DOI 10.1007/s11094-021-02395-z
Pharmaceutical Chemistry Journal, Vol. 55, No. 2, May, 2021 (Russian Original Vol. 55, No. 2, February, 2021)

SEARCH FOR NEW DRUGS

SYNTHESIS OF NEW N-MONO- AND N,N-DIALKYLATED IMIDAZOLE DERIVATIVES AND THEIR ANTIPLATELET AND ANTICOAGULATION ACTIVITY

K. G. Gurevich,[1] A. L. Urakov,[2,*] A. V. Basantsev,[3] A. V. Samorodov,[4] A. A. Danilin,[3] P. P. Purygin,[3] N. A. Klenova,[3] I. I. Bashirov,[2] L. I. Bashirova,[4] A. A. Golovanov,[5] V. I. Korunas,[4] and D. O. Lipatov[4]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 55, No. 2, pp. 8 – 12, February, 2021.

Original article submitted January 18, 2021.

Currently there are several effective synthetic methods for preparing various *N*-mono- and *N*,*N*-dialkylated imidazole derivatives with a very wide variety of biological activities including antiplatelet and anticoagulation effects. The present work reports *N*-mono- and *N*,*N*-dialkylation of 2-methyl-, 2-ethyl-, and 4-nitroimidazoles using (adamantyl-1)bromomethylketone. The synthesized compounds were identified by elemental analyses and PMR and IR spectroscopy. Five compounds having antiplatelet properties were found in *in vitro* investigations of the antiplatelet and anticoagulation activity in blood of healthy volunteers. The anticoagulation effects of these compounds on the maximum aggregation amplitude were comparable with that of acetylsalicylic acid and even exceeded it with respect to the duration of inhibition of the platelet release reaction.

Keywords: imidazole, (adamantyl-1)bromomethylketone, *N*-alkylation, antiplatelet effect, anticoagulation effect.

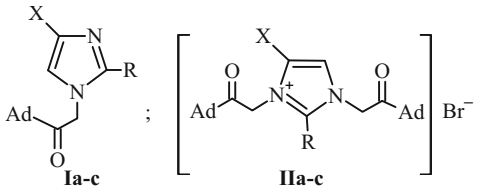
Imidazole derivatives and particularly its quaternary salts are N-containing heterocyclic scaffolds included in biologically active compounds with antitumor, antifungal, anticoagulant, and antiplatelet properties [1]. The presence of an *N*-monoalkylated imidazole in the hemostatic Ozagrel was reported to inhibit the synthesis of cyclooxygenase and thromboxane A_2 and to decrease the formation rate of thrombi [2]. Also, quaternary imidazolium salts were shown to inhibit plasma transglutaminase, an enzyme facilitating blood-clot formation [3].

[1] A. I. Evdokimov Moscow State University of Medicine and Dentistry, 20/1 Delegatskaya St., Moscow, 127473 Russia.
[2] Izhevsk State Medical Academy, Ministry of Health of the RF, 281 Kommunarov St., Izhevsk, Udmurtia, 426034 Russia.
[3] Institute of Natural Sciences, S. P. Korolev Samara National Research University, 34 Moskovskoe Shosse, Samara, 443086 Russia.
[4] Bashkir State Medical University, Ministry of Health of the RF, 3 Lenina St., Ufa, Bashkortostan, 450008 Russia.
[5] Togliatti State University, 14 Belorusskaya St., Togliatti, 445020 Russia.
[*] e-mail: urakoval@live.ru

Biologically active adamantyl-containing compounds affect human platelet aggregation and other processes. Addition of a highly lipophilic adamantyl moiety into compounds with a given biological activity helps to increase their affinity for membranes and creates a platform for additional modification via introduction of various substituents into the structure. The affinity of aggregants for serotonin 5-HT_2-receptors was shown to increase with a bulky adamantyl substituent in their molecules [4].

Considering the urgency of this work, the goal of the present research was to synthesize and study the antiaggregant and anticoagulation activity of a series of *N*-mono- and *N*,*N*-dialkylated imidazoles containing an adamantoylmethyl fragment in their structures.

