ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2011, Vol. 37, No. 6, pp. 758–764. © Pleiades Publishing, Ltd., 2011.
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Research in the Field of Imidazo[1,2-*a*]benzimidazole Derivatives. XXX. Synthesis and Properties of (Imidazo[1,2-*a*]benzimidazolyl-2)acetic Acids[1]

V. A. Anisimova[a, 2], I. E. Tolpygin[a], A. A. Spasov[b], T. S. Serdyuk[c], and A. G. Sukhov[c]

[a] *Institute of Physical and Organic Chemistry, Southern Federal University, pr. Stachki 194/2, Rostov-on-Don, 344090 Russia*
[b] *Volgograd State Medical University, Volgograd, Russia*
[c] *Kogan Research Institute of Neurocybernetics, Southern Federal University, Rostov-on-Don, Russia*

Received May 25, 2011; in final form, June 16, 2011

Abstract—Ethyl esters of (9-subtituted-imidazo[1,2-*a*]benzimidazolyl-2)acetic acids have been synthesized. The chemical properties of these esters have been studied: hydrolysis, decarboxylation, hydrazinolysis, and hydrazone formation. Some of these compounds demonstrate biological activity (fungicidal, antimicrobial, antiarrhythmic) and effects on brain rhythmogenesis.

Keywords: imidazo[1,2-a]benzimidazoles; acids; synthesis; biological activity

DOI: 10.1134/S1068162011060033

INTRODUCTION

Imidazo[1,2-*a*]benzimidazole derivatives, which contain a guanidine group, demonstrate a wide range of pharmacological activities, often modulated by the presence of known pharmacophore groups in their structures [2–6]. Residues of acetic acid and its derivatives belong to pharmacophores, and their presence in various heterocyclic systems endows them with various activities. For example, acetic acid residues are responsible for the anti-inflammatory and analgesic activities of many aromatic and heterocyclic acetic acids [7–10]. Antitumor activity has been found in various derivatives of indolyzine-3-acetic acid [11]. In this work, derivatives of (imidazo[1,2-*a*]benzimidazolyl-2)acetic acids were synthesized by interaction of 1-substituted 2-aminobenzimidazoles with esters of γ-halogenoacetoacetic acids and chemical and biological properties of the compounds synthesized were studied [12].

RESULTS AND DISCUSSION

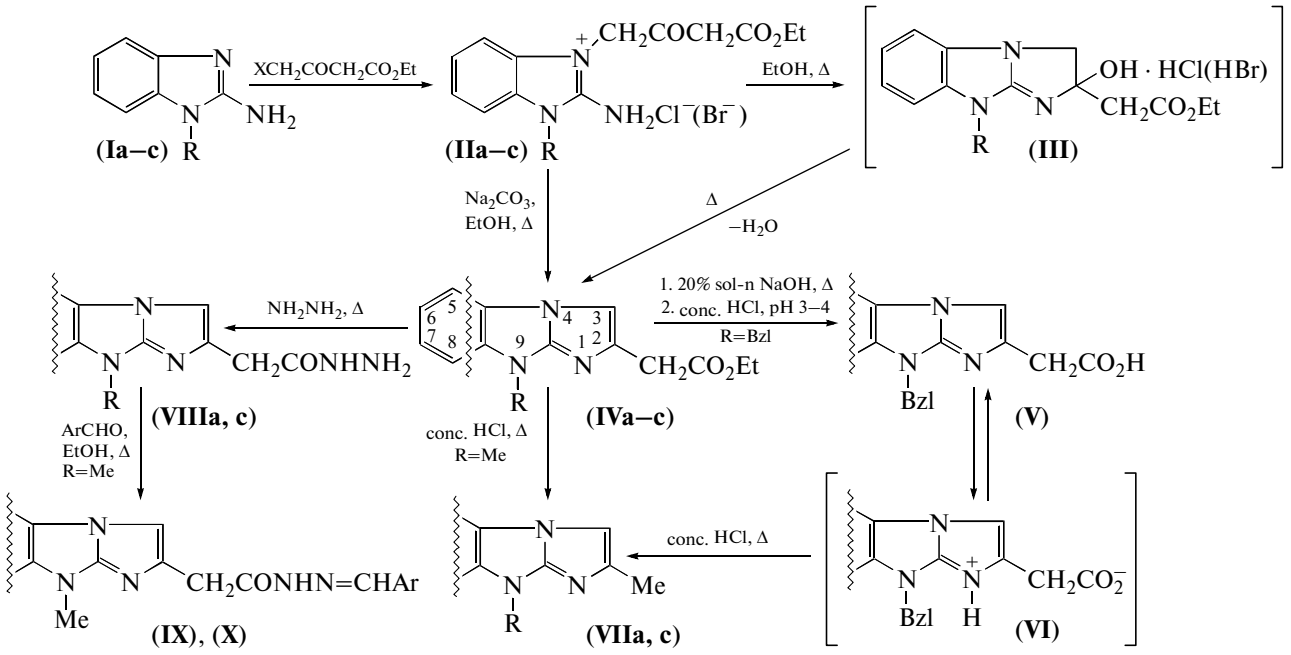
Many esters of hetarylacetic acids can be obtained in one step. An example is the interaction of 2-aminopyridines [13], 2-aminoisoquinolines [14], 2-aminothiazoles, and 2-aminobenzothiazoles [15] with ethyl γ-bromoacetate. However, 1-alkyl-2-aminobenzimidazoles (**Ia–c**) interacted with this ester to produce corresponding quaternary salts (**IIa–c**).

