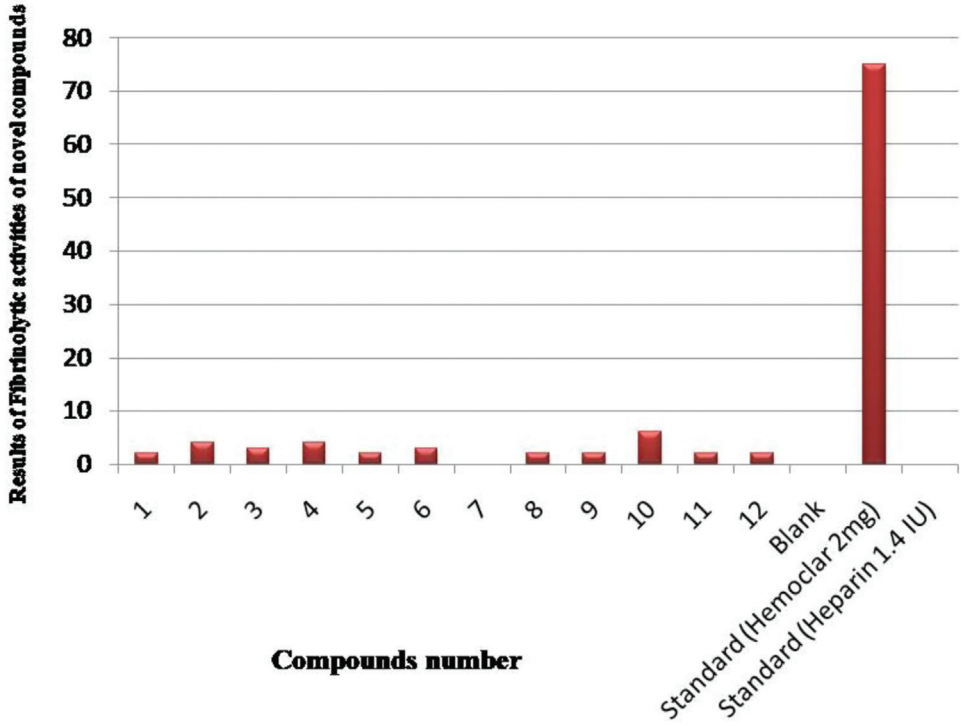
The DPPH free-radical scavenging activity of isatin derivatives was evaluated. From data described in Table 2, it was noticed that compound **9** exhibited highest antioxidant activity at 85% (Fig. 3). Then, compounds **3, 4, 11,** and **12** were considered also good antioxidant compounds as compared with other rest compounds. Despite these results, ascorbic acid standard has slightly higher activity than compound **9**.

Figure 1



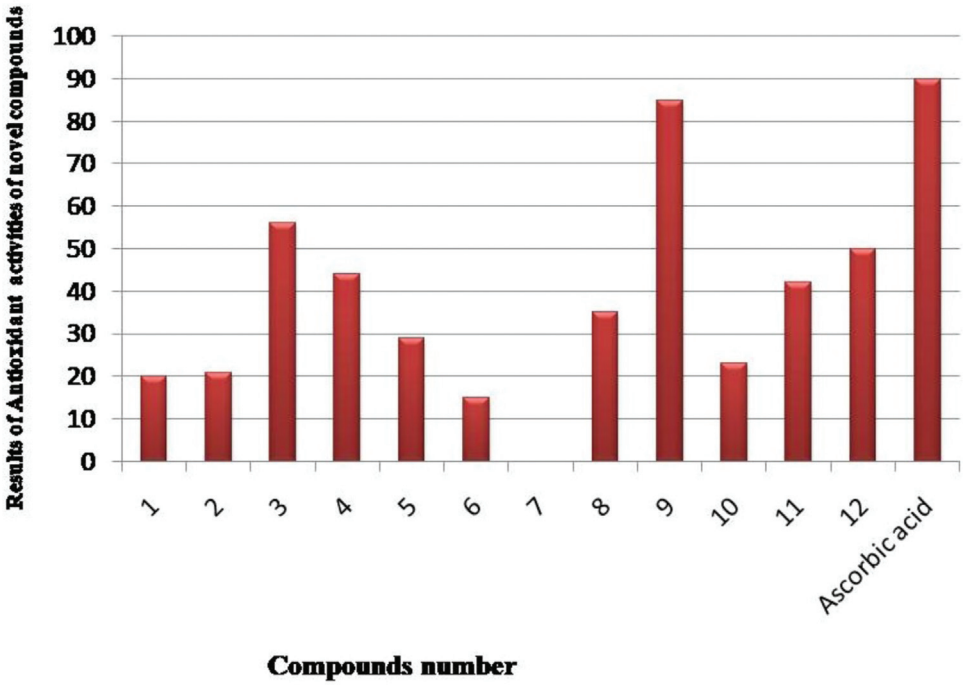
Results of fibrinolytic activities of novel compounds.

Figure 2



Results of anticoagulant activities of novel compounds.

Figure 3



Results of antioxidant activities of novel compounds.

