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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF AMIDES OF 2-AMINO-3-INDOLYLACRYLIC ACID

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Opening of the five-member ring of indolylmethyleneoxazolones with aliphatic amines was used to synthesize a series of amides of 2-amino-3-indolylacrylic acid. These compounds were studied in vitro in relation to the various types of pharmacological activity typical of this class, including receptor (5-HT_2-, 5-HT_3-, and $P2Y_1$-ergic, κ-opioid), antiaggregant, hemorheological, antiarrhythmic, and antioxidant activities.

Keywords: indole, indolylmethyleneoxazolones, amides of 2-amino-3-indolylacrylic acid, hemorheological activity, antiaggregant, antioxidant, antiarrhythmic, 5-HT_2 antagonist, 5-HT_3 antagonist, κ-opioid activity, $P2Y_1$ antagonist, ketanserin, ondansetron, basilen blue, Y50,488, quinidine, acetylsalicylic acid, pentoxifylline, dibunol.

Our previous studies identified a number of types of receptor (serotoninergic, purinergic, kappa-opioid), antiarrhythmic, antiaggregant, and antioxidant actions among compounds with heterocyclic systems containing nitrogen atoms in the molecular nucleus [1 – 4] and phenyl-containing substituents [5]. The indole fragment is present in an enormous number of biologically active compounds [6]. Given this, as well as the assignment of various therapeutic agents to the indole class, including rhynchophylline (an antiarrhythmic), indobufen (an antiaggregant), and 6′-GNTI (a kappa agonist) [7 – 9], leads to interest in the study of new indole derivatives for these types of specific pharmacological activity.

We have synthesized and tested substituted amides of 2-amino-3-indoleacrylic acid. The structures of these compounds include an amino group separated by two carbon atoms from the two-ring indole moiety. This structure is typical of many biologically active indole derivatives - the amino acid tryptophan, the neurotransmitter serotonin, and alkaloids of the β-carboline series [10]. We have previously shown that several amides of 2-amino-3-indolylacrylic acid have local anesthetic and antiarrhythmic properties [11]. With the aim of preparing analogous substances, we proposed a three-stage synthesis of such amides, compounds IVa-n. At the first stage, reaction of indole-3-aldehyde (I) with diethylsulfate or dimethylsulfamoyl chloride introduced substituents into position 1 of indole. The 1-substituted indole-3-aldehydes (II) were subjected to the Erlenmeyer-Plöchl reaction to obtain indolylmethyleneoxazolones (IIIa – h) and their reactions with amines were studied (Scheme 1). This led to the synthesis of a series of amides of 2-amino-3-indolylacrylic acids (IVa – n). Data on the substituents and melting temperatures of these compounds are presented in Table 1. The structures of oxazolones IIIa – h and amides IVa – n were confirmed by ^{1}H NMR spectroscopy (Tables 1 – 3). Compounds IVa-n were tested for biological activity as solutions of their hydrochlorides in water (melting points are given in Table 2).

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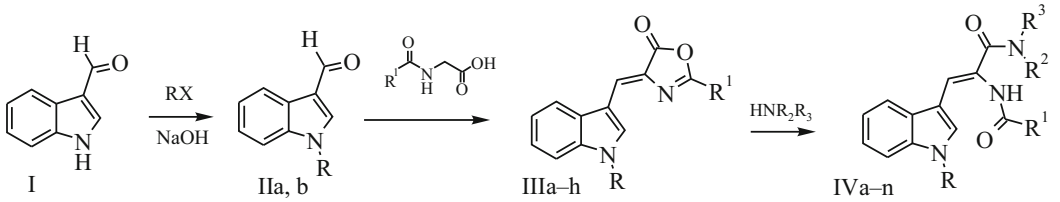
EXPERIMENTAL CHEMICAL SECTION

^{1}H NMR spectra were recorded on a Bruker DPX-250 instrument. N-Aroylglycines were prepared as described in [12]. Indole-3-carbaldehyde (I) was synthesized as described in [13]. Elemental analysis data (C, H, N) for the compounds synthesized here corresponded to theoretically calculated values.