The reaction of benzimidazoles (**Ia–c**) with esters of γ-halogenoacetoacetic acids (Scheme 1) was carried out in acetone, methyl ethyl ketone (at room temperature for the γ-brominated ester and with boiling for the γ-chlorinated one), or by brief fusion of the reagents. When conducted in alcohols (ethanol or 2-propanol), the reaction was accompanied by the formation of numerous by-products and tars. Therefore, the yields of target products (**IIa–c**) were poor. The IR spectra of the resulting salts had bands at 1780–1770 and 1750–1735 cm^{-1}, corresponding to acetyl and ester groups, respectively, and two bands at 3260 and 3090–3070 cm^{-1} corresponding to amino group, which confirmed the structures of 2-aminobenzimidazolium salts. The structures were also proven by ^1H NMR signals from N^+CH_2CO (singlet at ~3.90 ppm) and NH_2 (singlet at 9.28–9.54 ppm).

The resulting halogenides (**IIa–c**) were unstable, and their melting ranges changed notably after recrystallization from ethanol or after storage. Their IR spectra lost bands characteristic of carbonyl and carboxy group vibrations and acquired a band at 1745 cm^{-1}, which can be attributed to vibrations of the C=O group in intermediate hydrohalogenides of ethyl esters of 9-substituted (2-hydroxy-2,3-dihydroxyimidazo[1,2-*a*]benzimidazolyl-2)acetic acids (**III**). The ^1H NMR spectra of the cyclic products had additional signals in the weaker field range: OH protons (8.70–9.10 ppm) and N^+H (12.80–13.20 ppm) groups, and signals from protons of substituents at position 9 were doubled.

[1] For communication XXIX, see [1].

[2] Corresponding author: phone: +7(863)243-47-00, fax:+7(863)243-46-67, e-mail: anis@ipoc.sfedu.ru.



(**I**)–(**IV**), (**VII**), (**VIII**) **a** R=Me, **b** R=*n*-Bu, **c** R=Bzl; (**IX**) Ar=$C_6H_4NO_2$-4, (**X**) Ar=5-nitrofuryl

Scheme 1. Synthesis of (9-substituted imidazo[1,2-*a*]benzimidazolyl-2)acetic acids.

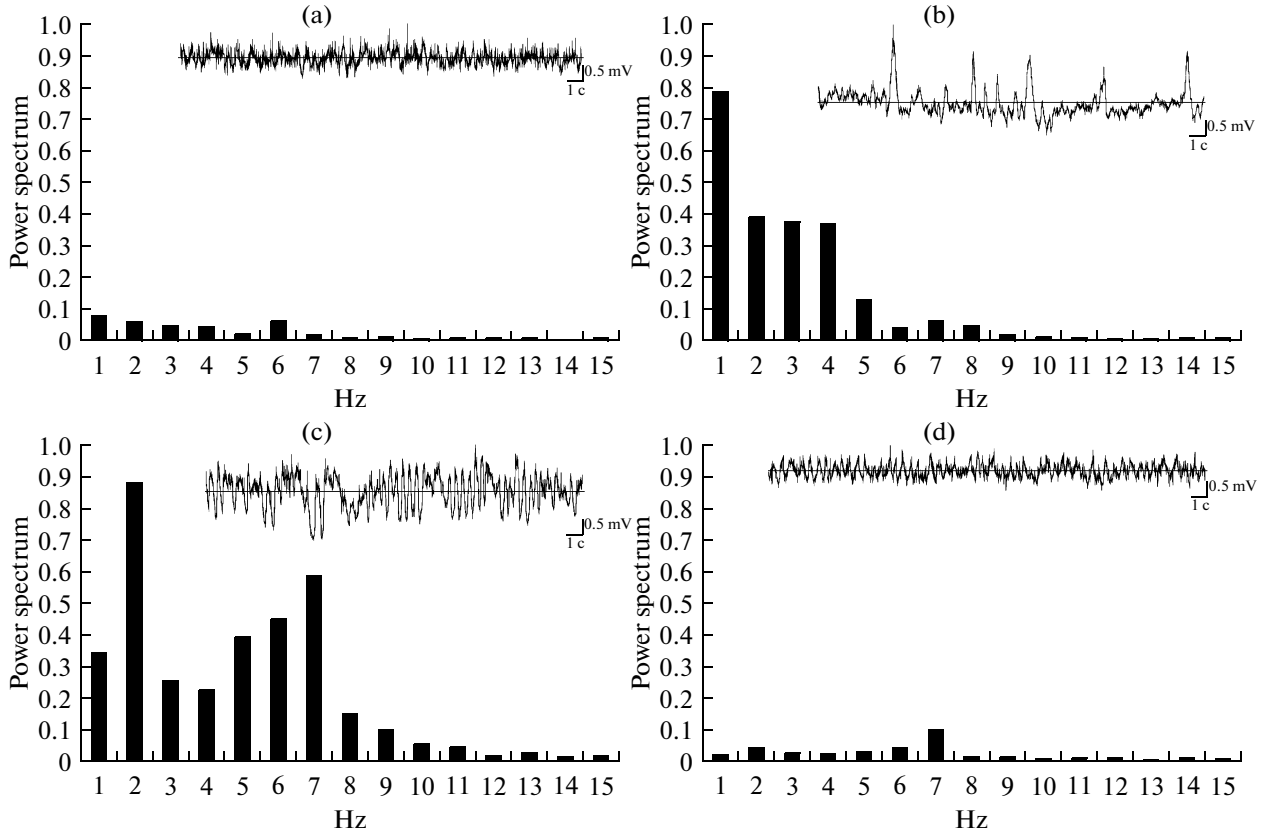
Dehydration reactions of hydroxy derivatives (**III**) yielding esters (**IVa**–**c**) were carried out by treatment with aqueous ammonia or $NaHCO_3$ at room temperature or in boiling ethanol. In the latter case, the process was accompanied by side reactions, and the yields of target products (**IVa**–**c**) were lower. For example, boiling of salt (**IIc**) in ethanol brought about a mixture of ester (**Vc**) and 9-benzyl-2-methylimidazo[1,2-*a*]benzimidazole (**VIIc**). The formation of the latter product can be explained by hydrolysis of the ester group followed by decarboxylation, catalyzed by the HX present in the original mixture.