Structural-activity relationship

Based on the structural-activity relationship of the prepared compounds, it was revealed that the presence of S groups in compound **10** exhibited highest fibrinolytic activity. However, the presence of $NHNH_2$ group in compound **12** increased the anticoagulant activity. Moreover, the presence of active methylene group in compound **9** resulted in the highest antioxidant activity.

Table 2 Antioxidant activities of novel compounds

Compound	%
1	20
2	21
3	56
4	44
5	29
6	15
7	NA
8	35
9	85
10	23
11	43
12	50
Ascorbic acid	90

Activity color key color: Higher activity. Moderate activity. Low activity.

Conclusion

Some new series of isatin derivatives were synthesized and screened for their antioxidant, anticoagulant, and fibrinolytic activities. Results showed that (Table 1) compound **10** exhibited the highest fibrinolytic activity (the lysis of plasma clot percentage was 80%), whereas compounds **2** and **4** exhibited higher fibrinolytic activity compared with those of 'rest compounds' (50% lysis of plasma clot) and standard 'Pentosan polysulfate (Hemoclar)' preparation (75% lysis). On the contrary, compound **12** exhibited highest anticoagulant activities at 29 min. Moreover, from Table 2, it was noticed that compound **9** exhibited highest antioxidant activity at 85%.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Manolov I, Maichle-Moessmer C, Nicolova I, Danchev N. Synthesis and anticoagulant activities of substituted 2,4-diketochromans, biscoumarins, and chromanocoumarins. Arch Pharm 2006; 339:319–326.

2 Hamdi N, Puerta MC, Valerga P. Synthesis, structure, antimicrobial and antioxidant investigations of dicoumarol and related compounds. Eur J Med Chem 2008; 43:2541–2548.

3 Nath S, Smitha S, Seshaiah J. Biological activities of isatin and its derivatives. Acta Pharm 2005; 55:27–46.

4 Chohan ZH, Pervez H, Rauf A. Isatin-derived antibacterial and antifungal compounds and their transition metal complexes. J Enz Inhib Med Chem 2004; 19:417.

5 Pandeya SN, Smitha S, Jyoti M, Sridhar SK. Biological activities of isatin and its derivatives. Acta Pharm 2005; 55:27.

6 Erdmann OL. Untersuchungen über den Indigo. J Prakti Chemie 1840 19:321–362.

7 Laurent AO. Recherches sur l'indigo. Annal Chimieet Physique 1840 3:393–434.

8 Silva JFM, Garden SJ, Pinto AC. The chemistry of isatins. J Braz Chem Soc 2001; 12:273.

9 Ischia M, Palumbo A, Prota G. Adrenalin oxidation revisited. New products beyond the adrenochrome stage. Tetrahedron 1988; 44:6441.

10 Glover V, Bhattacharya SK, Chakrabarti A, Sandler M. The psychopharmacology of isatin. Stress Med 1998; 14:225.

11 Glover V, Revely MA, Sandler M. Inhibitory potency of some isatin analogues on human monoamine oxidase A and B. Biochem Pharmacol 1980; 29:467.

12 Shvekhgeimer MGA. Synthesis of heterocyclic compounds by the cyclization of isatin and its derivatives. Chem Heterocycl Compd 1996; 32:249.

13 Sriram D, Yogeeswari P, Meena K. Synthesis, anti-HIV and antitubercular activities of isatin derivatives. Pharmazie 2006; 61:274–277.

14 Chiyan Zu I, Clarkson C, Smith P, Lehman J, Rosenthal J, Chibale PJK. Design, synthesis and anti-plasmodial evaluation in vitro of new 4-aminoquinoline isatin derivatives. Bioorg Med Chem 2005; 13:3249.

15 Verma M, Pandey S, Singh N, Stables KN. Anticonvulsant activity of Schiff bases of isatin derivatives. J P Acta Pharm 2004; 54:49.

16 Smitha S, Pandey SN, Stables JP. Anticonvulsant and sedative-hypnotic activities of N-substituted isatin semicarbazones. Acta Pharm 2002; 4:129–134.

17 Moustafa OS, Ahmed RA. Synthesis and antimicrobial activity of some new cyanopyridines, isoxazoles, pyrazoles, and pyrimidines bearing sulfonamide moiety. Phosph Sulf Silic Relat Elements 2003; 178:475–484.

18 Mahadevan KM, Basavaray KM, Mathias DAP, Vaidya VP. Synthesis of novel naphtho[2,1-b]furo pyrazolyl, isoxazolyl and pyridyl derivatives as potential antimicrobial agents. Indian J Chem 2005; 44:789–793.

19 Nizamuddin N, Khan MH, Srivastava MK, Tiwari S. Synthesis and herbicidal activity of 1, 3, 10-triaryl-imidazolo[4,5-e]pyrido[1, 2-a]-2, 3, 4, 10-tetrahydro-pyrimidin-2-thiones. Indian J Chem 2000; 39:853.