1-Ethyl-1*H*-indole-3-carbaldehyde (IIa). Compound I (29.03 g, 0.2 mol) was dissolved with gentle heating (~40°C)

Scheme 1



I: a) R = C_2H_5 ;
b) R = $SO_2N(CH_3)_2$
II: a) R = C_2H_5 ; R^1 = C_6H_5
b) R = C_2H_5 ; R^1 = 2-FC_6H_4
c) R = C_2H_5 ; R^1 = 2-ClC_6H_4
d) R = C_2H_5 ; R^1 = 2-$CH_3OC_6H_4$
e) R = C_2H_5 ; R^1 = 4-$CH_3OC_6H_4$
f) R = C_2H_5 ; R^1 = 3,4-$(CH_3O)_2C_6H_4$
g) R = $SO_2N(CH_3)_2$; R^1 = 4-$CH_3C_6H_4$
h) R = $SO_2N(CH_3)_2$; R^1 = 2-FC_6H_4

IIIa–i: R-R^3 – see Table. 2.

in 50 ml of dimethylsulfoxide (DMSO). The solution was cooled to room temperature and solutions of 13 g (0.325 mol) of NaOH in 13 ml of water and 33 ml of diethylsulfate (0.25 mol) were added simultaneously over 30 min. The temperature was kept below 30°C. After addition of these reagents, the mixture was held for 1 h at room temperature, and the temperature was then gradually (over 1 h) increased to 50°C and the mixture was poured into 600 ml of water. The resulting precipitate of pale pink color was collected by filtration. The yield was 33.35 g (96%). The melting temperature was 103 – 105°C. ^{1}H NMR spectral data were consisted with those reported in [14].

3-Formyl-*N*,*N*-dimethyl-1*H*-indole-1-sulfonamide (IIb). This was prepared by the same method from aldehyde I and dimethylsulfamoyl chloride. The yield was 97% and the melting temperature was 122 – 124°C. The ^{1}H NMR spectrum ($CDCl_3$), δ, ppm, was: 2.93 [s, 6H, $SO_2N(CH_3)_2$], 7.30 – 7.45 (m, 2H, 5-H, 6-H), 7.85 – 7.93 (m, 1H, 7-H), 8.08 (s, 1H, 2-H), 8.27 – 8.37 (m, 1H, 4-H), 10.08 (s, 1H, CHO).

(3*Z*)-3-[(2-Aryl-5(4*H*)-oxo-1, 3-oxazol-4-yl)methylene]-1-R-1*H*-indoles (IIIa – h) (general method). A mixture of 0.1 mol of the corresponding N-aroylglycine and 0.1 mol of IIa or IIb was heated in 40 ml of acetic anhydride for 3 h at 120°C. The resulting precipitate was washed with acetic acid and benzene and recrystallized from toluene, yielding red crystals. Yields, melting temperatures, and ^{1}H NMR spectra for compounds IIIa-h are shown in Table 1.

Amides of (2-aroylamino)-3-(1-R-1*H*-indol-3-yl)acrylic acid (IVa – n) and their hydrochlorides. General method. A suspension of 3 mmol of substance IIIa – h in a mixture of 3.5 ml of benzene and 3.5 ml of acetonitrile was supplemented with 4 mmol of the corresponding amine and boiled to disappearance of the color of the initial oxazolone (1 – 2 h). The precipitate forming on cooling was collected by filtration and recrystallized from isopropanol. Substituents and melting temperatures of compounds IVa – n are shown in Table 2, and yields and ^{1}H NMR spectra are shown in Table 3. Hydrochlorides were prepared by dissolving 0.001 mol of amide IVa – n in 5 ml of dry acetone and supplementing this solution with saturated HCl solution in isopropanol to an acid reaction. If the product did not precipitate on stirring with a glass rod, the mixture was diluted with diethyl ether. Colorless precipitates were collected by filtration and washed with diethyl ether. Hydrochloride yields were 90 – 99%. The melting temperatures of hydrochlorides are shown in Table 2.