Compounds **Ia-c** were synthesized via *N*-monoalkylation of corresponding imidazole derivatives **IIIa-c** by (adamantyl-1)bromomethylketone **IV** in the presence of NaH in hexamethylphosphoramide (HMPA) (Scheme 1). This sol-

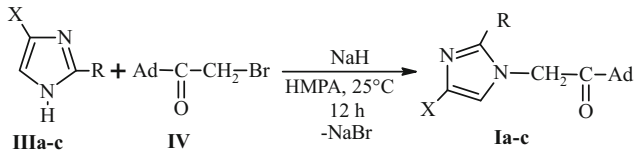


Ia-c **IIa-c**

Ad= ; **Ia**, **IIa:** R=CH_3, X=H; **Ib**, **IIb:** R=C_2H_5, X=H; **Ic**, **IIc:** R=H, X=NO_2.

vent was selected to increase the nucleophilicity and the solubility of the imidazolides.

Scheme 1



IIIa-c **IV** **Ia-c**

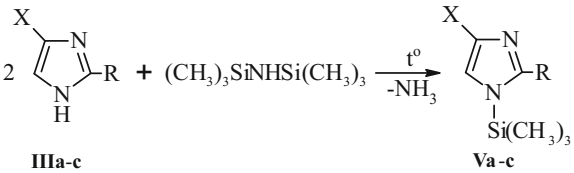
Ia, **IIIa**: R = CH_3, X = H; **IIb**, **IIIb**: R = C_2H_5, X = H; **Ic**, **IIIc**: R = H, X = NO_2.

PMR spectra of 1-(adamantoyl-1)methylimidazoles **Ia-c** exhibited singlets at 4.78 or 4.90 ppm for the corresponding methylene protons and multiplets at 1.67 – 2.15 ppm for the adamantyl protons.

IR spectra of **Ia-c** showed characteristic absorption bands for carbonyl stretching vibrations (1706 – 1714 cm^{-1}) and adamantyl C–H bonds (2908 – 2911) and lacked C–Br vibrational bands (629).

Derivatives of 1,3-*bis*[(adamantoyl-1)methyl]imidazolium bromide were prepared in two steps. Reaction of **IIIa-c** with hexamethyldisilazane (HMDS) produced trimethylsilyl derivatives **Va-c** that were purified by vacuum distillation (Scheme 2). Table 1 presents the characteristics of the 1-trimethylsilylimidazole derivatives.

Scheme 2



IIIa-c **Va-c**

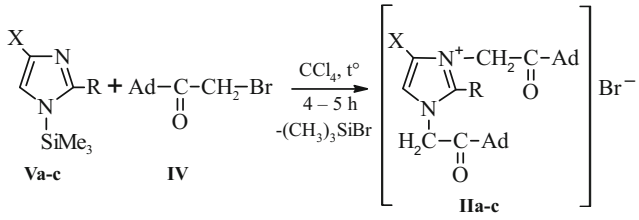
IIIa, Va: R = CH_3, X = H; **IIIb**, Vb: R = C_2H_5, X = H; **IIIc**: R = H, X = NO_2.

TABLE 1. Physicochemical Constants of 1-Trimethylsilylimidazole Derivatives

Compound	bp, °C/20 mm Hg	n_D^{20}
Va	130 – 131	1.4775
Vb	121 – 123	1.4735
Vc	–	1.4765

N,*N*-Dialkylation of **Va-c** followed Scheme 3. The reaction of (adamantyl-1)bromomethylketone (**IV**) with 1-trimethylsilylimidazole derivatives in CCl_4 led to formation of imidazolium bromides **IIa-c**.

Scheme 3



Va-c **IV** **IIa-c**

IIa, Va: R = CH_3, X = H; **IIb**, Vb: R = C_2H_5, X = H; **IIc**, Vc: R = H, X = NO_2.

The presence in the IR spectra in the ranges 1710 – 1713 and 2907 – 2908 cm^{-1} of characteristic absorption bands for carbonyl and adamantane C–H stretching vibrations indicated that alkylation of **Va-c** occurred.

PMR spectra of **IIa-c** contained singlets for protons of methylenes bonded to imidazole N-1 and N-3 at 5.55, 5.56, and 4.89 ppm, respectively. Multiplets for protons of the two adamantyl moieties included in **IIa-c** were observed at 1.71 – 2.12 ppm.

EXPERIMENTAL CHEMICAL PART

PMR spectra were recorded with TMS internal standard on a Bruker AM 400 instrument at operating frequency 400 MHz. IR spectra were taken on an FT-801 FT-IR spectrometer with an ATR accessory. Elemental analyses used an instrument for rapid gravimetric determination of elements [5]. Elemental analyses agreed with those calculated. TLC used Silufol UV-254 plates (Czech Rep.) with detection in I_2 vapor and UV light from a chromatographic UFS 254/365. Melting points of crystalline compounds were measured on a POTP-2 apparatus (Russia) in sealed capillaries.