Refluxing of salts (**IIa**–**c**) in ethanol with sodium bicarbonate caused their easy cyclization to esters (**IVa**–**c**) with 65–75% yields. The IR spectra of tricyclic compounds (**IVa**–**c**) showed bands at 1750 cm^{-1} (C=O) and 1150 cm^{-1} (C–O). The ^1H NMR spectra had signals characteristic of ethyl protons and protons of the CH_2CO group at 3.70–3.80 ppm in the form of a narrow doublet with $J \sim 1$ Hz. This shape of the signal appears to be related to the retarded rotation of these protons.

Cautious acidification of solutions of esters (**IVa**–**c**) to pH 3–4 with hydrochloric acid yielded water-soluble hydrochlorides (**IVa**–**c** · **HCl**). Esters (**IVa**–**c**) were inert to ammonia in the cold or with heating. No amides were obtained even by long heating of the esters with piperidine or morpholine. However, the reaction of (**IVa**, **c**) with hydrazine hydrate occurred readily to produce hydrazides (**VIIIa**, **c**) with high yields within 5–10 min. The IR spectra of (**VIIIa**, **c**) showed the absorption band of the carbonyl group and deformation vibrations of the NH group at 1680 and 1630 cm^{-1}, respectively. The stretch vibrations of the NH_2 group were indicated by two bands within 3500–3050 cm^{-1}. Hydrazide (**VIIIa**) was readily condensed to aromatic and heterocyclic aldehydes to form hydrazones (**IX**) and (**X**).

Hydrolysis of ester (**IVc**) with 20% NaOH produced the sodium salt of the acid. It proved to be hygroscopic. It emerged in the form of an oil, crystallizing after long desiccation over phosphorus pentoxide. Acidification of an aqueous solution of this salt to pH 3–4 yielded free acid (**V**) mixed with a small amount of the decarboxylation product (**VIIc**). The latter could be removed by washing with petroleum ether or benzene. Hydrolysis of esters (**IVa**, **c**) in concentrated hydrochloric acid yielded 2,9-disubstituted imidazo[1,2-*a*]benzimidazoles (**VIIa**, **c**).

Acid (**V**) readily decomposes during storage. Its IR spectrum showed vibrations of the C=O group at 1670 cm^{-1}, and the stretch vibrations of the N^+H group formed a broad band at 2700–2500 cm^{-1}. The shift of the carbonyl absorption to lower frequencies pointed to the existence of acid (**V**) as betaine (**VI**) [16]. The hydrolysis was accompanied by a shift of protons of the CH_2CO group about 0.6 ppm upfield and a shift of aromatic H5 proton about 0.2 ppm downfield. The readiness of decarboxylation of this acid may be related to its betaine structure, because there is evidence that decarboxylation of many heterocyclic carboxylic acids is mediated by zwitterions [17].



The activity patterns of a cortical column of rat somatic cortex before and after microapplication of compound (**IVa · HCl**) (50 nl, 0.75 mM) in isotonic NaCl solution. Power spectra and the focal baseline bioelectric activity of a cortical column (inset) are shown: (a) before microapplication; (b–d) after microapplication: (b) 5 min; (c) 10 min; (d) 30 min.

Investigation of biological properties of the compounds synthesized revealed a fungicidal activity in bromides (**IIa–c**, **X=Br**). These salts reduced the damage to wheat by rust and cucumber plants by powdery mildew by 48–50 and 54–56%, respectively, at a concentration of 0.02%. The compounds showed no activity against late blight of tomatoes. The reference compound Benomyl at 0.02% reduced the degree of wheat damage by rust by 97% and tomato damage by late blight, by 24% (by 43% at 0.1%), whereas the damage to cucumbers by a Benomyl-resistant powdery mildew was reduced by only 6%.

Large concentrations (300–1000 μg/ml) of hydrazones (**IX**) and (**X**) exerted bacteriostatic action on cocci. At 1×10^{-3} μg/ml, they were active against salmonellas and causative agents of dysentery and typhoid fever.

