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SYNTHESIS OF NEW 1-ARYL-6-BENZYLIMIDAZO[1,2-a][1,3,5]TRIAZINES WITH POTENTIAL PHARMACOLOGICAL ACTIVITY

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Abstract: In the reaction of ethyl N-(1-arylimidazolidine-2-ylidene) carbamic acid ester with 4-chlorobenzylamine new derivatives of 1-(1-arylimidazolidine-2-ylidene)-3-arylurea (**I–VI**) were obtained. Cyclic derivatives of dihydroimidazo[1,2-a][1,3,5]trizines were synthesized by condensation of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea with carbonyldiimidazole (CDI) (**VII–XII**). The effect of compounds **X**, **XI** on the central nervous system of mice in some behavioral tests was investigated.

Keywords: 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea derivatives, reaction of cyclization with carbonyldiimidazole, central nervous system activity

Pain is still considered a very complex process involving multiple neurotransmitters and neuromodulators. Some types of pain can be treated now with efficiency, but the side effect associated with use of these drugs makes the search for new approaches inevitable. Search for new biological targets (1) or new more selective drugs are the most important approaches.

Commonly, for the pharmacophore models of opioid-receptor activity some groups are important. The aminocarbonyl derivatives of 1-aryl-2-imidazolidine-2 have significant antinociceptive activity connected with activation of the μ opioid protein (MOP) receptor (2–4).

The synthetic derivatives of triazepine are various and important groups of medicines.

In the search for new derivatives with potential pharmacological activity, new 1-aryl-6-(chlorobenzyl)-5,7-(1H)dioxo-2,3-dihydroimidazo[1,2-a][1,3,5]triazines were obtained. This heterocyclic system was obtained in two-step reaction (5, 6). The reaction sequences leading to the formation of compounds **I–VI** and **VII–XII** are outlined in Scheme 1.

EXPERIMENTAL

Chemistry

Melting points were determined on a Böetius apparatus and are given uncorrected.

The ^{1}H NMR spectra were recorded on a Bruker Avance 300 apparatus in DMSO-d_6 with TMS as an internal standard. Chemicals were purchased from Aldrich or Merck Co. and were used without further purification. The purity of obtained compounds was checked by TLC on Merck plates silica gel F_{234} in $CHCl_3/CH_3OH$ (10:1, v/v) solvent system with UV visualization.

Elemental analyses were performed on Perkin-Elmer analyzer and were in the range of ± 0.535 for each analyzed element (C, H, N, Cl).

1-(1-Arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)ureas (I–VI)

General procedure

Ethyl N-(1-arylimidazolidine-2-ylidene)carbamic acid ester (0.02 mol) and 0.02 mol (2.8 g) of 4-chlorbenzylamine were dissolved in 50 mL of methanol and refluxed for 6 h. The solvent was removed. The precipitate was filtered off and crystallized from propan-2-ol.

1-Aryl-6-benzylimidazo[1,2-a][1,3,5]triazines (VII–XII)

General procedure

1-(1-Arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea (0.01 mol) (2) and 0.01 mol (1.6