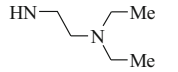
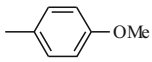
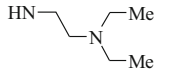
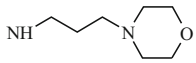
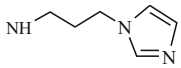
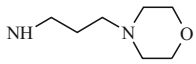
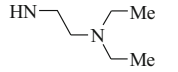
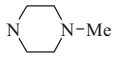
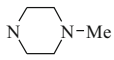
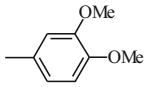
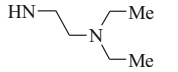
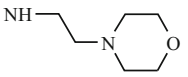
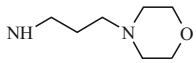
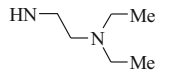
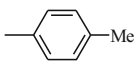
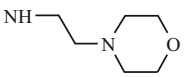
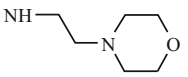
TABLE 1. Yields, Melting Temperatures, and ^{1}H NMR Spectra of Oxazolines IIa–h.

Compound	Yield, %	Melting temperature, °C	^{1}H NMR spectrum, δ, ppm, solvent $CDCl_3$
IIIa	45	145 – 147	1.75 (t, 3H, $CH_2\underline{CH_3}$), 4.30 (q, $\underline{CH_2}CH_3$), 7.26 – 8.25 (m, 10H, HAr), 8.52 (s, 1H, -CH=).
IIIb	36	143 – 145	1.80 (t, 3H, $CH_2\underline{CH_3}$), (q, $\underline{CH_2}CH_3$), 7.28 – 8.11 (m, 9H, HAr), 8.52 (s, 1H, -CH=).
IIIc	43	186 – 187	1.78 (t, 3H, $CH_2\underline{CH_3}$), 4.30 (q, $\underline{CH_2}CH_3$),7.28 – 8.13 (m, 9H, HAr), 8.64 (s, 1H, -CH=).
IIId	40	146 – 148	1.60 t, 3H, $CH_2\underline{CH_3}$), 4.05 (s, 3H, OCH_3), 4.30 (q, 4H, $\underline{CH_2}CH_3$), 7.00 – 8.20 m (9H, H_{arom}), 8.50 sec (1H, CH=).
IIIe	45	161 – 163	1.81 (t, 3H, $CH_2\underline{CH_3}$), 3.95 sec (3H, OCH_3), 4.28 (q, $\underline{CH_2}CH_3$), 6.83 – 8.14 (m, 9H, HAr), 8.43 (s, 1H, -CH=).
IIIf	60	185 – 187	1.57 (t, 3H, $CH_2\underline{CH_3}$), 3.96 sec (3H, OCH_3),4.05 (s, 3H, OCH_3), 4.32 (q, 4H, $\underline{CH_2}CH_3$), 6.94 – 8.06 m (8H, H_{arom}), 8.44 sec (1H, CH=).
IIIg	61	215 – 216	2.43 (s, 1H, CH_3), 2.95 [s, 6H, $SO_2N(CH_3)_2$], 7.27 – 8.12 (m, 9H, HAr), 8.72 (s, 1H, -CH=).
IIIh	55	207 – 209	2.93 [s, 6H, $SO_2N(CH_3)_2$], 7.29 – 8.13 (m, 9H, HAr), 8.75 (s, 1H, -CH=).

EXPERIMENTAL PHARMACOLOGICAL SECTION

Studies of antagonist activity in relation to serotonin 5-HT_2 receptors and purine $P2Y_1$ receptors and agonist activity in relation to γ-opioid receptors were performed using an in vitro platelet activation model [15] using narrow-angle light scattering. Studies were performed using rabbit platelets in a salt solution containing 140 mM NaCl, 10 mM Tris-HCl pH 7.4. Antagonist activity in relation to 5-HT_2 receptors was studied using 1 μM 5-hydroxytryptamine (Sigma, USA) as platelet activation inducer. $P2Y_1$ antagonist activity was assessed by addition of 5 mM EDTA to salt medium (to exclude the development of P_2X-mediated effects) and use of 70 nM ADP (Sigma, USA) as platelet activation inducer. Study compounds were tested at a concentration of 1 μM. Kappa-agonist activity was assessed in terms of the extent of platelet activation induced by study compounds at 0.1 mM; the specificity of the opioid nature of the actions of compounds was confirmed by tests using the opioid receptor antagonist naltrexone FV (OAO Moskovskaya Farmatsevticheskaya Fabrika, Russia). The following reference substances were used: the 5-HT_2 antagonist ketanserin (Sigma, USA), the $P2Y_1$ antagonist basilen blue (Sigma, USA), and the selective κ-opioid receptor agonist U-50,488 (Sigma,

TABLE 2. Substituents and Melting Temperatures of Amides IVa – n and their Hydrochlorides.