Experiments with isolated rat atria showed that esters (**IVa**, **c · HCl**) reduced heart rate at concentrations: (**IVa · HCl**), 7.2×10^{-4} M, and (**IVc · HCl**), 9.5×10^{-4} M. Procainamide had a similar effect at 3.7×10^{-3} M, and quinidine, at 3.4×10^{-4} M. Thus, the compounds under study overpower procainamide by factors of 5.1 and 3.9, respectively, but rank below quinidine. The most active compound (**IVa · HCl**), reduced heart rate by 33% and insignificantly, by 9%, elongated ventricular conduction at 45 mg/kg. Procainamide had a similar effect at 20 mg/kg.

Thus, the activity of (**IVa · HCl**) in the cardiac muscle excitation reduction test exceeded that of procainamide by a factor of 5.1, but had a lesser effect on ventricular conduction.

As the heart rate-reducing effect of many pharmaceuticals, such as β-adrenergic blocking or antiarrhythmic agents, is often related to the action on the pacemaking activity of sinoatrial node cells [18], it is reasonable to suppose a similar effect of compounds of similar types on rhythmogenesis in the brain, in particular, the effect on the excitation of pacemaking neurons characteristic of cholinesterase inhibitors and the development of epileptiform activity [19, 20].

Microapplication of (**IVa · HCl**) solution to cortical columns of the somatic cortex of rats induced a regular change in the focal baseline activity of the recorded cortical columns, which points to a significant rise of their excitability (figure).

Large-amplitude negative potentials appeared on the baseline 5 min after the microapplication, pointing to an increase in cortex excitability in the microapplication site (figure, b). Later, large-amplitude poten-

tials became more frequent, and they grouped into rhythmic discharges of high-amplitude θ activity (figure, c). The increase in amplitudes of baseline activity after (**IVa · HCl**) microapplication was spectrometrically confirmed by a significant, five- to tenfold, increase in the power of recorded focal activity at the frequencies 1–2 Hz and 5–7 Hz, that is, in the ranges of δ and θ activities (figure, b and c).

The effect of butylated derivative (**IVb · HCl**) on the baseline activity of cortical columns showed basically the same pattern, but the activation of focal rhythmic activity was more pronounced. The action of (**IVb · HCl**) manifested itself as an initial increase in the amplitudes of slow δ waves at the frequency 2–3 Hz, which turned to typical epileptiform activity 10 min after the microapplication.

The activating effect of (**IVa**, **b · HCl**) at the experimental concentrations lasted usually for no more than 30 min. The increase in the amplitude of negative potentials after microapplication of (**IVa**, **b · HCl**) was similar to the variation of the focal activity of cortical columns under the effect of acetylcholine agonists, such as carbachol or neostigmine. This fact is indicative of the involvement of choline receptors in cortex activation. The effect on cholinergic synapses was confirmed by investigation of antiarrhythmic activity. A decrease in rat atrial contraction rate was observed, which is typical of cholinergic reagents.

To sum up, experiments with microapplication of (**IVa**, **b · HCl**) solutions revealed the activating effect of these compounds, which manifested itself in an increase in the excitability of cortical columns mediated by choline receptors of cortical neurons. Esters of (9-alkylimidazo[1,2-*a*]benzimidazolyl-2)acetic acids can be utilized as reversible acetylcholine esterase inhibitors.

EXPERIMENTAL

^{1}H NMR spectra (δ, ppm; *J*, Hz) were recorded on a Unity-300 spectrometer (Varian, United States, 300 MHz) in DMSO-d_6, unless otherwise indicated. Signals from residual protons of the solvents were used as an internal reference. Infrared spectra (ν, cm^{-1}) were recorded in Nujol on a Specord-75-IR (Germany). Melting ranges were measured in glass capillary tubes on a PTP (M) device (Russia). The reaction monitoring and determination of the identity of synthesized compounds were done by TLC on plates with aluminum oxide. Chloroform was used as the mobile phase. Spots were visualized with iodine vapor in a humid chamber.

Ethyl-4-(1-methyl-2-aminobenzimidazolium-3)acetoacetate chloride (IIa, X=Cl). A. Ethyl γ-chloroacetoacetate (2 ml, ~15 mmol) was added to a stirred solution of amine (**Ia**) (1.5 g, 10 mmol) in 50 ml of acetone. The mixture was refluxed for 3 h. Fibrous matter began to precipitate after 15–20 min. The mixture darkened. After cooling, the matter was filtered and thoroughly washed with acetone. Yield: 2.5 g (79%). Melting range: 161–162°C (decomp., MeCN). IR: 3280, 3050 (NH_2), 1770, 1740 (C=O), 1670 (C=N), 1600, 1530, 1500 (C=C). Anal. Found: C, 54.02; H, 5.74; Cl, 11.28; N, 13.55%; Calcd. for $C_{14}H_{18}ClN_3O_3$: C, 53.94; H, 5.82; Cl, 11.37; N, 13.48%. ^{1}H NMR: 9.30 (2 H, s, NH_2), 7.13–7.62 (4 H, m, H_{Ar}), 5.52 (2 H, s, CH_2), 4.13 (2 H, q, *J* 7.2, CH_2), 3.90 (2 H, s, CH_2), 3.63 (3 H, s, CH_3), 1.21 (3 H, t, *J* 7.2, CH_3).

