Synthesis and Antimicrobial, Antiplatelet, and Anticoagulant Activities of New Isatin Deivatives Containing a Hetero-Fused Imidazole Fragment

**A. V. Bogdanov[a,b,*], A. R. Sirazieva[a], A. D. Voloshina[a], T. A. Abzalilov[c],
A. V. Samorodov[c], and V. F. Mironov[a]**

[a] *Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center, Russian Academy of Sciences,
Kazan, 420088 Russia*
[b] *Kazan (Volga region) Federal University, Kazan, 420008 Russia*
[c] *Bashkir State Medical University, Ministry of Health of the Russian Federation, Ufa, 450008 Russia*
*e-mail: abogdanov@inbox.ru

Abstract—A series of isatin derivatives containing an adenine or theophylline fragment have been synthesized. The corresponding *N'*-[2-(trimethylammonio)acetyl] and *N'*-(2-pyridinioacetyl) hydrazones have been found to exhibit neither cytotoxicity nor hemotoxicity. Quaternary salts based on adenine derivatives of 5-methyl- and 5-ethylisatins showed the highest antiplatelet activity which exceeded the activity of acetylsalicylic acid by a factor of 1.5.

Keywords: isatin, xanthines, hydrazones, antimicrobial activity, hemotoxicity, anticoagulants, antiplatelet activity

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INTRODUCTION

Imidazole derivatives exhibit a broad spectrum of biological activity [1–3] (Fig. 1). Among them, 4-nitroimidazoles like **A** have been studied most thoroughly. A particular position in the series of functionally substituted imidazoles is occupied by 4,5-hetero-fused derivatives of natural and synthetic origins, namely purines and xanthines. Adenine (9*H*-purin-6-amine) is a natural compound whose derivatives are potential scaffolds for the design of compounds possessing antiviral [4] and antimicrobial activities [5, 6] (e.g., structure **B** in Fig. 1). Another important and interesting

class of hetero-fused imidazoles is represented by xanthines that are referred to as privileged structures [7, 8]. Studies of xanthine derivatives containing methyl groups at different positions (theophylline, caffeine, theobromine) resulted in the discovery of pentoxifylline (**D**) which is an efficient drug in the therapy of cardiovascular diseases [9]. It has also been reported that combinations of caffeine (1,3,7-trimethylxanthine) with a number of antibiotics show enhanced antibacterial activity against *P. aeruginosa* and *S. aureus* [10].

The use of the isatin scaffold in searching for new antibacterial and antiviral drugs, including those active against SARS CoV-2 [11–16], and the synthesis of
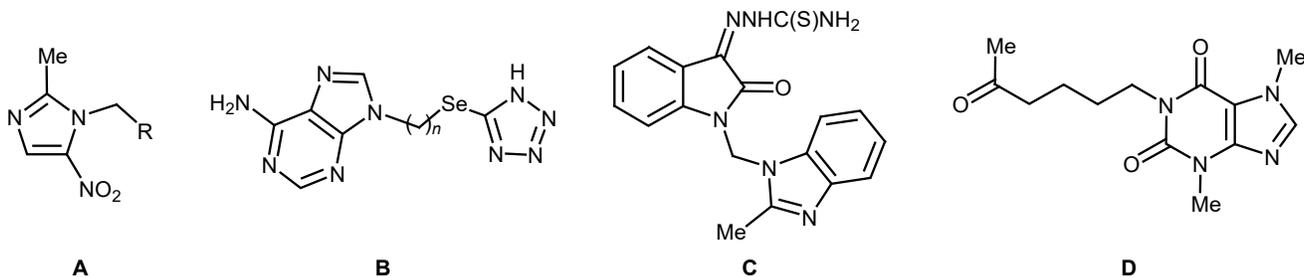

Fig. 1. Biologically active imidazole derivatives.

hybrid compounds based on isatin and imidazole remain important problems. It should be noted that we have found no published data on antimicrobial properties of isatin derivatives containing an imidazole moiety nor data on their effect on hemostasis. Only antiviral activity of isatin thiosemicarbazone (**C**) containing a benzimidazole fragment at N^1 has been reported [17].