Compound	R	R^1	NR^2R^3	Molecular formula	Melting temperature, °C	Melting temperature of hydrochloride, °C
IVa	C_2H_5	Ph		$C_{26}H_{32}N_4O_2$	171 – 173	208 – 210
IVb	C_2H_5			$C_{27}H_{34}N_3O_3$	178 – 179	156 – 157
IVc	$SO_2N(Me)_2$			$C_{27}H_{32}FN_5OS$	189 – 190	148 – 150
IVd	$SO_2N(Me)_2$			$C_{26}H_{27}FN_6O_4S$	204 – 206	189 – 191
IVe	C_2H_5			$C_{27}H_{31}ClN_4O_3$	182 – 183	148 – 150
IVf	C_2H_5			$C_{26}H_{31}ClN_4O_2$	170 – 172	179 – 181
IVg	$SO_2N(Me)_2$			$C_{25}H_{28}FN_5O_4S$	226 – 227	186 – 187
IVh	C_2H_5			$C_{26}H_{30}N_4O_3$	191 – 193	237 – 240
IVi	C_2H_5			$C_{29}H_{38}N_4O_5$	123 – 124	232 – 233
IVj	C_2H_5			$C_{27}H_{32}N_4O_4$	179 – 180	166 – 168
IVk	C_2H_5	Ph		$C_{27}H_{34}N_4O_3$	152 – 154	173 – 175
IVl	C_2H_5			$C_{26}H_{31}FN_4O_2$	123 – 124	204 – 205
IVm	$SO_2N(Me)_2$			$C_{27}H_{33}N_5O_5S$	177 – 178	216 – 218
IVn	C_2H_5			$C_{26}H_{29}FN_4O_3$	154 – 155	214 – 215

USA). Narrow-angle light scattering was recorded using a probe with angular coordinates of 12° on a LASKA-1K instrument (Lyumex, Russia). The magnitudes of the antagonist/agonist activities of substances were assessed in terms of changes in light scattering induced by activation of platelets (%) in relation to control values.

Evaluation of antagonist activity in relation to serotonin 5-HT_3 receptors was performed as described in [16]. The actions of substances were assessed in terms of their influences on the extent of the serotonin-induced spasmogenic effect on isolated guinea pig ileum (Krebs buffer solution, pH 7.4, t 37°C). The spasmogenic effect was induced using 3 μM 5-hydroxytryptamine (Sigma, USA). All substances were studied at equimolar concentrations. The reference agent was ondansetron (Lens-Farm, Russia). The level of 5-HT_3 serotoninergic activity was assessed in terms of changes in the spasmogenic effect in control and experimental measurements.

Effects on rabbit platelet aggregation were evaluated in vitro as described in [17]. Study compounds were incubated with plasma for 5 min. Aggregation was induced with ADP (Reanal, Hungary) at a concentration of 5 μM. Experiments were performed using a 230 LA dual-channel laser platelet aggregation analyzer (NPF Biola, Russia). Activity was assessed in terms of decreases in platelet aggregation compared with controls (%). The reference agent was acetylsalicylic acid (Sigma, USA).

Hemorheological activity was assessed in terms of changes in rabbit blood viscosity in a model of impaired rheological blood properties in vitro as described in [18], with incubation of blood at 42.5°C for 60 min. Blood samples were standardized to a single hematocrit value of 45 U. Study compounds were added to blood samples to a final concentration of 10 μM. The reference agent was pentoxifylline (Aventis, Germany). Blood viscosity was measured using an AKR-2 viscometer (Russia). The effects of substances on erythrocyte aggregation were assessed in terms of changes in the index of aggregation calculated as the ratio of blood viscosity at a shift rate of 3 sec^{-1} to the viscosity of blood at 100 sec^{-1}.

The antiarrhythmic properties of compounds were assessed in terms of their effects on the assimilation of an imposed rhythm [19]. Experiments were performed on isolated rat atria in Krebs nutrient medium at 25°C with oxygenation. Activity was assessed in terms of the minimal effective concentration (MEC) of substances preventing the imposed rhythm (3 Hz, impulse duration 0.5 msec, current potential 2 times threshold; ÉSL-2 electrostimulator, Russia) over a

TABLE 3. Yields and ^{1}H NMR Spectra of Compounds IVa – n.