B. A finely pound mixture of 1.5 g (10 mmol) of amine (**Ia**) and 2.7 ml (20 mmol) of ethyl γ-chloroacetoacetate was melted at 70–80°C for 15 min. The melt was cooled and treated with acetone. The sediment was filtered and thoroughly washed with acetone. Yield: 2.1 g (68%). The salt was identical to that obtained by protocol A.

Ethyl-4-(1-methyl-2-aminobenzimidazolium-3)acetoacetate bromide (IIa, X=Br). Ethyl γ-bromoacetoacetate (3.6 g, 15 mmol) was added to a solution of amine (**Ia**) (2.2 g, 15 mmol) in 50 ml of acetone. The mixture was thoroughly mixed and left at room temperature overnight. The quaternary salt sediment formed on the next day was filtered and washed with acetone, benzene, and diethyl ether. Yield: 4.6 g (87%). Melting range: 199–200°C (decomp., MeCN). IR: 3260, 3070 (NH_2), 1780, 1735 (C=O), 1685 (C=N), 1600, 1530, 1505 (C=C). Anal. Found: C, 47.15; H, 5.16; Br, 22.35; N, 11.88%; Calcd. for $C_{14}H_{18}BrN_3O_3$: C, 47.21; H, 5.09; Br, 22.43; N, 11.80%.

Ethyl-4-(1-butyl-2-aminobenzimidazolium-3)acetoacetate chloride (IIb, X=Cl) was synthesized similar to (IIa, X=Cl). Yield: 89%. Melting range: 163–164°C (decomp., MeCN). IR: 3200, 3080 (NH_2), 1760, 1725 (C=O), 1675 (C=N), 1605, 1530, 1505 (C=C). Anal. Found: C, 57.75; H, 6.78; Cl, 10.11; N, 11.80%; Calcd for $C_{17}H_{24}ClN_3O_3$: C, 57.70; H, 6.84; Cl, 10.02; N, 11.88%. ^{1}H NMR: 9.28 (2 H, s, NH2), 7.20–7.68 (4 H, m, H_{Ar}), 5.50 (2 H, s, CH_2), 4.24 (2 H, t, *J* 7.4, CH2), 4.12 (2 H, q, *J* 7.2, CH2), 3.90 (2 H, s, CH_2), 1.57–1.75 (2 H, m, CH_2), 1.26–1.42 (2 H, m, CH_2), 1.20 (3 H, t, *J* 7.4, CH_3), 0.89 (3 H, t, *J* 7.4, CH_3).

Ethyl-4-(1-benzyl-2-aminobenzimidazolium-3)acetoacetate chloride (IIc, X=Cl) was synthesized similarly to (IIa, X=Cl). Yield: 46%. Melting range: 157–158 (decomp., MeCN). IR: 3230, 3050 (NH_2), 1770, 1730 (C=O), 1680 (C=N), 1600, 1540, 1500 (C=C). Anal. Found: C, 61.85; H, 5.80; Cl, 9.07; N, 10.90%; Calcd. for $C_{20}H_{22}ClN_3O_3$: C, 61.93; H, 5.72; Cl, 9.14; N, 10.83%. ^{1}H NMR: 9.54 (2 H, s, NH_2), 7.10–7.60 (9 H, m, H_{Ar}), 5.53 (2 H, s, CH_2), 5.47 (2 H, s, CH_2), 4.13 (2 H, q, *J* 7.2, CH2), 3.91 (2 H, s, CH_2), 1.20 (3 H, t, *J* 7.1, CH_3).

Ethyl-4-(1-benzyl-2-aminobenzimidazolium-3)acetoacetate bromide (IIc, X=Br). A mixture of amine (**Ic**) (2.2 g, 10 mmol), ethyl γ-bromoacetoacetic acid

(2.5 ml, 10 mmol), and 5 ml of acetone was thoroughly mixed and, after the completion of the spontaneous exothermic reaction, heated in a water bath at 60–70°C for 15 min. The mixture was cooled, and the colorless sediment was filtered and washed on the filter with acetone and diethyl ether. Yield: 2.3 g (53%). Melting range: 184–185°C (decomp., MeCN). IR: 3260, 3090 (NH_2), 1760, 1730 (C=O), 1665 (C=N), 1605, 1535, 1500 (C=C). Anal. Found: C, 55.52; H, 5.04; Br, 18.55; N, 9.80%; Calcd. for $C_{20}H_{22}BrN_3O_3$: C, 55.57; H, 5.13; Br, 18.48; N 9.72%.

Ethyl-(9-methylimidazo[1,2-*a*]benzimidazolyl-2) acetate (IVa). Bromide (**IIa**) (2.1 g, 6 mmol) and NaHCO3 (0.5 g, 6 mmol) were suspended in 10 ml of ethanol and refluxed for 2 h. The ethanol was evaporated, and the residue was dissolved in a small volume of chloroform. The insoluble inorganic salts were filtered out, and the filtrate was run through a column with aluminum oxide. Chloroform was used as the mobile phase. Chloroform was evaporated from the eluate, and the residue was dried over phosphorus pentoxide. Yield: 1.1 g (73%). Melting range: 68–69°C (MeCN). IR: 1750 (C=O), 1630, 1605, 1500 (C=C, C=N), 1450 (CH_2), 1150 (C–O). Anal. Found: C, 65.42; H, 5.96; N, 16.25%. Calcd. for $C_{14}H_{15}N_3O_2$: C, 65.36; H, 5.88; N, 16.33%. ^{1}H NMR ($CDCl_3$): 7.45 (1 H, d, *J* 7.9, H5), 7.10–7.32 (4 H, m, H_{Ar}), 4.19 (2 H, q, *J* 7.4, OCH_2), 3.71–3.78 (5 H, m, NCH_3, CH_2), 1.27 t (3 H, *J* 7.2, CH_3).

