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SYNTHESIS AND ANALGESIC ACTIVITY OF [*b*]-ANNELATED 4-QUINOLONES

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Considering the side effects of existing drugs used for pain relief, the search for new analgesics with high efficacy and safety remains relevant. A series of imidazopyrrolo[3,4-*b*]quinoline-5,11-diones and pyrimidopyrrolo[3,4-*b*]quinoline-6,12-diones II were synthesized, whose analgesic activity at a dose of 0.05 mmol/kg was studied in the hotplate test in rats after i.p. administration. One compound was found with analgesic activity comparable to that of reference compound diclofenac sodium. Compounds II were less toxic than diclofenac sodium.

Keywords: [*b*]-annelated 4-quinolones, analgesic activity, antinociceptive action.

Pain is a psychophysical reaction in animals and humans to harmful stimuli inducing organic or functional impairments [1]. It is regarded as the world's main clinical, social, and economic problem [2]. Nociceptive pain most commonly arises on activation of peripheral pain receptors (nociceptors) in response to heat, cold, mechanical, and chemical stimuli, as well as in inflammation [2, 3]. All existing analgesics – both peripherally and centrally acting – can produce serious undesirable side effects [4]. Thus, the widely used group of non-steroidal anti-inflammatory drugs (NSAID), some of which are non-prescription drugs, provoke damage to the gastrointestinal tract and kidneys, along with bleeding [5 – 7]. The side effects of opioid drugs are sedation, vertigo, nausea, vomiting, constipation, physical dependence, addiction, and respiratory suppression [3, 8]. The fact of undesirable side effects of existing drugs used for ameliorating pain maintains a continuous search for novel chemical compounds with analgesic actions which might provide the basis for creating new drugs with high efficacy and safety.

Compounds in the 4-quinolones series, particularly their [*b*]-annelated derivatives (scheme 1) are known to be able to have analgesic effects working by a variety of mechanisms of action. Tetrahydropyridazino[4, 5-*b*]quinoline-1,10-dione methylsulfonate (A) inhibits pain sensations induced in rats by administration of 1% formalin beneath the skin of the hindpaw. The authors identified the mechanism of action of 4-quinolone A on glutamate receptors as operating via ligand-controlled ion channels in the neuron cell membrane and selective binding of N-methyl-D-aspartate (NMDA) [9, 10].

Compounds of the pyrazoloquinolinone series of general formula **B** are effective in the treatment of neuropathic pain, as well as acute and chronic pain of inflammatory origin due to inhibition of protein kinase C, which is a therapeutic target for relieving pain [11 – 13].

These examples do not exhaust the list of compounds containing the 4-quinolone fragment and having analgesic actions. The compounds addressed by our studies are tetracyclic [*b*]-annelated 4-quinolones prepared by interaction of 2,3-diacyl-4-quinolones (**I**) with N,N-binucleophiles. The methyl esters of 3-aroyl-4-oxo-1,4-dihydro-2-quinolinecarboxylic acids **I** were synthesized using the known reaction decarbonylating 1-aryl-3-aroyl-2,5-dioxo-4,5-dihydro-1*H*-pyrrole-2-carboxylates [14]. Interaction of the alkoxycarbonyl and aroyl groups of compounds I with ethylenediamine led to formation of tetrahydro-1*H*-imidazo[1′,2′:1,5]pyrrolo-[3,4-*b*]quinoline-5,11-diones (**IIa-d**), while interaction with propylenediamine produced hexahydropyrimido[1′,2′:1, 5]-pyrrolo[3,4-*b*]quinoline-6,12-diones **IIe-h** (scheme 2). Reac-

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tions were run by boiling in 1,4-dioxane at a ratio of reagents of 1:1.

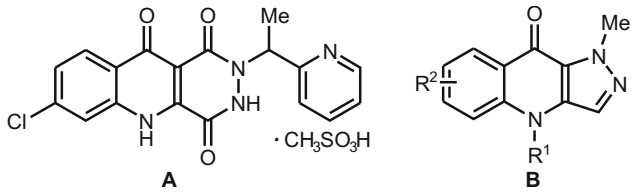
EXPERIMENTAL CHEMICAL SECTION

The melting temperatures of the compounds synthesized here were measured on a PTP-M instrument (Russia) without correction. Reactions were monitored by TLC on Sorbfil PTSKh-AF-A-UF and MerckART 60 F_{254} plates. IR spectra were recorded on a Specord-80 spectrometer (Russia). ^1H and ^{13}C NMR spectra were recorded on Avance DRX-500 and Avance DRX-400 spectrometers (Bruker BioSpin, Germany) in DMSO-d_6 (with TMS as the internal standard). Elemental analysis was performed using a PE-2400, c. II analyzer (Perkin Elmer, USA), with values for C, H, and N within 0.3% of theoretical.