Compound	Yield, %	^{1}H NMR spectrum, δ, ppm, solvent $CDCl_3$
IVa	78	0.95 [t, 6H, $N(CH_2\underline{CH_3})_2$], 1.32 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 2.45 [q, 4H, $N(\underline{CH_2}CH_3)_2$], 2.52 (t, 2H, CH_2), 3.26 – 3.42 (m, 2H, CH_2), 4.05 (q, 2H, $N_{ind}\underline{CH_2}CH_3$], 6.95 (broad s, 1H, NH), 7.10 – 8.05 (m, 12H, ArH, -CH=, NH).
IVb	80	0.95 [t, 6H, $N(CH_2\underline{CH_3})_2$], 1.36 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 2.45 [q, 4H, $N(\underline{CH_2}CH_3)_2$], 2.50 (t, 2H, CH_2), 3.25 – 3.40 (m, 2H, CH_2), 3.90 (s, 3H, OCH_3), 4.07 (q, 2H, $N_{ind}\underline{CH_2}CH_3$], 6.83 – 8.03 (m, 12H, ArH, -CH=, NH).
IVc	70	1.75 – 1.90 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.40 – 2.60 [m, 6H, $N(CH_2)_3$], 2.92 [s, 6H, $SO_2N(CH_3)_2$], 3.45 – 3.70 [m, 6H $O(CH_2)_2$, $N_{ind}\underline{CH_2}CH_3$], 7.95 – 8.25 (m, 12H, ArH, -CH=, 2NH).
IVd	75	2.10 – 2.25 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.88 [s, 6H, $SO_2N(CH_3)_2$], 3.18 – 3.30 (m, 2H, NCH_2), 4.06 (t, 2H, $N_{imidazole}CH_2$), 6.80 – 8.05 (m, 13H, ArH, -CH=), 8.23 (t, 1H, $\underline{NH}CH_2$), 9.80 (broad s, 1H, NHCO).
IVe	76	1.43 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 1.72 – 1.88 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.36 – 2.60 [m, 6H, $N(CH_2)_3$], 3.42 – 3.58 (m, 2H, $NHCH_2$), 3.60 – 3.70 [m, 4H, $O(CH_2)_2$], 4.16 [q, 2H, $N_{ind}\underline{CH_2}CH_3$], 7.10 – 7.90 (m, 10H, ArH, -CH=).
IVf	88	1.00 [t, 6H, $N(CH_2\underline{CH_3})_2$], 1.43 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 2.52 [q, 4H, $N(\underline{CH_2}CH_3)_2$], 2.62 (t, 2H, CH_2), 3.42 – 3.55 (m, 2H, CH_2), 4.05 (q, 2H, $N_{ind}\underline{CH_2}CH_3$), 7.10 – 7.90 (m, 12H, ArH, -CH=, 2NH).
IVg	85	2.15 (s, 3H, CH_3), 2.28 – 2.32 [m, 4H, $(CH_2)_2$], 2.88 [s, 6H, $SO_2N(CH_3)_2$], 3.75 – 3.90 [m, 4H, $(CH_2)_2$], 6.30 (s, 1H, -CH=), 7.07 – 8.28 (m, 10H, ArH, -CH=, NH).
IVh	72	1.43 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 2.28 (s, 3H, CH_3), 2.45 – 2.37 [m, 4H, $(CH_2)_2$], 3.75 (s, 3H, OCH_3), 3.78 – 3.97 [m, 4H, $(CH_2)_2$], 4.20 [q, 2H, $N_{ind}\underline{CH_2}CH_3$], 6.37 (s, 1H, -CH=), 6.93 – 8.35 (m, 9H, ArH,), 9.78 (broad s, 1H, NH).
IVi	76	0.98 [t, 6H, $N(CH_2\underline{CH_3})_2$], 1.36 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 2.35 – 2.55 [m, 6H, $N(\underline{CH_2})_2$], 3.20 – 3.35 (m, 2H, CH_2), 3.88 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.08 [q, 2H, $N_{ind}\underline{CH_2}CH_3$], 6.80 – 7.80 (m, 10H, ArH, -CH=, NH), 8.12 (broad s, 1H, NH).
IVj	81	1.40 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 2.33 – 2.50 [m, 4H, $N(CH_2)_2$, 2.55 (t, 2H, $N\underline{CH_2}$), 3.40 – 3.63 [m, 6H, $NH\underline{CH_2}$, $O(CH_2)_2$], 3.95 (s, 3H, OCH_3), 4.13 [q, 2H, $N_{ind}\underline{CH_2}CH_3$], 6.88 (broad t, 1H, $\underline{NH}CH_2$) 7.00 – 8.35 (m, 11H, ArH, -CH=, NH).
IVk	74	1.38 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 1.70 – 1.85 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.35 – 2.60 [m, 6H, $N(CH_2)_3$], 3.40 – 3.56 (m, 2H, $NH\underline{CH_2}$), 3.60 – 3.80 [m, 4H, $O(CH_2)_2$], 4.16 [q, 2H, $N_{ind}\underline{CH_2}CH_3$], 7.18 – 8.12 (m, 13H, ArH, -CH=, 2NH).
IVl	74	0.95 [t, 6H, $N(CH_2\underline{CH_3})_2$], 1.42 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 2.52 [q, 4H, $N(\underline{CH_2}CH_3)_2$], 2.63 (t, 2H, CH_2), 3.40 – 3.55 (m, 2H, CH_2), 4.13 (q, 2H, $N_{ind}\underline{CH_2}CH_3$), 6.90 (broad t, 1H, $\underline{NH}CH_2$), 7.15 – 8.26 (m, 11H, ArH, -CH=, NH).
IVm	73	2.33 – 2.45 [m, 7H, CH_3, $N(CH_2)_2$], 2.50 (t, 3H, NCH_2), 2.68 [s, 6H, $SO_2N(CH_3)_2$], 3.35 – 3.48 (m, $NH\underline{CH_2}$), 3.48 – 3.60 [m, 4H, $O(CH_2)_2$], 6.96 (broad t, 1H, $\underline{NH}CH_2$), 7.20 – 7.95 (m, 11H, ArH, -CH=, NH).
IVn	80	1.43 (t, 3H, $N_{ind}CH_2\underline{CH_3}$), 2.37 – 2.52 [m, 4H, $N(CH_2)_2$], 2.55 (t, 2H, $N\underline{CH_2}$), 3.45 – 3.63 [m, 6H, $NH\underline{CH_2}$, $O(CH_2)_2$], 4.15 [q, 2H, $N_{ind}\underline{CH_2}CH_3$], 6.85 (broad t, 1H, $\underline{NH}CH_2$) 7.17 – 8.28 (m, 11H, ArH, -CH=, NH).