Ethyl-(9-methylimidazo[1,2-*a*]benzimidazolyl-2) acetate hydrochloride (IVa · HCl) was obtained by careful acidification of a solution of (IVa) in acetone to pH 2–3 with hydrochloric acid. Yield: 90%. The salt was purified either by recrystallization from a small volume of ethanol or precipitation from ethanol solution with diethyl ether. Melting point: 197°C (decomp.) Anal. Found: C, 57.18; H, 5.55; Cl, 12.13; N, 14.23%; Calcd. for $C_{14}H_{15}N_3O_2$ HCl: C, 57.24; H, 5.49; Cl, 12.07; N, 14.30%.

Ethyl-(9-butylimidazo[1,2-*a*]benzimidazolyl-2) acetate hydrochloride (IVb · HCl). Base (**IVb**) was obtained similarly to (**IVa**) in the form of an oil by cyclization of salt (**IIb**). Acidification of a solution of base (**IVb**) in acetone to pH 2–3 with concentrated hydrochloric acid produced hydrochloride (**IVb · HCl**). Yield: 72%. Melting range: 133–134°C (decomp., MeCN). IR: 1760 (C=O), 1630, 1600, 1505 (C=C, C=N), 1455 (CH_2), 1150 (C–O). Anal. Found: C, 60.72; H, 6.54; Cl, 10.65; N, 12.58%. Calcd. for $C_{17}H_{21}N_3O_2$ HCl. C, 60.80; H, 6.60; Cl, 10.56; N, 12.51%. ^{1}H NMR: 12.80 (1 H, wid. s, N^+H), 7.30–8.20 (5 H, m, H_{Ar}), 4.52 (2 H, t, *J* 6.9, CH_2), 4.20 (2 H, q, *J* 7.2, CH_2), 3.86 (2 H, s, CH_2), 1.80–2.00 (2 H, m, CH_2), 1.38–1.56 (2 H, m, CH_2), 1.33 (3 H, t, *J* 7.4, CH_3), 0.97 (3 H, t, *J* 7.2, CH_3).

Ethyl-(9-benzylimidazo[1,2-*a*]benzimidazolyl-2) acetate (IVc) was obtained similarly to (**IVb**) by cyclization of the corresponding salt (**IIc**). Yield: 83%. Melting point: 110°C (petroleum ether). IR: 1750 (C=O), 1630, 1600, 1500 (C=C, C=N), 1450 (CH_2), 1150 (C–O). Anal. Found: C, 72.14; H, 5.65; N, 12.68%; Calcd. for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60%. ^{1}H NMR ($CDCl_3$): 7.46 (1 H, d, *J* 8.1, H5), 7.20-7.35 (9 H, m, H_{Ar}), 5.36 (2 H, s, NCH_2), 4.20 (2 H, q, OCH_2), 3.77 (2 H, d, *J* 1.0, CH_2), 1.27 (3 H, t, *J* 7.2, CH_3).

Ethyl-(9-benzylimidazo[1,2-*a*]benzimidazolyl-2)acetate hydrochloride (IVc · HCl) was obtained similarly to (**IVa · HCl**). Yield: 95%. Melting point: 204°C (decomp., EtOH–Et_2O). Anal. Found: C, 65.02; H, 5.40; Cl, 9.50; N, 11.44; Calcd. for $C_{20}H_{19}N_3O_2$ HCl: C, 64.95; H, 5.45; Cl, 9.59; N, 11.36%.

(9-Benzylimidazo[1,2-*a*]benzimidazolyl-2)acetic acid (V). A suspension of ester (**IVc**) (2.0 g, 6 mmol) in 20 ml of 20% NaOH was refluxed for 2 h. The mixture was cooled, and the sodium salt precipitated in the form of an oil was separated. It crystallized after a prolonged standing in air. The salt was dissolved in 30 ml of water and carefully acidified to pH 3–4 with concentrated hydrochloric acid. The mixture was evaporated to a small volume and cooled. The sediment was filtered and washed with cold water. After drying at room temperature, the product was suspended in 30 ml of benzene, thoroughly mixed, and the undissolved acid (**VI**) was filtered and washed with petroleum ether. Yield: 1.2 g (65%). Melting range: 228–229°C (decomp.) IR: 2700-2500 (N^+H), 1670 (C=O), 1620, 1600, 1510 (C=C, C=N). Anal. Found: C, 70.74; H, 5.02; N, 13.69%; Calcd. for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76%. ^{1}H NMR: 7.68 (1 H, d, *J* 7.5, H5), 7.05–7.50 (9 H, m, H_{Ar}), 5.37 (2 H, s, NCH_2), 3.21 (2 H, s, CH_2).