Our studies in the field of isatin-based ammonium salts indicated high potential of these compounds as efficient and nontoxic antimicrobial agents and hence the necessity of more profound structural modification of their molecules [18–23].
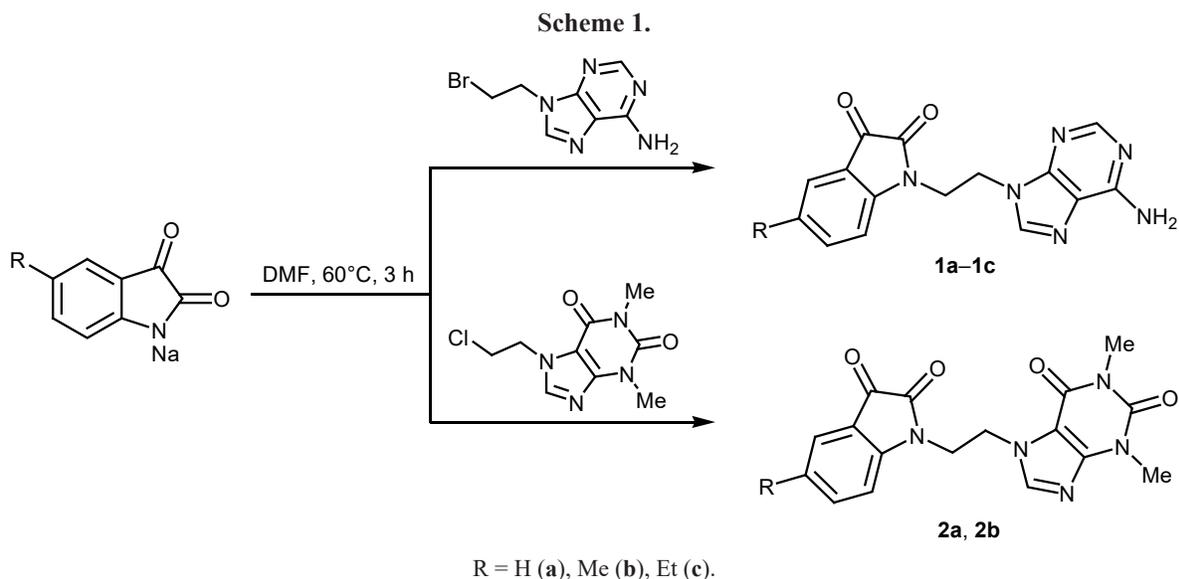
RESULTS AND DISCUSSION

Herein we describe the synthesis of a new series of isatin derivatives linked to a purine fragment through an ethylene bridge. The alkylation of isatins containing a substituent in the aromatic ring was carried out according to the procedure described in [22] using 9-(2-bromoethyl)adenine and 7-(2-chloroethyl)theophylline as alkylating agents (Scheme 1). Compounds **1a**–**1c**, **2a**, and **2b** were obtained in high yields, and they required no further purification. The yields of theophylline derivatives **2a** and **2b** were slightly lower, presumably due to their better solubility in aqueous DMF; we failed to isolate additional amounts of these compounds therefrom. The structure of isatin derivatives **1a**–**1c**, **2a**, and **2b** was unambiguously confirmed by IR and ^1H and ^{13}C NMR spectra.