10-Bromo-12b-phenyl-1,2,3,4,7,12b-hexahydropyrimido[1′,2′:1,5]pyrrolo[3,4-*b*]quinoline-6,12-dione (IIe). 3-Benzoyl-6-bromo-4-oxo-1,4-dihydroquinoline-2-carboxylic acid methyl ester (0.5 g, 1.3 mmol) dissolved in 40 ml of 1,4-dioxane was supplemented with 0.1 g (1.3 mmol) of propylenediamide. The reaction was boiled with a reflux condenser for 2 h (monitored by TLC) and evaporated in vacuo. The resulting precipitate was collected by filtration and recrystallized from 1,4-dioxane. The yield was 55%, T_m > 300°C (dioxane). The IR spectrum (Vaseline oil), ν, cm^{-1}, was: 3230, 3180, 3150, 3115 (NH), 1700 (C^6=O), 1644 (C^{12}=O), 1604 (C=C). The ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm, was: 12.97 (s, 1H, NH), 8.11 (d, J 2.4 Hz, 1H, 11-H), 7.83 (dd, J 9.2 Hz, J 2.4 Hz, 1H, 9-H), 7.74 (d, J 8.4 Hz, 8-H), 7.58 (d, J 7.2 Hz, 2H, 2′-H, 6′-H), 7.37 – 7.33 (m, 2H, 3′-H, 5′-H), 7.28 – 7.27 (m, 1H, 4′-H), 4.29 (d, J 12.8 Hz, 1H, 4-H), 2.98 – 2.87 (m, 2H, 3-H), 2.67 – 2.65 (m, 1H, 4-H), 1.50 – 1.48 (m, 2H, 2-H). The ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm, was: 169.7 (12-C), 161.2 (6-C), 140.5, 138.8, 136.5, 134.8, 129.2, 128.2 (3′-C, 5′-C), 127.6, 127.5 (2′-C, 6′-C), 127.2, 126.2, 121.9, 116.5, 78.5 (12b-C), 40.1 (2-C), 37.0 (4-C), 25.8 (3-C). The atomic formula was $C_{23}H_{23}N_3O_2$.

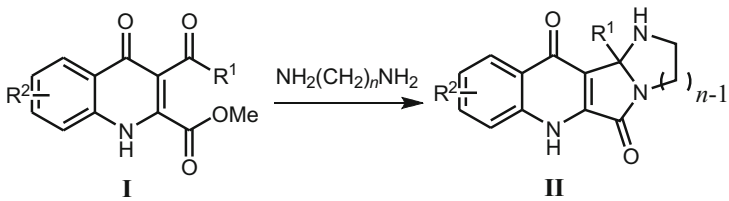
10-Methyl-12b-phenyl-1,2,3,4,7,12b-hexahydropyrimido[1′,2′:1,5]pyrrolo[3,4-*b*]quinoline-6,12-dione (IIf). This compound was prepared as described for IIe. The yield was 77%, T_m was 301 – 302° C (with degradation) (dioxane). The IR spectrum (Vaseline oil), ν, cm^{-1}, was: 3208, 3188, 3130, 3090, 3040 (NH), 1694 (C^6=O), 1640 (C^{12}=O), 1608 (C=C). The ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm, was: 12.11 (broad s, 1H, NH), 7.81 (broad s, 1H, 11-H), 7.68 (d, J 8.5 Hz, 1H, 8-H), 7.58 (d, J 7.5 Hz, 2H, 2′-H, 6′-H), 7.49 (d.d, J 8.5, 2.0 Hz, 1H, 9-H), 7.36 – 7.32 (m, 2H, 3′-H, 5′-H), 7.27 – 7.24 (m, 1H, 4′-H), 4.31 – 2.27 (m, 1H, 4-H), 2.97 – 2.87 (m, 2H, 3-H), 2.69 – 2.63 (m, 1H, 4-H), 2.37 (s, 3H, CH_3), 1.56 – 1.46 (m, 2H, 2-H). The ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm, was: 170.8 (12-C), 161.6 (6-C), 139.8, 138.0, 137.0, 133.4, 133.0, 128.1 (3′-C, 5′-C), 127.8, 127.5 (2′-C, 6′-C), 127.4, 125.7, 124.3, 119.4, 78.5 (12b-C), 40.0 (2-C), 36.9 (4-C), 25.8 (3-C), 20.8 (CH_3). The atomic formula was $C_{21}H_{19}N_3O_2$.