20 Ganjee A, Yuan M, Queener SF. 6-substituted 2,4-diaminopyrido[3,2-d] pyrimidine analogues of piritrexim as inhibitors of dihydrofolate reductase from rat liver, pneumocystis carinii, and toxoplasma gondii and as antitumor agents. J Med Chem 1998; 41:4533.

21 Markourtz JS, Brown CS, Moare TR. Atypical antipsychotics part I: pharmacology, pharmacokinetics, and efficacy. Ann Pharm Cotha 1999; 33:73.

22 Quan ML, Liquw AY, Pruitty JR, Carini DS, Bostrom L, Harrison KR, Knabb M. Design and synthesis of isoxazoline derivatives as factor Xa inhibitor. J Med Chem 1999; 42:170287.

23 Kedar RM, Vidhale NN, Chincholkar MM. Synthesis of new heterocyclics and their antimicrobial study. Orient J Chem 1999; 13:143.

24 Zhang H, Li, Chung JC, Costello TD, Valcis I, Ward R. The enantiospecific synthesis of an isoxazoline. A RGD mimic platelet GPIIb/IIIa antagonist. J Org Chem 1997; 62:2466.

25 Mahmoud MR, El-Ziaty AK, Abu El-Azm FSM, Ismail MF, Shiba SA. Utility of cyano-N-(2-oxo-1,2-dihydroindol-3-ylidene)acetohydrazide in the synthesis of novel heterocycles. J Chem Res 2013; 37:80–85.

26 Bondock S, Tarhoni AEG, Fadda AA. Utility of cyanoacetic acid hydrazide in heterocyclic synthesis. Arkivoc 2006; ix:113.

27 Marcos APM, Dayse NM, Clarissa PF, Kelvi LNZ, Helio GB. Reaction of β-alkoxyvinyl halomethyl ketones with cyanoacetohydrazide. J Braz Chem Soc 2008; 19: 1361.

28 Mahmoud MR, E1-Ziaty AK, Ismail MF, Shiba SA. Synthesis of novel pyrimidine and fused pyrimidine derivatives. Eur J Chem 2011; 2:347.

29 Mahmoud MR, El-Shahawi MM, Abu El-Azm FSM. Synthesis of novel quinazolinone and fused quinazolinones. Eur J Chem 2011; 2:404.

30 Mahmoud MR, Madkour HMF, El-Bordany EAA, Soliman SA. Synthesis and reactions of (Z)-2-imino-5-(3,4,5-trimethoxy benzylidene)thiazolidin-4 (H)one. Eur J Chem 2011; 2:475.

31 Mahmoud MR, El-Shahawi MM, Abu El-Azm FSM, Farahat SE. Synthesis and spectral characterization of novel pyridotriazolo-, pyridothiazolo-, pyridotetrazolopyrimidines and pyridopyrimidotriazepine derivatives for potential pharmacological activities. Am J Org Chem 2011; 1:14.

32 Mahmoud MR, Abou-Elmagd WSI, Derbala HA, Hekal MH. Novel synthesis of some phthalazinone derivatives. Chin J Chem 2011; 29: 1446.

33 Mahmoud MR, Abou-Elmagd WSI, Abd El-Wahab SS, Soliman SA. Synthesis and spectral characterisation of novel 2,3-disubstituted quinazolin-4(3H)-one derivatives. J Chem Res 2012; 66.

34 Mahmoud MR, Abou-Elmagd WSI, Derbala HA, Hekal MH. Synthesis and spectral characterisation of some phthalazinone derivatives. J Chem Res 2012; 75–82.

35 Mahmoud MR, Madkour HMF, Habashy MM, El-Shwaf AM. Heteroannulation of pyrido[2,3-d]pyrimidines. Synthesis and spectral

characterization of pyridotriazolopyrimidines, pyridopyrimidotriazine and pyridopyrimidotriazepine derivatives. Am J Org Chem 2012; 2:39.

36 Mahmoud MR, E1-Ziaty AK, Hussein AM. Utility of S-Benzylthiuronium chloride in the synthesis of heterocyclic systems. World Appl Sci J 2012; 17:101.

37 Mathur G, Nain S. Recent advancement in synthesis of isatin as anticonvulsant agents. Med chem 2014; 4:4.

38 Carlos BS, Oliveira B, Isabel F, Fernandes G, Silva BV, Oliveira-Brett PA. Isatin nitro-derivatives redox behaviou. CA J Electroanalyt Chem 2013; 689:207–215.

39 Lee D, Long SA, Murray XX, DeWolf WE Jr. Potent and selective nonpeptide inhibitors of caspases 3 and 7. J Med Chem 2001; 44:2015.

40 Calvery HO, Noller CR, Adam R. Arsonophenyl-cinchoninic acid (arsonocinchophen) and derivatives II. J Am Chem Soc 1925; 47:3058–3060.

41 Cane A, Tournaire M, Barritault D,, CrumeyrolleArias M. The endogenous oxindoles 5-hydroxyoxindole and isatin are antiproliferative and proapoptotic. Biochem Biophys Res Commun 2000; 276:379.

42 Pharmacopeia of the United States of America. 16th Revised. U.S.P. XVI. Board of Trustees 1960; 317.