Compounds **1a**–**1c**, **2a**, and **2b** were then reacted with acetohydrazides containing a trimethylammonium, pyridinium, or 2,3-dimethylpyridinium cationic center
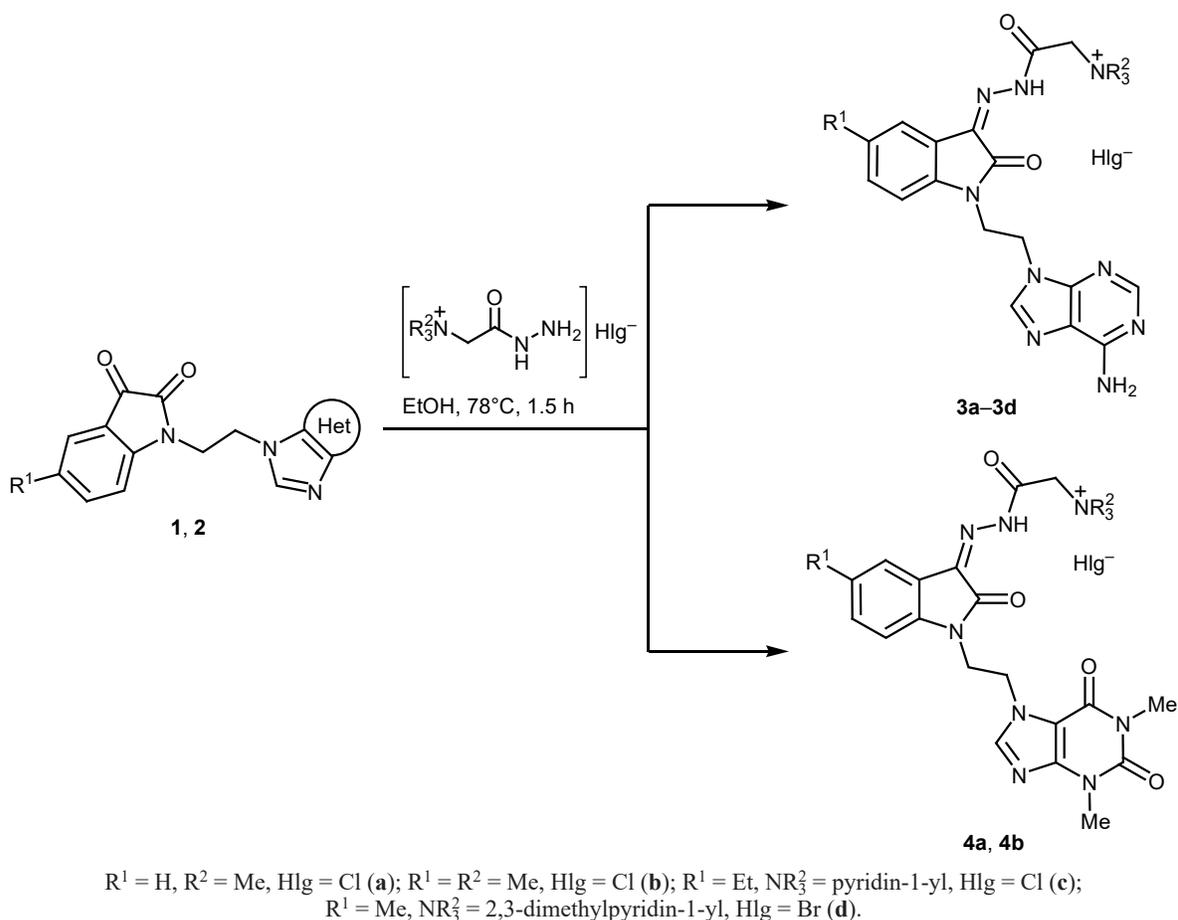
(Scheme 2). The resulting ammonium salts **3a**–**3d**, **4a**, and **4b** (yield 80–96%) were yellow solid powders. Their structure was confirmed by IR and NMR spectra. The IR spectra of all compounds **3** and **4a** showed a broadened absorption band in the region 3318–3446 cm^{-1} due to stretching vibrations of the hydrazone N–H bond. The IR spectra of theophylline derivatives **4a** and **4b** contained an additional medium-intensity band at about 3540 cm^{-1}, which indicated their existence in crystal as mixtures of two isomers with respect to the hydrazone C=N bond. Furthermore, in the far IR region of the spectra of adenine derivatives **3a**–**3d** (3137–3205 cm^{-1}) we observed narrow absorption peaks corresponding to N–H stretchings of the primary amino group. The presence of two isomers of hydrazones **4a** and **4b** in solution was also confirmed by their ^1H NMR spectra recorded from solutions in DMSO-d_6–D_2O, where the signals from methylene protons of the ethylene bridge (δ 4.16, 4.52 ppm) and methylene group linked to the quaternary nitrogen atom (δ 4.88 ppm) were broadened.

Biological testing of new hydrazones **3a**–**3d**, **4a**, and **4b** against both sensitive and methicillin-resistant (MRSA-1, MRSA-2) strains of *S. aureus* showed the absence of antimicrobial activity in combination with very low cytotoxicity and hemotoxicity (IC_{50}, HC_{50} > 600 μM). The cytotoxicity and hemotoxicity assays were carried out on human blood erythrocytes and normal liver cells (Chang liver cell line).

Being purine derivatives, adenine- and theophylline-based isatin hydrazones containing an ammonium moiety offer a significant potential as thrombolytic agents and anticoagulants [7, 24–29]. The presence in their molecules of an oxindole fragment which is con-

Scheme 1.

R = H (**a**), Me (**b**), Et (**c**).

Scheme 2.

R^1 = H, R^2 = Me, Hlg = Cl (**a**); R^1 = R^2 = Me, Hlg = Cl (**b**); R^1 = Et, NR$_3^2$ = pyridin-1-yl, Hlg = Cl (**c**);
R^1 = Me, NR$_3^2$ = 2,3-dimethylpyridin-1-yl, Hlg = Br (**d**).

sidered a privileged structure enhances this potential [30–33]. Therefore, in this work we studied the effect of hydrazones **3a–3d**, **4a**, and **4b** on the parameters of platelet aggregation and coagulation in hemostasis (Table 1). As follows from the data in Table 1, the examined compounds showed different antiplatelet aggregation activities. Adenine derivatives **3b** and **3c** containing an alkyl group at the 5-position of the indole fragment proved to be 1.5 times more active than acetylsalicylic acid with respect to platelet aggregation level, and the latent period of aggregation was prolonged by more than 30%. In addition, theophylline-based trimethylammonium salt **4b** exhibited platelet aggregation activity which exceeded the activity of etamsylate more than twofold without affecting the latent period.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-400 (400 MHz for ^{1}H and 100.6 MHz) and Bruker Avance-600 (600 MHz for ^{1}H and 150.9 MHz for ^{13}C) spectrometers (Germany) using DMSO-d_6, CDCl$_3$–DMSO-d_6, or D$_2$O–DMSO-d_6 as solvent; the chemical shifts were measured relative to the residual proton and carbon signals of the deuterated solvent. Signals from hydrogen-bearing carbons were assigned on the basis of ^{13}C DEPT spectra. The mass spectra (MALDI) were run on a Bruker UltraFlex III TOF/TOF mass spectrometer (Germany). The IR spectra were recorded on a Bruker Vector-22 spectrometer (Germany) from samples dispersed in mineral oil and placed between KBr plates. Elemental analysis was performed on a Euro Vector 2000 CHNS-O analyzer (Italy). The melting points were measured with a Stuart SMP10 melting point apparatus (UK).