2,9-Dimethylimidazo[1,2-*a*]benzimidazole (VIIc). A mixture of ester (**IVa**) (0.77 g, 3 mmol) and 10 ml of concentrated hydrochloric acid was refluxed for 2 h. The solution was cooled and alkalized to pH 9–10 with 22% NH_4OH. Base (**VIIa**) was extracted with chloroform (3 × 10 ml). The extract was dried over sodium sulfate, evaporated to a small volume, and run through aluminum oxide with chloroform as the mobile phase. The solvent was evaporated from the extract, and the residue was recrystallized from ethanol. Yield: 0.53 g (95%). Melting point: 94°C (decomp., EtOH). IR: 1635 (C=N), 1610, 1500, 1470, 1460 (C=C). Anal. Found: C, 78.17; H, 5.84; N, 15.99%; Calcd. for $C_{11}H_{11}N_3$: C, 71.33; H, 5.99; N, 22.69%. ^{1}H NMR: 7.44 (1 H, d, *J* 8.0, H5), 7.14–7.37 (4 H, m, H_{Ar}), 3.78 (2 H, s, NCH_2), 2.40 (3 H, s, CH_3).

9-Benzyl-2-methylimidazo[1,2-*a*]benzimidazole (VIIc) was obtained similarly to tricyclic compound (**VIIa**) by decarboxylation of acid (**Vc**) with concentrated hydrochloric acid. Yield: 76%. Melting point: 111°C (decomp., EtOH). IR: 1640 (C=N), 1600, 1500, 1470, 1460 (C=C). Anal. Found: C, 78.17; H, 5.84; N, 15.99%; Calcd. for $C_{17}H_{15}N_3$: C, 78.13;

H, 5.79; N, 16.08%. ^{1}H NMR: 7.43 (1 H, d, *J* 7.8, H5), 7.04–7.35 (9 H, m, H_{Ar}), 5.35 (2 H, s, NCH_2), 2.38 (3 H, s, CH_3).

(9-Methylimidazo[1,2-*a*]benzimidazolyl-2)acetohydrazide (VIIIa). Ester (**IVa**) (0.26 g, 1 mmol) and hydrazine hydrate (2 ml) were refluxed for 5 min. At first, a solution formed, and it immediately gave rise to white needles. The sediment was filtered and washed with cold ethanol and diethyl ether. Yield: 0.22 g (92%). Melting range: 189–190°C (decomp., EtOH). IR: 3500-3030 (NH_2, two bands), 1680 (C=O), 1630 (NH). Anal. Found: C, 59.19; H, 5.48; N, 28.72%; Calcd. for $C_{12}H_{13}N_5O$: C, 59.25; H, 5.39; N, 28.79%. ^{1}H NMR: 9.09 (1 H, s, NH), 7.72 (1 H, d, *J* 7.8, H5), 7.42-7.53 (2 H, m, H_{Ar}), 7.28 (1 H, t, *J* 7.5, H_{Ar}), 7.15 (1 H, t, *J* 7.5, H_{Ar}), 4.23 (2 H, s, NH_2), 3.67 (3 H, s, NCH_3), 3.38 (2 H, s, CH_2).

(9-Benzylimidazo[1,2-*a*]benzimidazolyl-2)acetohydrazide (VIIIc) was obtained from ester (**IVc**) similarly to (**VIIIa**). Yield: 91%. Melting point: 165°C (decomp., EtOH). IR: 3500–3030 (NH_2, two bands), 1675 (C=O), 1628 (NH). Anal. Found: C, 67.62; H, 5.45; N, 22.00%; Calcd. for $C_{18}H_{17}N_5O$: C, 67.70; H, 5.37; N, 21.93%. ^{1}H NMR: 9.11 (1 H, s, NH), 7.75 (1 H, d, *J* 7.8, H5), 7.45–7.56 (2 H, m, H_{Ar}), 7.10–7.40 (7 H, m, H_{Ar}), 5.37 (2 H, s, NCH_2), 4.24 (2 H, s, NH_2), 3.39 (2 H, s, CH_2).

***N*'-(4-Nitrobenzylidene)-(9-methylimidazo[1,2-*a*]benzimidazolyl-2)acetohydrazide (IX).** Hydrazide (**VIIIa**) (0.2 g, 0.8 mmol) and 4-nitrobenzaldehyde (0.12 g, 0.8 mmol) were refluxed in 4 ml of ethanol for 2 h. The mixture was cooled and the resulting sediment was filtered. Yield: 0.3 g (98%). Melting range: 213–214°C (decomp., H_2O–DMF). IR: 3455, 3100 (NH, two bands), 1685 (C=O), 1605 (NH). Anal. Found: C, 60.55; H, 4.34; N, 22.26%; Calcd. for $C_{19}H_{16}N_6O_3$: C, 60.63; H, 4.28; N, 22.33%. ^{1}H NMR: 11.82 and 11.72 (1 H, 2 s, NH), 9.09 and 8.87 (1 H, 2 s, CH), 7.07–8.42 (9 H, m, H_{Ar}), 4.04 (2 H, s, CH_2), 3.67 (3 H, s, NCH_3).