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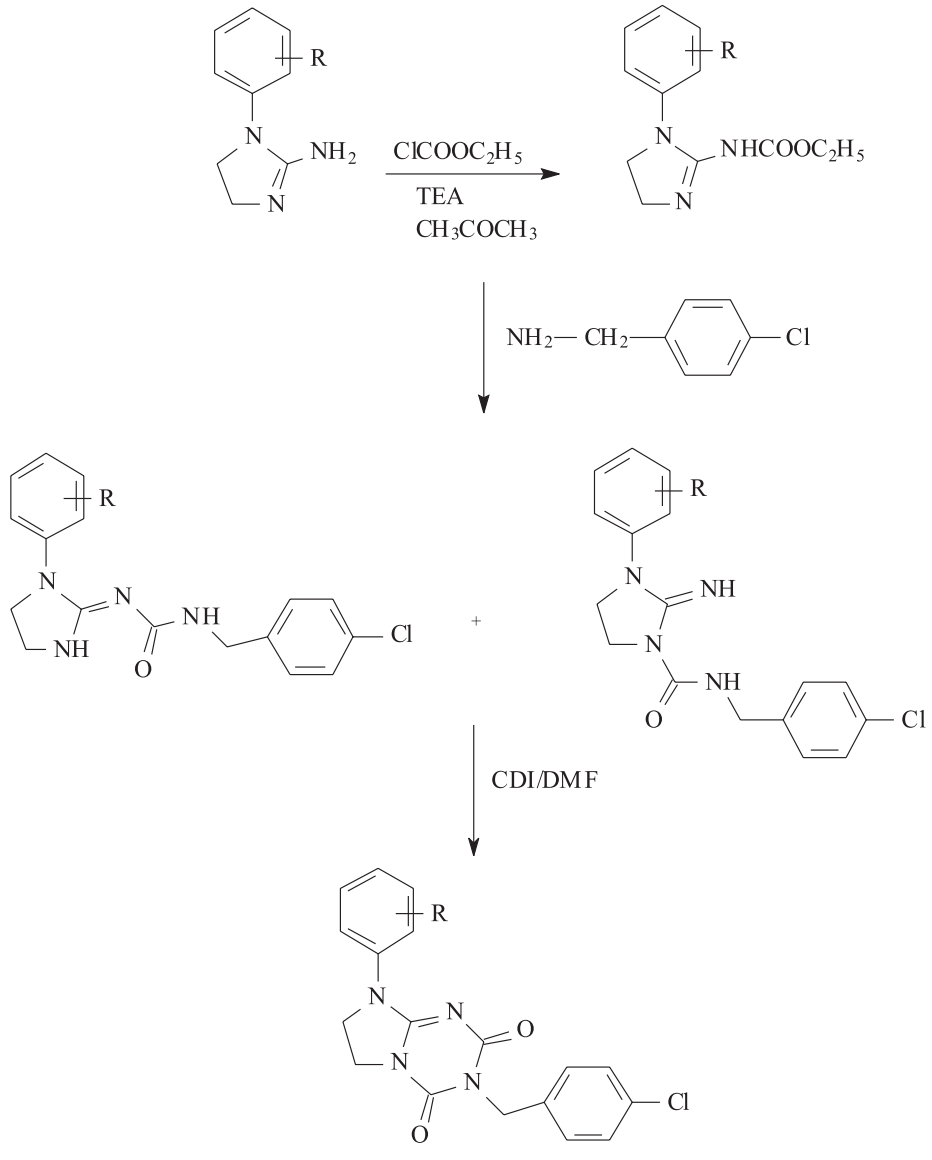


Scheme 1.

g) of carbonyldiimidazole (CDI) were dissolved in 50 mL of DMF and refluxed for 6 h. The solvent was removed by the low-pressure evaporation and the resulting solid residue was crystallized from methanol: propan-2-ol (1:1, v/v) mixture.

PHARMACOLOGY

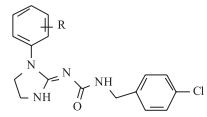
Materials and metods

Behavioral experiments

The experiments were performed on male Albino Swiss mice (18–30 g) between 8 a.m. and 3 p.m., in accordance with the opinion of Local Ethics Committee for Animal Experimentation. The animals were kept 8–10 to a cage, at room temp. of 20 ± 1°C, on a 12:12 h dark-light cycle. Standard food (laboratory pellets, Bacutil, Motycz) and water were available *ad libitum*. The investigated substance, marked as **XI** was administered intraperitoneally (*i.p.*) in volume of 10 mL/kg as suspension in aqueous solution of 0.5% methylcellulose (tylose) in the dose of 200, 100 and 50 mg/kg. The compound was injected 60 min before the tests. The controls received the equivalent volume of the solvent. Each experimental group consisted of eight to ten animals.

All tests performed, suggested by Vogel and Vogel (7), are generally accepted as basic in investigation of the central activity by behavioral methods.