Citrate blood was centrifuged on an OPN-3.02 centrifuge (*DASTAN*, Kyrgyzstan). Platelet aggregation was studied according to the procedure described in [34] using an AT-02 aggregometer (*Medtekh*, Russia). Anticoagulant activity was estimated using a Solar CGL 2110 turbidimetric hemocoagulometer (*SOLAR*, Russia). The results were processed using Statistica 10.0 (StatSoft, USA).

Table 1. Effect of compounds **3a**–**3d**, **4a**, and **4b** and reference drugs (in percent of control) on the parameters of platelet aggregation and coagulation in hemostasis[a]

Compound no.	Latent period	Maximum amplitude	Platelet aggregation rate	Time of achieving maximum amplitude	APTT[b]
3a	+3.1 (2.4–6.4)[c]	−5.8 (4.7–6.4)[c]	−11.4 (10.5–16.7)[c]	+14.7 (12.9–15.3)[c,d]	+3.7 (2.9–5.4)[e]
3b	+5.6 (4.3–8.5)[c]	−19.3 (17.4–21.8)[c]	−28.3 (24.5–30.1)[d,f]	+21.3 (15.3–24.7)[c,d]	+6.5 (4.9–8.3)[c,e]
3c	+9.4 (7.2–12.4)[c]	−20.2 (18.4–23.5)[c]	−31.5 (26.9–34.3)[d,f]	+1.5 (0.8–3.4)[e,f]	+5.3 (4.1–9.6)[c,e]
3d	+6.3 (4.5–8.9)[c]	−4.2 (3.7–5.6)[c]	−3.1 (2.4–4.5)[f]	+2.7 (1.3–4.5)[c]	+2.5 (1.8–4.3)[e]
4a	+3.2 (1.5–5.6)[c]	−6.3 (5.4–7.1)[c]	−15.4 (11.7–19.4)[c]	+21.6 (17.4–25.3)[c,d]	+8.4 (7.3–10.4)[c,e]
4b	+1.1 (0.4–2.3)[c,g]	+8.3 (7.4–10.5)[c,f,g]	+12.7 (10.6–15.3)[c]	−10.5 (7.4–13.3)[c,g]	+7.3 (5.6–9.4)[c,e]
Acetylsalicylic acid	−2.1 (1.1–2.6)	−13.7 (10.8–16.4)[c]	−10.5 (7.6 – 12.3)[c]	+10.5 (8.7–13.4)[c]	+1.1 (0.5–1.9)[e]
Etamsylate	−16.7 (13.5–18.4)[d,f]	+3.4 (2.7–4.4)[c,f]	+16.7 (14.3–20.1)[d,f]	+14.2 (10.3–15.6)[c,f]	–
Sodium heparin	–[h]	–	–	–	+20.3 (19.7–21.4)[d]

[a] The latent period is given for collagen-induced platelet aggregation, and the other parameters correspond to ADP-induced platelet aggregation.
[b] APTT is activated partial thromboplastin time.
[c] $p \leq 0.05$.
[d] $p \leq 0.001$ (compared to control).
[e] $p \leq 0.05$ (compared to sodium heparin).
[f] $p \leq 0.001$ (compared to acetylsalicylic acid).
[g] $p \leq 0.05$ (compared to etamsylate).
[h] "–" stands for no data; $n = 6$.

Commercially available DMSO-d_6 (99.9 atom % D, Acros Organics), CDCl$_3$ (99.8 atom % D, Acros Organics), D$_2$O (99.8 atom % D, Acros Organics), trifluoroacetic acid (98%, Acros Organics), isatin (98%, Acros Organics), 5-methylisatin (95%, Acros Organics), 7-(2-chloroethyl)theophylline (≥98.0%, TCI Chemicals), and adenine (>99.0%, TCI Chemicals) were used. 5-Ethylisatin [35] and 9-(2-bromoethyl)-adenine [36] were synthesized according to literature procedures.