12b-Phenyl-8-ethyl-1,2,3,4,7,12b-hexahydropyrimido-[1′,2′:1,5]pyrrolo[3,4-*b*]quinoline-6,12-dione (IIg). This was prepared as described for compound IIe. The yield was 55%, T_m was 292–293°C (with degradation) (acetonitrile). The IR spectrum (KBr), ν, cm^{-1}, was: 3342, 3303 (NH), 1698 (C^6=O), 1636 (C^{12}=O), 1596 (C=C). The ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm, was: 11.44 (s, 1H, NH), 7.94 (d, J 8.1 Hz, 1H, 11-H), 7.59 – 7.57 (m, 2H, 2′-H, 6′-H), 7.54 – 7.52 (m, 1H, 9-H), 7.37 – 7.34 (m, 2H, 3′-H, 5′-H), 7.30 – 7.25 (m, 2H, 10-H, 4′-H), 4.30 (d, J 13.2 Hz, 1H, 4-H), 3.19 – 3.04 (m, 2H, CH_2), 2.97 – 2.87 (m, 2H, 2-H), 2.67 – 2.64 (m, 1H, 4-H), 1.54 – 1.48 (m, 2H, 3-H), 1.21 (t, J



R^1 = H, Alk, NHAlk, Het; R^2 = H, Alk, Hal, Bz

Scheme 1. Examples of [*b*]-annelated 4-quinolones with analgesic activity.



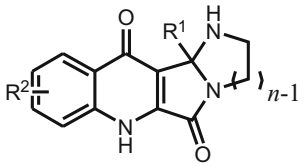
II: *n* = 2, R^1 = Ph, R^2 = H (a), R^2 = 9-Br (b), R^2 = 9-Me (c), R^2 = 7-Et (d),
n = 3, R^1 = Ph, R^2 = 10-Br (e), R^2 = 10-Me (f), R^2 = 8-Et (g), R^1 = *n*-MeC_6H_4, R^2 = 8-Et (h).

Scheme 2. Synthesis of tetracyclic pyrrolo[3,4-*b*]quinolinediones.

7.4 Hz, 3H, CH_3). The ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm, was: 171.3 (12-C), 167.6 (6-C), 161.2, 140.1, 137.7, 136.8, 133.9, 131.7, 128.3, 128.1 (3′-C, 5′-C), 127.5 (2′-C, 6′-C), 127.5, 126.5, 123.7, 123.0, 90.3, 77.8 (12b-C), 40.1 (2-C), 36.8 (4-C), 25.8 (3-C), 23.2 (CH_2), 14.5 (CH_3). The atomic formula was $C_{22}H_{21}N_3O_2$.

8-Ethyl-12b-(*p*-tolyl)-1,2,3,4,7,12b-hexahydropyrimido[1′,2′:1,5]pyrrolo[3,4-*b*]quinoline-6,12-dione (IIh). This was prepared as described for compound IIe. The yield was 85%, T_m was 250 – 251°C (with degradation) (dioxane). The IR spectrum, ν, cm^{-1}, was: 3270, 3230 (NH), 1699 (C^6=O), 1638 (C^{12}=O), 1617 (C=C). The ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm, was: 11.24 (broad s, 1H, NH), 7.93 (d.d, J 8.2, 1.6 Hz, 1H, 11-H), 7.52 (dd, J 7.2, 1.6 Hz, 1H, 9-H), 7.47 – 7.42 (m, 2H, 2′-H, 6′-H), 7.29 – 7.26 (m, 1H, 10-H), 7.15 (d, J 8.0 Hz, 2H, 3′-H, 5′-H), 4.33 – 4.24 (m, 1H, 4-H), 3.19 – 3.03 (m, 2H, CH_2), 2.98 – 2.83 (m, 2H, 3-H), 2.72 – 2.66 (m, 1H, 4-H), 2.27 (s, 3H, CH_3), 1.56 – 1.42 (m, 2H, 2-H), 1.21 (t, J 7.5 Hz, 3H, CH_3). The ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm, was: 171.6 (12-C), 162.0 (6-C), 141.1, 138.8, 137.1, 134.3, 131.9, 129.3 (3′-C, 5′-C), 128.7, 128.0 (2′-C, 6′-C), 127.5, 127.0, 124.1, 123.4, 78.3, 40.2 (2-C), 37.3 (4-C), 26.4 (3-C), 23.8 (CH_2), 21.1 (CH_3), 15.1 (CH_3). The atomic formula was $C_{23}H_{23}N_3O_2$.