1-(Adamantoyl-1)methylimidazoles Ia-c (general method). A solution of imidazole **III** (1.94 mmol) in HMPA (3 mL) cooled to 0°C was stirred and treated in portions with NaH (2.13 mmol) that was rinsed beforehand with dry hexane. The stirring was continued at the same temperature for 30 min and then at 25°C for 5 h. The mixture was cooled to 0°C, treated dropwise with a solution of (adamantyl-1)bromomethylketone (**IV**, 1.94 mmol) in HMPA (2 mL), held at 25°C for 12 h, and poured into a beaker with ice. The resulting white precipitate was filtered off, rinsed with H_2O, dried in air, and recrystallized from an appropriate solvent.

1-(Adamantoyl-1)methyl-2-methylimidazole (Ia). Yield 0.30 g (60%). mp 203 – 205°C (C_6H_6). IR spectrum, ν_{max}, cm^{-1}: 1714 (C=O), 2910 (Ad). PMR spectrum ($CDCl_3$), δ, ppm: 1.69 – 2.10 (m, 15H, H-Ad), 2.25 (c, 3H, CH_3), 4.78 (s, 2H, CH_2), 6.97 – 6.99 (d, arom H, CH=), 7.26 – 7.28 (d, arom H, CH=). Found, %: C 74.59; H 8.33. $C_{16}H_{22}N_2O$. Calc., %: C 74.38; H 8.58.

1-(Adamantoyl-1)methyl-2-ethylimidazole (Ib). Yield 0.13 g (28%). mp 200 – 202°C (CCl_4). IR spectrum, ν_{max}, cm^{-1}: 1706 (C=O), 2908 (Ad). PMR spectrum ($CDCl_3$), δ, ppm: 1.28 – 1.33 (t, 3H, CH_3), 1.67 – 2.15 (m, 15H, H-Ad), 2.45 – 2.51 (m, 2H, CH_2), 4.78 (s, 2H, CH_2), 6.69 – 6.70 (d, arom H, CH=), 6.98 – 7.00 (d, arom H, CH=). Found, %: C 74.77; H 8.69. $C_{17}H_{24}N_2O$. Calc., %: C 74.96; H 8.88.

1-(Adamantoyl-1)methyl-4-nitroimidazole (Ic). 1-Trimethylsilylimidazoles **Va-c** were prepared according to literature methods [6, 7].

Yield 0.44 g (77%). mp 250 – 252°C (dioxane). IR spectrum, ν_{max}, cm^{-1}: 1710 (C=O), 2911 (Ad). PMR spectrum (DMSO-d_6), δ, ppm: 1.70 – 2.10 (m, 15H, H-Ad), 4.90 (s, 2H, CH_2), 7.30 – 7.32 (d, arom H, CH=), 7.78 – 7.80 (d, arom H, CH=). Found, %: C 62.01; H 6.91. $C_{15}H_{19}N_3O_3$. Calc., %: C 62.27; H 6.62.

1.3-*bis*[(Adamantoyl-1)methyl]imidazolium bromides IIa-c (general method). 1-Trimethylsilylimidazole derivative **V** (1.95 mmol) was added under a stream of Ar to a 25-mL round-bottomed flask containing anhydrous CCl_4 (5 mL). The resulting solution was treated dropwise with stirring with (adamantyl-1)bromomethylketone (**IV**, 1.95 mmol) in anhydrous CCl_4 (5 mL). The mixture was stirred at room temperature for 5 h. The solid that formed during the reaction was filtered off, rinsed with Et_2O and hot CCl_4, dried in air, and recrystallized from an appropriate solvent.

1.3-*bis*[(Adamantoyl-1)methyl]-2-methylimidazolium bromide (IIa). Yield 0.42 g (42%). mp 370°C (EtOH–THF, 1:1, dec.). R_f 0.1 (EtOH–MeCN, 3:1). IR spectrum, ν_{max}, cm^{-1}: 1710 (C=O), 2908 (Ad). PMR spectrum (DMSO-d_6), δ, ppm: 1.76 – 2.12 (m, 30H, H-Ad), 3.34 (c, 3H, CH_3), 5.55 (c, 4H, CH_2), 7.50 (c, arom H, CH=). Found, %: C 65.41; H 7.78; Br 15.29. $C_{28}H_{39}N_2O_2Br$. Calc., %: C 65.23; H 7.62; Br 15.49.