Isatin derivatives 1a–1c, 2a, and 2b (*general procedure*). Isatin, 5-methylisatin, or 5-ethylisatin (10 mmol) was dissolved in 20 mL of DMF, and 0.42 g of sodium hydride (10 mmol, 60% suspension in mineral oil) was added with stirring on a magnetic stirred at 10°C. The mixture was allowed to warm up to 25°C over a period of 30 min, and 7-(2-chloroethyl)-theophylline (2.43 g, 10 mmol) or 9-(2-bromoethyl)-adenine (2.42 g, 10 mmol) was added. The resulting solution was stirred at 60°C for 3 h and poured into a mixture of 100 g of ice and 50 mL of water. The mixture spontaneously warmed up to 25°C, and the solid product was filtered off, washed with diethyl ether, and dried under reduced pressure (18 mm Hg, water-jet pump).

1-[2-(6-Amino-9*H*-purin-9-yl)ethyl]-2,3-dihydro-1*H*-indole-2,3-dione (1a). Yield 2.74 g (89%), orange powder, mp 242°C (decomp.). IR spectrum, ν, cm^{-1}: 3442, 3318, 3160, 3104, 1732, 1652, 1611, 1596, 1473, 1362, 1300, 1186, 1150, 1096. ^{1}H NMR spectrum (CDCl$_3$–DMSO-d_6, 1:1), δ, ppm: 4.13 t (2H, CH$_2$, $^3J = 5.7$ Hz), 4.50 t (2H, CH$_2$, $^3J = 5.7$ Hz), 6.83 d (1H, $^3J = 8.4$ Hz), 7.01 d.d (1H, $^3J = 7.5$, 7.5 Hz), 7.44 d.d (1H, $^3J = 7.5$, $^4J = 1.4$ Hz), 7.43 d.d (1H, $^3J = 7.5$, 7.5 Hz), 7.82 s (2H, NH$_2$), 8.10 s (1H), 8.20 s (1H). Mass spectrum: m/z 309 [M + H]$^+$. Found, %: C 58.30; H 3.78; N 27.09. C$_{15}$H$_{12}$N$_6$O$_2$. Calculated, %: C 58.44; H 3.92; N 27.26.

1-[2-(6-Amino-9*H*-purin-9-yl)ethyl]-5-methyl-2,3-dihydro-1*H*-indole-2,3-dione (1b). Yield 2.54 g (79%), orange powder, mp 254°C (decomp.). IR spectrum, ν, cm^{-1}: 3564, 3360, 3160, 3108, 2925, 1732, 1682, 1656, 1618, 1601, 1492, 1420, 1345, 1302, 1157, 1130. ^{1}H NMR spectrum (CDCl$_3$–DMSO-d_6, 1:1), δ, ppm: 2.21 s (3H, CH$_3$), 4.09 t (2H, CH$_2$, $^3J = 5.8$ Hz), 4.48 t (2H, CH$_2$, $^3J = 5.8$ Hz), 6.62 d (1H, $^3J = 8.0$ Hz), 7.20 br.d (1H, $^3J = 8.4$ Hz), 7.22 br.s (1H, 4-H), 7.49 s (2H, NH$_2$), 8.06 s (1H), 8.10 s (1H). Mass spectrum: m/z 323 [M + H]$^+$. Found, %: C 59.50; H 4.23; N 25.89. C$_{16}$H$_{14}$N$_6$O$_2$. Calculated, %: C 59.62; H 4.38; N 26.07.

1-[2-(6-Amino-9*H*-purin-9-yl)ethyl]-5-ethyl-2,3-dihydro-1*H*-indole-2,3-dione (1c). Yield 2.65 g (79%), orange powder, mp 228°C. IR spectrum, ν, cm^{-1}: 3363, 3166, 2929, 1742, 1723, 1653, 1616, 1488, 1418, 1342, 1306, 1255, 1128, 1081. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.12 t (3H, CH$_3$, ^3J = 7.7 Hz), 2.54 q (2H, CH$_2$, ^3J = 7.6 Hz), 4.08 t (2H, CH$_2$, ^3J = 5.3 Hz), 4.41 t (2H, CH$_2$, ^3J = 5.3 Hz), 6.82 d (1H, ^3J = 8.0 Hz), 7.09 s (2H, NH$_2$), 7.32 br.s (1H, 4-H), 7.35 br.d (1H, ^3J = 7.8 Hz), 7.96 s (1H), 8.13 s (1H). Mass spectrum, *m/z* 337 [*M* + H]$^+$. Found, %: C 60.52; H 4.63; N 24.79. C$_{17}$H$_{16}$N$_6$O$_2$. Calculated, %: C 60.71; H 4.79; N 24.99.