TABLE 1. Analgesic Activity of Pyrrolo[3,4-*b*]quinolinediones **II**



Compound	Time period, sec	Increase in time period, %	p_1	p_2
IIa	8.8 ± 0.7	24	> 0.05	< 0.05
Control	7.1 ± 0.9			
Diclofenac	15.0 ± 2.0		< 0.05	
IIc	17.8 ± 1.3	97	< 0.0001	> 0.05
Control	9.0 ± 0.6			
Diclofenac	15.6 ± 1.1		< 0.05	
IId	11.4 ± 1.5	61	< 0.05	> 0.05
Control	7.1 ± 0.9			
Diclofenac	15.0 ± 2.0		< 0.05	
IIe	12.7 ± 1.1	64	< 0.002	> 0.05
Control	7.7 ± 0.4			
Diclofenac	13.1 ± 0.8		< 0.05	
IIg	10.2 ± 0.5	13	> 0.05	< 0.0006
Control	9.0 ± 0.5			
Diclofenac	14.6 ± 0.7		< 0.05	
IIh	10.0 ± 0.7	28	> 0.05	< 0.05
Control	7.9 ± 0.7			
Diclofenac	14.9 ± 1.4		< 0.05	

p_1 compared with controls; p_2 compared with diclofenac.

EXPERIMENTAL BIOLOGICAL SECTION

Experiments were performed in compliance with standard ethical norms for the humane treatment of animals [15, 16]. Animals were kept in polycarbonate cages (Bioskape, Germany) on Rehofix MK 2000 litter (JRS, Germany). Animals received Chara feed for conventional small laboratory rodents (Assortment Agro, Russia). Animals had free access to water in standard drinking bottles.

The analgesic actions of compounds **II** were studied in the hotplate test, evaluating changes in pain sensitivity by comparison of pain responses in groups of animals given drug with a control group [17]. The test was performed using a Hotplate 60200 (TSE Systems, Germany). Each study group consisted of six outbred Sprague Dawley rats aged three months. Substances were given i.p. as suspensions in 2% starch paste at a dose of 0.05 mmol/kg. The control group received 2% starch paste only. Reference drug was diclofenac sodium (Chemofarm A. D., Serbia) and was given at an equimolar dose. After 1 h, animals were placed on a hotplate at 50°C with a Plexiglas cylinder. The time from the moment of placing the animal on the hotplate to the behavioral response to nociceptive stimulation (licking or shaking the paw, jumping) was measured; the maximum time spent by the animal on the plate was 30 sec regardless of the response, to prevent inevitable damage to the experimental animals′ skin. The analgesic activity of the compounds was expressed in terms of the increase in the latent period (%) in groups of animals receiving drugs as compared with the control group and was calculated as:

$$AA, \% = 100 \times (t_d - t_c)/t_c,$$

where t_d is the time of the response to nociceptive stimulation in animals given drug and t_c is the time of the response the nociceptive stimulation in control animals given 2% starch paste.

Results (Table 1) were processed in GraphPadPrism 6 by Multiple *t* tests. Differences between latent periods for the experimental and control groups were regarded as significant at $p \le 0.05$. The analgesic activity of the compounds was expressed as the increase in the time of the response to nociceptive stimulation (%) in groups of animals receiving drug as compared with the control group (Table 1).

Compounds **IIa** and **IIg** had no antinociceptive action. Compounds **IId** and **IIe** had significant analgesic activity, in-

creasing the time of the reaction to thermal stimulation by 61% and 64% respectively. The greatest analgesic effect was seen with compound **IIc**.

Acute toxicity for three compounds (**IIa**, **e**, **g**) was assessed in outbred three-month-old white CD-1 mice using i.p. administration. The experimental group consisted of three individuals of both sexes (two males, one female). Study compound was given to mice i.p. as suspension in 2% starch paste at a dose of 500 mg/kg. The total duration of observations of the animals was 14 days; animals were under continuous observation on the first day after drug administration. The overall state of the animals was assessed once a week, along with behavioral features, the intensity and nature of movement activity, the presence and type of convulsions, impairments to movement coordination, responses to tactile stimulation, respiratory rate, the state of the fur and skin, the color of the mucosa, and food and water intake. The LD_{50} value of substances was greater than 500 mg/kg and substances were assigned to toxicity class 4, i.e., compounds with low toxicity [19].

Thus, one compound (**IIc**) was identified whose analgesic activity was comparable with that of reference agent diclofenac sodium given at the equimolar dose. Members of this class of compounds were found to be less toxic than diclofenac sodium given by the same route (LD_{50} 42 – 132 mg/kg, mice, i.p.) [20]. The results confirm the potential for seeking substances with analgesic actions among compounds **II**.

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