Table 1. Physical and analytical data for compounds **I–VI.**

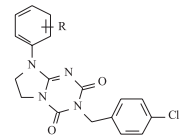


No.	R	Formula Molecular weight	Yield % M.p. (°C)	Analysis Calculated/Found [1] %C	%H	%Cl	H NMR (δ, ppm) DMSO-d_6
I	H	$C_{17}H_{17}N_4OCl$ 328.81	59 100–102	62.10 62.22	5.21 5.55	10.78 10.72	3.40–3.59 (dd, 4H, H2/H3) 4.20 (d, 2H, CH_2 benzyl) 7.45–7.76 (m, 8H, CH aromat.) 8.60, 8.78 (2s, 2H, 2×NH)
II	2-CH_3	$C_{18}H_{19}N_4OCl$ 342.84	58 118–120	63.06 63.16	5.59 5.66	10.34 10.01	2.26 (s, 3H CH_3) 3.66–3.80 (dd, 4H, H2/H3) 4.11 (m, 2H, CH_2 benzyl) 6.40–7.68 (m, 8H, CH aromat) 6.48–6.55 (s, 1H, N8) 8.52–8.63 (s, 1H, N3)
III	4-CH_3	$C_{118}H_{19}N_4OCl$ 342.84	53 103–105	63.06 63.40	5.59 5.72	10.34 10.11	2.45 (s, 3H, CH_3) 3.56–3.79 (dd, 4H, H2/H3) 4.21 (d, 2H, CH_2 benzyl) 7.20–7.66 (m,8H, CH aromat.) 8.60, 8.80 (2s, 2H, 2×NH)
IV	2-OCH_3	$C_{18}H_{19}N_4O_2Cl$ 358.84	74 115–117	60.25 60.35	5.34 5.61	9.88 9.69	2.20 (s, 3H, OCH_3) 3.46–3.89 (dd, 4H, H2/H3) 4.18 (d, 2H, CH_2 benzyl) 7.05–7.58 (m, 8H, CH aromat.) 8.40, 8.70 (2s, 2H, 2×NH)
V	4-OCH_3	$C_{18}H_{19}N_4O_2Cl$ 358.84	40 108–110	60.25 60.55	5.34 5.51	9.88 9.95	2.11 (s, 3H, OCH_3) 4.15 (dd, 4H, H2/H3) 4.10 (d, 2H, CH_2 benzyl) 7.20–7.46 (m, 8H, CH aromat.) 9.60, 9.80 (2s, 2H, 2×NH)
VI	2-Cl	$C_{17}H_{16}N_4OCl_2$ 363.26	88 82–84	56.21 56.65	4.44 4.65	19.52 19.40	3.50–3.91 (dd, 4H, H2/H3) 4.21 (d, 2H, CH_2 benzyl) 7.11–7.40 (m, 8H, CH aromat) 7.79–7.91 (s, 1H, N8), 8.60–8.84 (s, 1H, N3)

The acute toxicity of the compound was assessed in mice acc. to Litchfield and Wilcoxon method (8), as the ED_{50} calculated on the loss of the righting reflex within 48 h. In addition, the activity of compounds was assessed in the following tests:

- locomotor activity: measured in photoresistor actometers for a single mouse for 30 min as: a) spontaneous activity; b) amphetamine-induced hyperactivity: mice received subcutaneusly (*s.c.*) 5 mg/kg of amphetamine 30 min before the test;
- nociceptive reactions: studied in the acetic acid (0.6%)-induced writhing test (9); the number of writhing episodes was measured for 10 min starting 5 min after *i.p.* administration of acid solution;
- motor coordination: evaluated in the rota rod test (10);
- body temperature in normothermic mice: measured in the rectum of animals with a thermistor thermometer and recorded 30, 60, 90, 120, 150 and 180 min after the injection of investigated compounds in the doses of 200 and 100 mg/kg *i.p.*;
- pentylenetetrazole (110 mg/kg, *s.c.*)-induced convulsions: evaluated as the number of mice with clonic seizures, tonic convulsions and dead animals;
- "head twitch" responses after 5-hydroxytryptophan (L-5-HTP): recorded acc. to Corne et al. (11); mice received 5-HTP (180 mg/kg, *i.p.*) and

Table 2. Physical and analytical data for compounds **VII-XII**.



No.	R	Formula Molecular weight	Yield % M.p. (°C)	Analysis Calculated/Found			^{1}H NMR (δ, ppm) DMSO-d_6
				%C	%H	%Cl	
VII	H	$C_{18}H_{15}N_4O_2Cl$ 354.81	44 263–265	60.95 60.65	4.26 4.53	9.99 9.80	3.69–4.10 (dd, 4H, H2/H3) 4.69 (d, 2H, CH_2 benzyl) 6.31–7.45 (m, 9H, CH_{ar})
VIII	2-CH_3	$C_{19}H_{17}N_4O_2Cl$ 368.83	52 247–249	61.87 61.72	4.65 4.78	9.61 9.50	2.45 (s, 3H, CH_3) 3.99–4.33 (dd, 4H, H2/H3) 4.92 (d, 2H, CH_2 benzyl) 6.31–7.45 (m, 8H, CH_{ar})
IX	4-CH_3	$C_{19}H_{17}N_4O_2Cl$ 368.83	43 225–227	61.87 61.94	4.65 4.35	9.61 9.52	2.80 (s, 3H, CH_3) 4.05–4.12 (dd, 4H, H2/H3) 4.90 (d, 2H, CH_2 benzyl) 7.34–7.62 (m, 8H, CH_{ar})
X	2-OCH_3	$C_{19}H_{17}N_4O_2Cl$ 384.83	54 258–260	59.30 59.65	4.45 4.61	9.21 9,20	3.55 (s, 3H, OCH_3) 3.69–4.22 (dd, 4H, H2/H3) 4.65 (d, 2H, CH_2 benzyl) 6.60–7.41 (m, 8H, CH_{ar})
XI	4-OCH_3	$C_{19}H_{17}N_4O_2Cl$ 384.83	58 260–261	59.30 59.56	4.45 4.68	9.21 9.17	3.53 (s, 3H, OCH_3) 3.90–4.30 (m, 4H, H2/H3) 4.46 (d, 2H, CH_2 benzyl) 6.83–7.14 (m, 8H, CH_{ar})
XII	2-Cl	$C_{18}H_{14}N_4O_2Cl_2$ 389.26	64 196–197	55.54 55.69	3.63 3.89	18.22 18.20	4.15–4.24 (m, 4H, H2/H3) 4.96 (d, 2H, CH_2 benzyl) 7.24–7.80 (m, 8H, CH_{ar})