1.3-*bis*[(Adamantoyl-1)methyl]-2-ethylimidazolium bromide (IIb). Yield 0.53 g (70%). mp 375°C (EtOH, dec.). IR spectrum, ν_{max}, cm^{-1}: 1710 (C=O), 2908 (Ad). PMR spectrum (DMSO-d_6), δ, ppm: 0.87 – 0.93 (t, 3H, CH_3), 1.70 – 2.05 (m, 30H, H-Ad), 2.47 – 2.50 (m, 2H, CH_2), 5.56 (s, 4H, CH_2), 7.49 (c, arom H, CH=). Found, %: C 65.59; H 7.59; Br 15.27. $C_{29}H_{41}N_2O_2Br$. Calc., %: C 65.77; H 7.80; Br 15.08.

1.3-*bis*[(Adamantoyl-1)methyl]-4-nitroimidazolium bromide (IIc). Yield 0.14 g (13%). mp 330 – 332°C (Et_2OH–EtOH, 1:1, dec.). IR spectrum, ν_{max}, cm^{-1}: 1713 (C=O), 2907 (Ad). PMR spectrum (DMSO-d_6), δ, ppm: 1.71 – 2.10 (m, 30H, H-Ad), 4.89 (s, 4H, CH_2), 6.19 (s, arom H, CH=), 6.57 (s, arom H, CH=). Found, %: C 59.03; H 7.16; Br 14.82. $C_{27}H_{36}N_3O_4Br$. Calc., %: C 59.34; H 6.64; Br 14.62.

EXPERIMENTAL BIOLOGICAL PART

Experiments were conducted according to requirements of Rules of Good Laboratory Practice of the Eurasian Economic Union in the Area of Circulation of Medicines.

Antiaggregant and anticoagulant activity was assessed *in vitro* on isolated blood samples from 64 healthy male donors aged 18 – 24 years. The studies were approved by the Ethics Committee of Bashkir State Medical University, Ministry of Health of Russia (No. 1 of Feb. 20, 2019). Informed consent was obtained from all study participants before collecting blood from them.

The effects of the compounds on platelet aggregation were studied by the method of Born [8] on an AT-02 aggregometer (NPF Medtech, Russia). Antiaggregant activity of the tested compounds and reference drug was assessed at a final concentration of 1×10^{-3} M with incubation for 5 min. The aggregation inductor was adenosine diphosphate (ADP) at a concentration of 20 μg/mL and collagen at a concentration of 5 mg/mL (Tekhnologiya-Standart, Russia). The effects of the compounds on the maximum aggregation amplitude (MA), the aggregation rate, and the time to reach MA during ADP-induced platelet aggregation were studied. The latent period of aggregation in the collagen-induced aggregation test of platelets was evaluated and corresponded to the platelet-release response. The reference drug was acetylsalicylic acid (substance-powder, Shandong Xinhua Pharmaceutical Co. Ltd., China) [9].

Anticoagulant activity was determined by clotting tests [10] in a Solar CGL 2110 turbidimetric hemocoagulometer (ZAO SOLAR, Belarus). The final concentration of the tested compounds and reference drug was 5×10^{-4} g/mL. The activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen concentration were studied according to Clauss. The reference drug was heparin sodium (heparin sodium, 5000 IU/mL, solution for injection, 1-mL ampuls; OAO Sintez, Russia).

Statistical analysis used the Statistica 10.0 software (StatSoft Inc., USA). A check for normal distributions was made using the Shapiro–Wilk criterion. The median, 25 and 75 percentiles, minima, and maxima were calculated to describe variational series. One-factor dispersion analysis (if all data obeyed normal distribution laws and the dispersions of all sets were equal; *F*-criterion) or a Kruskal–Wallis test (if all data did not obey normal distribution laws; *H*-criterion) was performed. The critical significance level *P* for statistical criteria was set at 0.05.

RESULTS AND DISCUSSION

A series of laboratory studies focused on ADP-induced platelet aggregation found that compounds **Ia**, **Ib**, and **IIa-c** exhibited antiaggregant activity at the level of acetylsalicylic acid (Table 2). Compounds **Ia** and **Ic** (*N*-adamantoylmethylated imidazole derivatives) decreased statistically signifi-

TABLE 2. Effects of **Ia-c** and **IIa-c** and Reference Drug on Platelet Aggregation and Coagulation and Plasma Stage of Hemostasis, Me (25 – 75%)