7-[2-(2,3-Dioxo-2,3-dihydro-1*H*-indol-1-yl)ethyl]-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (2a). Yield 2.58 g (73%), orange powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3466, 3107, 2961, 1746, 1700, 1659, 1611, 1550, 1474, 1407, 1373, 1350, 1233, 1157. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.14 s (3H, CH$_3$), 3.38 s (3H, CH$_3$), 4.10 t (2H, CH$_2$, ^3J = 5.4 Hz), 4.51 t (2H, CH$_2$, ^3J = 5.4 Hz), 6.82 d (1H, ^3J = 8.1 Hz), 7.05 d.d (1H, ^3J = 7.5, 7.5 Hz), 7.52–7.48 m (2H), 8.10 s (1H). Mass spectrum: *m/z* 376 [*M* + Na]$^+$. Found, %: C 57.58; H 4.03; N 19.72. C$_{17}$H$_{15}$N$_5$O$_4$. Calculated, %: C 57.79; H 4.28; N 19.82.

1,3-Dimethyl-7-[2-(5-methyl-2,3-dihydro-1*H*-indol-1-yl)ethyl]-1*H*-purine-2,6(3*H*,7*H*)-dione (2b). Yield 2.75 g (75%), orange powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3467, 3135, 2923, 2855, 1739, 1699, 1659, 1619, 1598, 1543, 1494, 1447, 1349, 1227, 1136. ^{1}H NMR spectrum (DMSO-d_6), δ ppm: 2.23 s (3H, CH$_3$), 3.14 s (3H, CH$_3$), 3.38 s (3H, CH$_3$), 4.07 t (2H, CH$_2$, ^3J = 5.5 Hz), 4.49 t (2H, CH$_2$, ^3J = 5.5 Hz), 6.82 d (1H, ^3J = 8.1 Hz), 6.73 d (1H, ^3J = 8.7 Hz), 7.33–7.31 m (2H), 8.08 s (1H). Mass spectrum: *m/z* 368 [*M* + H]$^+$. Found, %: C 58.72; H 4.50; N 18.93. C$_{18}$H$_{17}$N$_5$O$_4$. Calculated, %: C 58.85; H 4.66; N 19.06.

Ammonium salts 3a–3d, 4a, and 4b (*general procedure*). Isatin derivative **1a–1c**, **2a**, or **2b** (0.28 mmol) was dissolved in 10 mL of anhydrous ethanol, the corresponding hydrazide (0.28 mmol) was added in one portion, and 2 drops of trifluoroacetic acid were added. The mixture was refluxed for 1.5 h and allowed to cool down to room temperature, and the precipitate was filtered off, washed with anhydrous diethyl ether, and dried under reduced pressure (12 mm Hg).

2-(2-{1-[2-(6-Amino-9*H*-purin-9-yl)ethyl]-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene}hydrazinyl)-*N,N,N*-trimethyl-2-oxoethan-1-aminium chloride (3a). Yield 0.12 g (96%), yellow powder, mp 270°C (decomp.). IR spectrum, ν, cm^{-1}: 3386, 3157, 3016, 2909, 1719, 1684, 1615, 1592, 1471, 1408, 1375, 1298, 1224, 1146, 1078. ^{1}H NMR spectrum (D$_2$O–DMSO-d_6, 1:3), δ, ppm: 3.28 br.s (9H, N$^+$Me$_3$), 4.15 t (2H, CH$_2$, ^3J = 5.8 Hz), 4.47 t (2H, CH$_2$, ^3J = 5.8 Hz), 4.85 br.s [2H, CH$_2$C(O)], 6.85 d (1H, ^3J = 8.4 Hz), 6.99 br.s (2H, NH$_2$), 7.06 d.d (1H, ^3J = 7.8, 7.5 Hz), 7.29 d.d (1H, ^3J = 7.9, 7.4 Hz), 7.52 br.d (1H, ^3J = 7.4 Hz), 7.89 s (1H), 8.07 s (1H). Mass spectrum: *m/z* 422 [*M* − Cl]$^+$. Found, %: C 52.31; H 5.04; N 27.40. C$_{20}$H$_{24}$ClN$_9$O$_2$. Calculated, %: C 52.46; H 5.28; N 27.53.