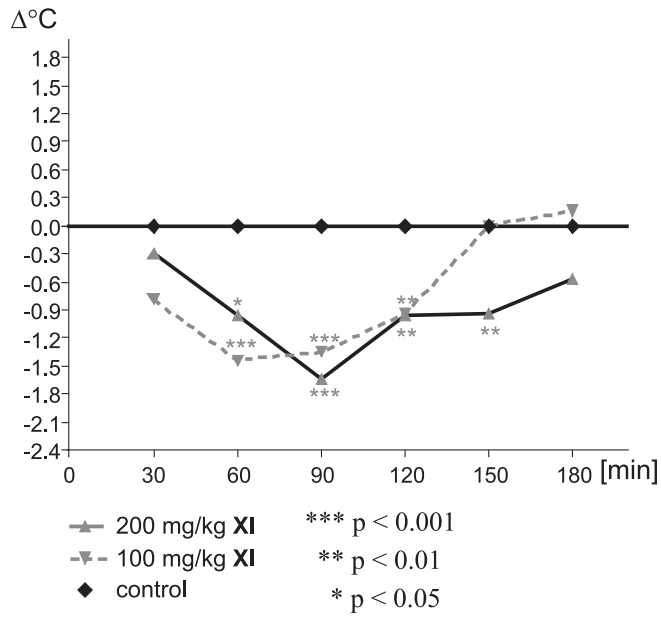


Figure 1. The influence of the tested compound on the body temperature of mice. Each point represents the mean for a group of 8–10 mice

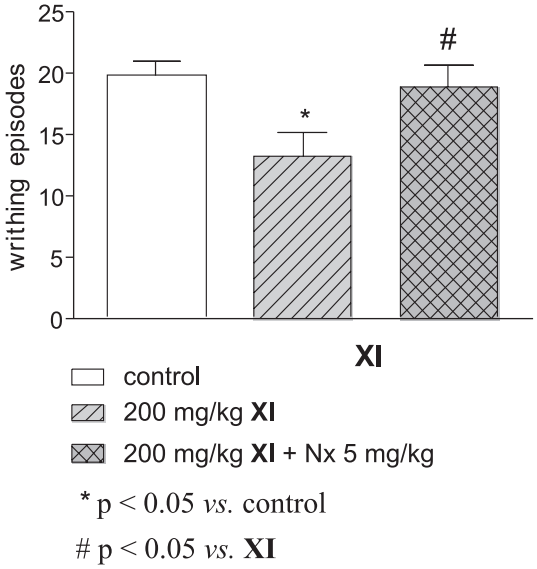


Figure 2. The influence of the tested compound on the spontaneous locomotor activity of mice. Numer of movements in the control group was 338 ± 26. The results are expressed as the mean ± SEM of groups of 6–8 mice

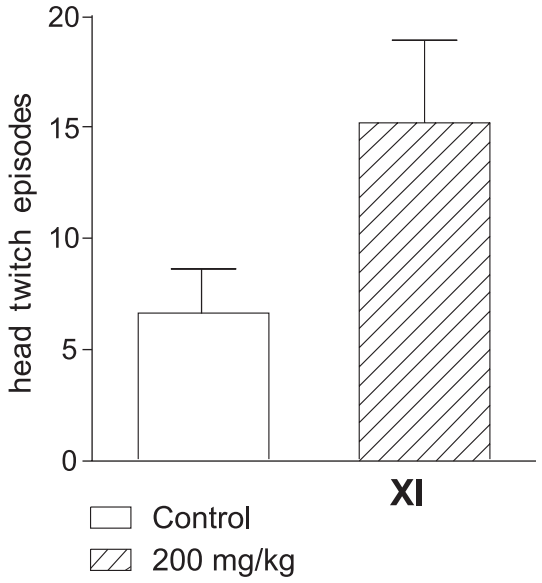


Figure 3. The influence of the tested compound on the head twitch responses evoked by 5-HTP (230 mg/kg, *i.p.*) Number of „head twitch" in the control group was 6.6 ± 2. The results are expressed as the mean ± SEM of group of 8 mice