TABLE 4. Serotoninergic, Purinergic, and κ-Opioidergic Activities of Amides of 2-Amino-3-indolylacrylic acids IVa – n.

Compound	Antagonist activity (1 μM), Δ% ($M \pm m$)			Kappa-opioid activity (0.1 mM) Δ% ($M \pm m$)
	5-HT_2	5-HT_3	$P2Y_1$	
IVa	–0.6 ± 5.3#	–25.6 ± 6.5*#	8.6 ± 1.7*#	3.0 ± 0.4#
IVb	–18.8 ± 4.8*#	–4.7 ± 1.9#	–12.9 ± 7.9	5.4 ± 0.8#
IVc	3.0 ± 5.7#	–27.1 ± 2.2*#	–24.9 ± 1.2*	2.1 ± 0.3#
IVd	–34.8 ± 7.7*#	–19.7 ± 0.9*#	13.7 ± 2.6*	4.1 ± 1.5#
IVe	–20.4 ± 11.2#	–13.5 ± 7.3#	–21.2 ± 2.8*	2.8 ± 0.5#
IVf	–31.4 ± 11.2*#	–31.6 ± 7.2*#	–16.8 ± 2.8*	1.7 ± 0.3#
IVg	…	…	–6.8 ± 8.1#	–1.0 ± 0.5#
IVh	–7.6 ± 9.7#	–24.1 ± 1.9*#	–13.8 ± 6.8	3.4 ± 1.0#
IVi	–21.6 ± 4.5*#	–11.0 ± 3.1*#	5.6 ± 3.2#	–2.1 ± 1.0#
IVj	–4.7 ± 5.1#	–20.9 ± 2.3*#	–7.5 ± 1.4*#	2.0 ± 0.5#
IVk	…	–6.7 ± 6.1#	–7.3 ± 6.8	10.6 ± 1.1#
IVll	–5.1 ± 6.1#	–30.3 ± 6.5*#	6.5 ± 9.3	1.8 ± 1.5#
IVm	–7.6 ± 5.4#	–5.0 ± 2.4#	…	–3.2 ± 1.0#
IVn	–26.6 ± 6.5*#	…	–3.6 ± 5.8#	2.4 ± 0.9#
Ketanserin	–81.4 ± 1.5*	…	…	…
Ondansetron	…	–80.1 ± 2.1*	…	…
Basilen blue	…	…	–20.8 ± 2.3*	…
U50.488	…	…	…	22.8 ± 1.6*