2-(2-{1-[2-(6-Amino-9*H*-purin-9-yl)ethyl]-5-methyl-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene}hydrazinyl)-*N,N,N*-trimethyl-2-oxoethan-1-aminium chloride (3b). Yield 0.12 g (91%), yellow powder, mp 270°C (decomp.). IR spectrum, ν, cm^{-1}: 3446, 3205, 3149, 2941, 1723, 1691, 1665, 1603, 1491, 1440, 1350, 1308, 1242, 1153, 1086. ^{1}H NMR spectrum (D$_2$O–DMSO-d_6, 1:3), δ, ppm: 2.26 s (3H, CH$_3$), 3.31 br.s (9H, N$^+$Me$_3$), 4.16 t (2H, CH$_2$, ^3J = 5.0 Hz), 4.52 t (2H, CH$_2$, ^3J = 5.0 Hz), 4.88 br.s [2H, CH$_2$C(O)], 6.83 d (1H, ^3J = 8.1 Hz), 7.15 br.d (1H, ^3J = 8.1 Hz), 7.37 br.s (1H), 8.18 s (1H), 8.33 s (1H). ^{13}C NMR spectrum (D$_2$O–DMSO-d_6, 1:3), δ$_C$, ppm: 20.7 (CH$_3$), 40.0 (CH$_2$), 41.8 (CH$_2$), 54.1 (CH$_3$), 62.3 (CH$_2$), 109.7 (CH), 118.3, 118.8, 121.7 (CH), 132.9 (CH), 133.0, 135.5, 141.1, 143.6 (CH), 147.5 (CH), 149.5, 151.9, 161.2, 166.4. Mass spectrum: *m/z* 436 [*M* − Cl]$^+$. Found, %: C 53.29; H 5.41; N 26.58. C$_{21}$H$_{26}$ClN$_9$O$_2$. Calculated, %: C 53.44; H 5.55; N 26.71.

1-[2-(2-{1-[2-(6-Amino-9*H*-purin-9-yl)ethyl]-5-ethyl-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene}hydrazinyl)-2-oxoethyl]pyridinium chloride (3c). Yield 0.13 g (95%), yellow powder, mp 187°C (decomp.). IR spectrum, ν, cm^{-1}: 3318, 3137, 3050, 2968, 1690, 1635, 1514, 1488, 1418, 1366, 1260, 1201, 1133, 1057. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.17 t (3H, CH$_3$, ^3J = 7.6 Hz), 2.62–2.59 m (2H, CH$_2$), 4.23 t (2H, CH$_2$, ^3J = 5.5 Hz), 4.52 t (2H, CH$_2$, ^3J = 5.5 Hz), 6.20 br.s [2H, CH$_2$C(O)], 6.97 d (1H, ^3J = 6.6 Hz), 7.23 d (1H, ^3J = 7.7 Hz), 7.38 br.s (1H), 7.99 br.s (2H, NH$_2$), 8.18 s (1H), 8.27 d.d (1H, ^3J = 6.9, 6.7 Hz), 8.30 s (1H), 8.73 d.d (1H, ^3J = 7.9, 7.9 Hz), 9.12–9.09 m (2H), 12.53 s (1H, NH). Mass spectrum: *m/z* 470 [*M* − Cl]$^+$. Found, %: C 56.83; H 4.59; N 24.80. C$_{24}$H$_{24}$ClN$_9$O$_2$. Calculated, %: C 56.97; H 4.78; N 24.92.

1-[2-(2-{1-[2-(6-Amino-9*H*-purin-9-yl)ethyl]-5-methyl-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene}hy-

drazinyl)-2-oxoethyl]-2,3-dimethylpyridinium bromide (3d). Yield 0.14 g (90%), yellow powder, mp 218°C (decomp.). IR spectrum, ν, cm^{-1}: 3368, 3179, 3019, 2971, 1691, 1655, 1626, 1596, 1492, 1364, 1249, 1215, 1147, 1127, 1050. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.30 s (3H, CH$_3$), 2.54 s (3H, CH$_3$), 2.65 s (3H, CH$_3$), 4.21 t (2H, CH$_2$, ^3J = 5.3 Hz), 4.51 t (2H, CH$_2$, ^3J = 5.3 Hz), 6.18 br.s [2H, CH$_2$C(O)], 6.92 d (1H, ^3J = 7.2 Hz), 7.20–7.19 m (1H), 7.38 br.s (1H), 7.77 br.s (2H, NH$_2$), 7.96 d.d (1H, ^3J = 7.2, 6.7 Hz), 8.13 s (1H), 8.23 s (1H), 8.49 d (1H, ^3J = 7.1 Hz), 8.87 br.s (1H), 12.54 s (1H, NH). ^{13}C NMR spectrum (D$_2$O–DMSO-d_6, 1:3), δ, ppm: 17.1 (CH$_3$), 19.6 (CH$_3$), 20.7 (CH$_3$), 40.0 (CH$_2$), 41.5 (CH$_2$), 59.3 (CH$_2$), 109.7 (CH), 118.4 (CH), 118.9, 121.6, 124.9 (CH), 132.8, 132.9 (CH), 135.8, 138.9, 141.1 (CH), 142.6 (CH), 144.9, 147.0 (CH), 149.7 (CH), 153.5, 161.2, 167.1. Mass spectrum: m/z 484 $[M - Br]^+$. Found, %: C 53.02; H 4.49; N 22.17. C$_{25}$H$_{26}$BrN$_9$O$_2$. Calculated, %: C 53.20; H 4.64; N 22.33.