Compound	Latent period, % of control	Maximum amplitude, % of control	Aggregation rate, % of control	APTT, % of control
	collagen-induced aggregation	ADP-induced aggregation		
Ia	– 14.5 (10.2 – 19.7)$^{*,\#\#}$	– 9.6 (8.3 – 11.9)*	– 2.9 (2.1 – 4.3)$^{\#}$	2.8 (2.1 – 4.5)
Ib	– 1.3 (1.1 – 2.3)	– 15.9 (13.4 – 16.7)*	– 23.2 (22.1 – 26.7)$^{**,\#}$	1.7 (1.1 – 3.4)
Ic	– 11.3 (9.8 – 12.4)$^{*,\#\#}$	– 8.9 (7.7 – 9.1)$^{*,\#}$	– 1.6 (1.4 – 2.3)$^{\#}$	8.7 (8.1 – 10.3)*
IIa	– 16.3 (11.7 – 18.3)$^{*,\#\#}$	– 11.4 (9.5 – 13.7)*	– 9.4 (6.3 – 12.1)*	3.2 (3.1 – 4.9)*
IIb	– 7.6 (6.5 – 9.1)$^{*,\#}$	– 14.3 (12.7 – 15.8)*	– 24.1 (21.4 – 25.7)$^{**,\#}$	9.1 (8.7 – 10.3)*
IIc	– 4.7 (3.9 – 6.2)$^{*,\#}$	– 9.5 (7.4 – 11.2)*	– 21.9 (18.5 – 23.6)$^{**,\#}$	3.1 (2.4 – 5.8)*
Acetylsalicylic acid	– 2.1 (1.1 – 2.6)	– 13.7 (10.8 – 16.4)*	– 10.5 (7.6 – 12.3)*	–
Heparin sodium	–	–	–	20.3 (19.7 – 21.4)*

Note: * $P \le 0.05$; ** $P \le 0.001$ vs. the control; $^{\#}$ $P \le 0.05$, $^{\#\#}$ $P \le 0.001$ vs. acetylsalicylic acid; data vs. heparin sodium are statistically significant ($P \le 0.05$); $n = 6$.

cantly the platelet aggregation rate as compared to acetylsalicylic acid. The tested compounds significantly shortened the latent period as compared to the control. Compound **Ib** was inactive.

Compounds **Ic** and **IIa-c** caused hypocoagulation, increased the APTT by 3.1 – 9.2% as compared to the control, and did not affect the fibrinogen concentration and prothrombin time (Table 2). The strength of the effects of the tested compounds were significantly inferior to that of heparin, which increased the APTT by 20.3%.

Thus, compounds **Ia**, **Ib**, and **IIa-c** were found to have antiaggregant effects on the MA that were comparable to that of acetylsalicylic acid and had superior effects on the inhibition time of the prothrombin-release response. Therefore, compounds **Ia**, **Ib**, and **IIa-c** were most promising for further studies.

REFERENCES

1. X.-Q. Wang, P.-T. Ye, M.-J. Bai, et al., *Bioorg. Med. Chem. Lett.*, **30**, 127210 (2020).
2. M. Nakazawa, K. Iizuka, A. Ujiie, et al., *J. Pharm. Soc. Jpn.*, **114**(12), 911 – 933 (1994).
3. J. J. Baldwin, D. C. Remy, and A. Claremon, US Pat. 5,098,707, Merck & Co., Inc., Mar. 24, 1992.
4. A. J. Bojarski, M. J. Mokrosz, et al., *Bioorg. Med. Chem.*, **50**(1), 87 – 95 (2002).
5. V. A. Klimova, *Principal Microanalytical Methods for Organic Compounds* [in Russian], Khimiya, Moscow (1975), pp. 51 – 56.
6. A. F. Pozharskii, V. A. Anisimova, and E. B. Tsupak, *Practical Works in the Chemistry of Heterocycles* [in Russian], RGU (1988) p. 98.
7. D. Li, H. Bao, Q. Tan, et al., *Synth. Commun.*, **35**(8), 1017 – 1026 (2005).
8. G. V. R. Born, *J. Physiol.*, **162**, 67 – 68 (1962).
9. D. A. Abaimov, L. R. Spavronskaya, A. A. Shabalina, et al., *Khim.-farm. Zh.*, **53**(1), 52 – 57 (2019); *Pharm. Chem. J.*, **53**(1), 65 – 70 (2019); https: // doi.org/10.1007/s11094-019-01957-6.
10. A. N. Mironov (ed.), *Handbook for Preclinical Drug Trials* [in Russian], Vol. 1, Grif i K, Moscow (2013).