Note. *Significant differences compared with controls, $p < 0.05$; #significant differences compared with reference agent;... = not studied.

TABLE 5. Antiarrhythmic, Antiaggregant, Hemorheological, and Antioxidant Activity of Amides of 2-Amino-3-indolylacrylic acids IVa–n.

Compound	Antiarrhythmic activity, MEC, M	Effects on ADP-induced platelet aggregation (0.1 mM), Δ% ($M \pm m$)	Changes in index of erythrocyte aggregation (0.1 mM), Δ% ($M \pm m$)	Antioxidant activity (ascorbate-dependent LPO, 10 μM), % inhibition
IVa	7.0×10^{-5}	–55.2 ± 2.3*#	–8.4 ± 3.0*	37.2 ± 5.2*
IVb	5.0×10^{-4}	–21.25 ± 5.2*	–2.9 ± 4.6#	47.6 ± 3.7*
IVc	9.0×10^{-5}	–44.2 ± 2.3*	–7.9 ± 4.1	23.4 ± 2.9*#
IVd	1.0×10^{-4}	–42.4 ± 0.8*	–5.0 ± 1.9#	10.6 ± 3.2#
IVe	2.0×10^{-4}	–24.25 ± 4.6*	–7.3 ± 2.8#	33.1 ± 6.2*#
IVf	1.3×10^{-4}	–37.97 ± 2.5*	–1.8 ± 0.7#	56.4 ± 7.8*
IVg	1.2×10^{-4}	–42.53 ± 3.8*	1.5 ± 1.7#	50.3 ± 4.9*
IVh	2.2×10^{-4}	–53.52 ± 1.1*#	–7.2 ± 1.9*#	44.1 ± 3.8*#
IVi	3.8×10^{-4}	–29.14 ± 3.6*	–4.8 ± 1.1#	51.2 ± 6.1*
IVj	1.3×10^{-4}	–30.49 ± 1.0*	2.8 ± 2.8#	21.8 ± 3.3*#
IVk	7.0×10^{-5}	–41.1 ± 2.2*	–0.81 ± 1.4#	34.7 ± 2.8*#
IVl	2.0×10^{-4}	–52.46 ± 7.2*	…	42.5 ± 3.7*#
IVm	5.5×10^{-5}	–45.2 ± 2.8*#	–3.1 ± 1.5#	13.7 ± 2.6#
IVn	1.1×10^{-4}	–41.05 ± 6.3*	–2.7 ± 2.7#	21.7 ± 3.4*#
Ethmozine	5.1×10^{-5}	…	…	…
Quinidine	3.4×10^{-4}	…	…	…
Acetylsalicylic acid	…	–32.8 ± 4.6*	…	…
Pentoxifylline	…	…	–18.6 ± 2.5*	…
Dibunol	…	…	…	87.1 ± 7.2*

* Significant differences compared with controls, $p < 0.05$;... = not studied; #significant differences compared with reference substance, $p < 0.05$.

15-sec period of time. The activity of substances was compared with the actions of quinidine (Sigma, USA) and ethmozine (Zakusov Science Research Institute of Pharmacology, Russian Academy of Medical Sciences, Russia).

The antioxidant activity of compounds was determined in vitro using an ascorbate-dependent lipid peroxidation (LPO) model in rat liver as described in [20]. Substances were studied at a concentration of 10 μM. The rate of oxidation was assessed in terms of the accumulation of malondialdehyde, measured with thiobarbituric acid, with calculation of the percentage inhibition of LPO. The actions of substances were compared with that of dibunol (Merck, Germany).