2-[2-(1-{2-[1,3-Dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl]ethyl}-2-oxo-2,3-dihydro-1H-indol-3-ylidene)hydrazinyl]-N,N,N-trimethyl-2-oxoethan-1-aminium chloride (4a). Yield 0.11 g (80%), yellow powder, mp 246°C (decomp.). IR spectrum, ν, cm^{-1}: 3434, 3238, 3027, 2961, 1705, 1686, 1666, 1615, 1557, 1481, 1377, 1289, 1235, 1153, 1055. ^{1}H NMR spectrum (D$_2$O–DMSO-d_6, 1:5), δ, ppm: 3.09 s (3H, CH$_3$), 3.30 s (3H, CH$_3$), 3.34 br.s (9H, N$^+$Me$_3$), 4.16 t (2H, CH$_2$, ^3J = 5.1 Hz), 4.53 t (2H, CH$_2$, ^3J = 5.1 Hz), 4.88 br.s [2H, CH$_2$C(O)], 6.79 d (1H, ^3J = 8.0 Hz), 7.09 d.d.d (1H, ^3J = 8.0, 7.5, ^4J = 0.5 Hz), 7.31 d.d.d (1H, ^3J = 8.0, 7.5, ^4J = 1.0 Hz), 7.57 d (1H, ^3J = 8.0 Hz), 7.98 s (1H, NH). Mass spectrum: m/z 468 $[M - Cl]^+$. Found, %: C 52.39; H 5.30; N 22.04. C$_{22}$H$_{27}$ClN$_8$O$_4$. Calculated, %: C 52.54; H 5.41; N 22.28.

2-[2-(1-{2-[1,3-Dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl]ethyl}-5-methyl-2-oxo-2,3-dihydro-1H-indol-3-ylidene)hydrazinyl]-N,N,N-trimethyl-2-oxoethan-1-aminium chloride (4b). Yield 0.13 g (89%), yellow powder, mp >300°C (decomp.). IR spectrum, ν, cm^{-1}: 3541, 3401, 3057, 2966, 1704, 1662, 1621, 1552, 1479, 1457, 1360, 1278, 1233, 1156, 1051. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.26 s (3H, CH$_3$), 3.20 s (3H, CH$_3$), 3.30 s (3H, CH$_3$), 3.31 br.s (9H, N$^+$Me$_3$), 4.16 t (2H, CH$_2$, ^3J = 5.0 Hz), 4.52 t (2H, CH$_2$, ^3J = 5.0 Hz), 4.88 br.s [2H, CH$_2$C(O)], 6.83 d (1H, ^3J = 8.0 Hz), 7.15 br.d (1H, ^3J = 8.0 Hz), 7.37 br.s (1H), 8.18 s (1H, NH), 8.33 s (1H). ^{13}C NMR spectrum (D$_2$O–DMSO-d_6, 1:4), δ_C, ppm: 20.6 (CH$_3$),

27.7 (CH$_3$), 29.7 (CH$_3$), 40.7 (CH$_2$), 44.3 (CH$_2$), 53.8 (CH$_3$), 56.2 (CH$_2$), 106.8 (CH), 109.4 (CH), 118.7, 121.3, 132.5 (CH), 132.8 (CH), 141.2, 143.1, 148.6, 151.1, 154.7, 160.8, 166.9, 169.7. Mass spectrum: m/z 481 $[M - Cl]^+$. Found, %: C 53.30; H 5.47; N 21.52. C$_{23}$H$_{29}$ClN$_8$O$_4$. Calculated, %: C 53.43; H 5.65; N 21.67.

CONCLUSIONS

The proposed approach makes it possible to synthesize in two steps isatin derivatives containing an adenine or theophylline fragment in high yields. All newly synthesized compounds showed antiplatelet aggregation activity, and the activity of adenine derivatives of 5-methyl- and 5-ethylisatins exceeded the activity of acetylsalicylic acid by a factor of 1.5. The low toxicity of the obtained hydrazones to human erythrocytes and normal liver cells suggests prospects of using then as antiplatelet aggregation agents, as well as for preventing and terminating bleeding.

AUTHOR INFORMATION

A.V. Bogdanov, ORCID: https://orcid.org/0000-0002-2483-4742

A.D. Voloshina, ORCID: https://orcid.org/0000-0002-3540-8554

T.A. Abzalilov, ORCID: https://orcid.org/0000-0002-7044-2117

A.V. Samorodov, ORCID: https://orcid.org/0000-0001-9302-499X

V.F. Mironov, ORCID: https://orcid.org/0000-0002-4198-3774

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CONFLICT OF INTEREST

The authors declare the absence of conflict of interest.

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