***N*'-(4-Nitrofurfurylidene)-(9-methylimidazo[1,2-*a*]benzimidazolyl-2)acetohydrazide (X)** was obtained similarly to hydrazone (**IX**) from hydrazide (**VIIIa**) and 5-nitrofuran-2-carbaldehyde. Yield: 97%. Melting point: 143°C (decomp., EtOH). IR: 3470, 3125 (NH, two bands), 1690 (C=O), 1600 (NH). Anal. Found: C, 55.80; H, 3.92; N, 22.85%; Calcd. for $C_{17}H_{14}N_6O_4$: C, 55.74; H, 3.85; N, 22.94%. ^{1}H NMR: 11.86 and 11.75 (1 H, 2 s, NH), 8.25 and 7.91 (1 H, 2 s, CH), 7.00–7.70 (7 H, m, H_{Ar}), 3.95 (2 H, s, CH_2), 3.72 (3 H, s, NCH_3).

Fungicidal activity. The fungicidal activities of the compounds under study against late blight of tomato, brown leaf rust of wheat, and powdery mildew of cucumbers, were tested in a greenhouse.

The effects on late blight of tomato were tested on tomato plants with 5 to 7 leaves. Plants were sprinkled with suspensions of the compounds and inoculated with *Phytophthora* zoospores. Their efficacy was assessed on day 7 after the infection from the decrease in the degree of leaf injury with reference to control plants infected without sprinkling.

In tests of activity against wheat leaf rust, wheat plants of age 10–12 days were sprinkled with suspensions of compounds to be tested and three days later inoculated with *Puccinia* uredospores. Their efficacy was assessed on day 10 after the infection from the decrease in the degree of leaf injury with reference to unsprinkled control plants.

In experiments with powdery mildew of cucumbers, seed leaves of cucumber plants were inoculated with the fungus 24 h after sprinkling with aqueous suspensions of compounds to be tested. The degree of leaf injury was assessed from the area covered by the powdery coating on day 9 or 10 after the infection.

The results were compared with the fungicide Benomyl (methyl [1-(butylcarbamoyl)benzimidazolyl-2]carbamate).

Antimicrobial activity was evaluated by the agar diffusion test in vitro from the measurement of the zone of microbial growth inhibition around samples. A well was cut in an agar layer with a target microbial strain with a sterile 8-mm cork borer down to the plate bottom. To each of the wells, 0.1 ml of test solution was added. The plates were incubated in a temperature-controlled cabinet at 37°C for 16–18 h. Then the growth inhibition zones forming around the wells were measured.

The target microorganisms were Gram negative (typhoid fever agent, salmonella, pathogenic *Escherichia coli*) and Gram positive (various staphylococci species). The microbial load was 3×10^6 bacteria in 1 ml of washing from an 18-h agar culture. The assessment was done with reference to Furadonin, a pharmaceutical active at 100 μg/ml (except for pathogenic *Escherichia coli*).

Antiarrhythmic action. The antiarrhythmic properties of the compounds under study were assessed from their action on cardiac muscle excitability [21]. Experiments were carried out with isolated rat atria superfused with Krebs–Ringer solution (composition, mM: NaCl, 124; KCl, 3; $MgSO_4$ – 2, $CaCl_2$ – 2, $NaHCO_3$ – 26, KH_2PO_4, 1.24; glucose, 10; pH 7.4) at a flow rate of 2.4 ml/min, temperature 32°C, with oxygenation (95% oxygen and 5% carbon dioxide). The activity of compounds was judged from the minimum effective concentration (MEC) of substances preventing paced rhythm (3 Hz, pulse duration 0.5 ms, and voltage twice as high as the threshold value; pacemaker ESL-3) within 15 s, which was indicative of an increase in the refraction time of the cardiac muscle. The activities were compared with the action of procainamide and quinidine [22].

Activation of the excitability of cortical columns. The tests were carried out by acute experiments with unanesthetized immobilized genetically heteroge-

neous white rats, males and females, weighing 200–250 g [23]. The protocol met the international regulations for experiments with animals [24]. It had been approved by the Biomedical Ethics Committee of the Russian Academy of Sciences [protocol 98 of March 11, 2002]. Burr holes 3 mm in diameter were drilled for recording bioelectrical activity and stimulating brain regions: 2 mm caudal to bregma and 5.5 mm lateral to the sagittal suture above the vibrissa cortical representation. Deflection of focal activity from particular columns was done with glass microelectrodes filled with 2.5 M NaCl, resistance 2–5 MΩ, tip diameter 2–3 μm.

Microapplication of solutions of compounds (**IVa**, **b** · **HCl**) (volume 50 nl, concentration 0.75 mM in isotonic NaCl solution) was done by local injection with pressure using noncapillary glass microelectrodes with tip diameters 5–10 μm. The volume of the solution injected was determined by recording the position of the top of the meniscus in the thick portion of the electrode with an MBS-2 microscope (Russia) equipped with an eyepiece micrometer.

Analysis of the baseline activity and construction of averaged power spectra (range 1–30 Hz, step 1 Hz, baseline time 30–60 s, averaging epoch 1 s) were done by time series analysis using the Fourier transform (DataView software, Yu.I. Gusach, Kogan Research Institute of Neurocybernetics).

ACKNOWLEDGMENTS

This work was supported by the Ministry of Education and Science of the Russian Federation, grant AVTsP RNP VSh No. 2.2.1.1/11794.

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