Data were analyzed statistically using Student's *t* test and regression analysis.

RESULTS AND DISCUSSION

Studies of serotonin-induced platelet activation showed that the study amides of 2-amino-3-indolylacrylic acid IVa – n had insignificant 5-HT_2 antagonist activity (Table 4). The most marked effects were seen with substances IVd, IVf, IVi, and IVn, which suppressed serotonin-induced platelet activation by 21.6 – 34.8% relative to controls; substances were less active than ketanserin.

The highest levels of antagonist activity in relation to 5-HT_3 receptors was seen with compounds IVa, IVf, and IVl, where the substituents at the R^1 position were phenyl, *o*-chlorophenyl, and *o*-fluorophenyl respectively and a diethylaminoethyl fragment was present as the R^2 substituent (R^3 = H). Substitution of the R^2 substituent with morpholinopropyl or morpholinoethyl (IVc, IVe, IVl) produced some decrease in the pharmacological response. At the same time, the effect of ondansetron as significantly greater than those of all study compounds.

None of the study compounds, IVa – n, had κ agonist activity. The levels of activity of the compounds were not significantly different from controls.

Studies using the model of ADP-induced platelet activation in calcium-free medium showed that substance IVf also showed high $P2Y_1$ antagonist activity with a level close to that of basilen blue. This effect of the compound correlated with data showing its high antiaggregant activity (Table 5) and is in all probability associated with its antipurinergic and antiserotoninergic properties. In addition, a further two compounds, IVc and IVe, had a common morpholinopropyl substituent at R^2 (R^3 = H) and high purine-blocking actions, no less than that of basilen blue. Shortening of the aliphatic chain in the structure of the substituent, replacing it with morpholinoethyl (IVj, IVn), led to sharp reductions in this type of activity.

These experiments established that most of the study amides of 2-amino-3-indolylacrylic acids, IVa, IVc, IVd, IVf–h, and IVk–n, had antiaggregant properties greater than or equal to that of acetylsalicylic acid. This fact may be evidence for the potential of this class of compounds in the search for and development of new antiaggregants. The structures of most of the compounds with low antiaggregant properties (IVb, IVi, and IVj), with methoxyphenyl or dimethoxyphenyl R^1 substituents in combination with different R^2R^3 substituents, with the exception of the 4-methylpiperazinyl substituent (IVh), were of interest.

In terms of their influences on the processes of erythrocyte aggregation, virtually all the indole derivatives studied had low levels of activity. There were two exceptions: compounds IVa and IVh, the first containing a phenyl group (R^1) and a diethylaminoethyl (R^2) and the second an *o*-methoxyphenyl substituent (R^1) in combination with a 4-methylpiperazinyl (R^2R^3) group, which had greater activity than all the other study compounds, though they were less active than pentoxifylline in terms of the absolute value of the index of erythrocyte aggregation.

Moderate antioxidant activity, associated with influences on the processes of ascorbate-dependent lipid peroxidation, was seen with compounds IVf, IVg, and IVi, though they were less active than dibunol.

An interesting characteristic of the pharmacological actions of the study compounds was their marked antiarrhythmic effect, associated with influences on the duration of the effective refractory period of isolated rat atria. Thus, the levels of activity of substances with phenyl or tolyl substituents in the R^1 position (IVa, IVk, IVm) were comparable with that of ethmozine and were significantly greater than the action of quinidine. Introduction of a fluorine or chlorine atom into the benzene ring of the R^1 phenyl group and substitution of the latter for a hydroxymethyl group led to some decrease in the antiarrhythmic effect of the compounds (IVd, IVf-h, IVj, IVl, IVn). These latter compounds were less active than ethmozine but had smaller minimum effective concentrations than quinidine.

Thus, amides of 2-amino-3-indolylacrylic acid IVa-n had a quite wide spectrum of pharmacological activity. The most significant was their marked antiaggregant actions. In addition, there is the possibility of antiarrhythmic and purinergic activities. Some of the compounds were able to produce significant suppression of the effects of serotonin, to decrease blood viscosity, and to influence lipid peroxidation processes.

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