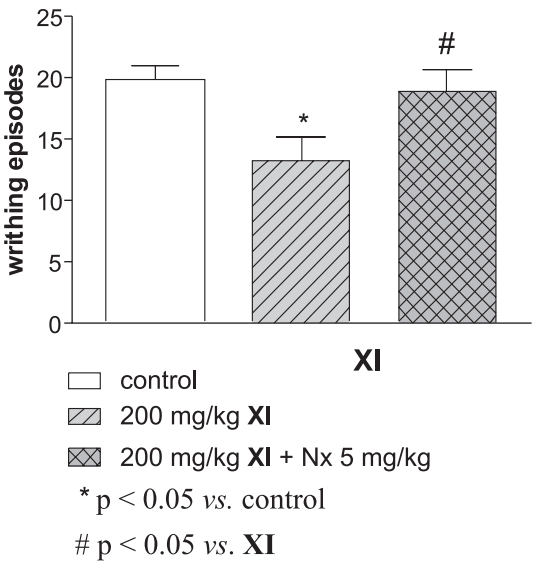


Figure 4. The influence of naloxone (Nx, 5 mg/kg *s.c.*) on the antinociceptive activity of the tested compound **XI** in the writhing test. Number of writhing episodes of control mice was 19.77 ± 1.2. The results are expressed as the mean ± SEM of group of 8–10 mice

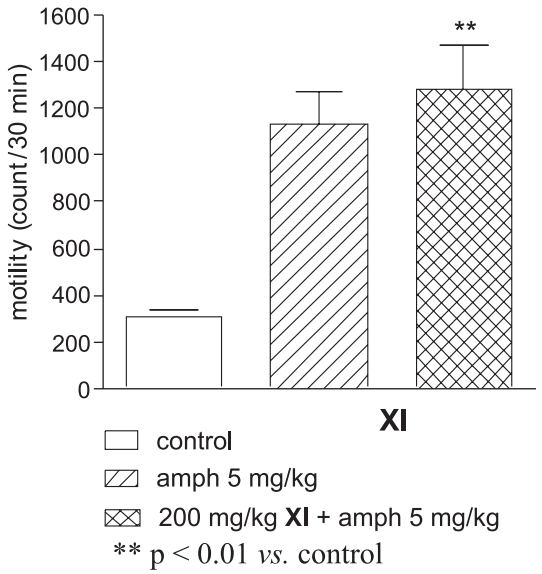


Figure 5. The influence of the tested compound on amphetamine (amph) (5 mg/kg)-induced hyperactivity of mice. The results are expressed as the mean ± SEM of group of 10 mice

the number of head twitches was recorded in 6 two-minutes intervals (4–6, 14–16, 24–26, 34–36, 44–46, 54–56 min) during 1 h;

- influence of naloxone (5 mg/kg, *s.c.*) on the antinociceptive effect of the compounds, assessed in the writhing test.

Statistical analysis

The obtained data were evaluated by Fisher exact test (pentylenetetrazole-induced seizures), and one-way ANOVA (other tests); subsequent comparisons between treatment and control groups were carried out using a *post-hoc* Dunnett's test, when $p < 0.05$.

RESULTS AND DISCUSSION

The title derivatives of 1-aryl-6-(4-chlorobenzyl)-5,7-(1H)dioxo-2,3-dihydroimidazo[1,2-a][1,3,5] triazine [**VII-XII**] were synthesized from the isomeric 1-aryl-2-iminoimidazolidine derivatives containg urea moiety: 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorbenzyl)ureas [**I-VI**] or 1-aryl-2-imino-3-(4-chlorbenzyl)aminocarbonyloimidazolidines (2) and carbonyldiimidazole (CDI) in DMF (Scheme 1).

New compounds were characterized by elemental analysis as well as by the ^{1}H NMR spectra. NMR spectral characteristics of the dihydroimidazotriazine-diones revealed two double-doublet signals of the H2 and H3 hydrogen atoms in the 4.00–4.21 ppm range and have the same value for the urea derivatives.

More detailed data related to compounds **I-VI** and **VII-XII** are listed in Tables 1 and 2, respectively.

Taking into account the possibility of two isomeric forms of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)ureas **I-VI** or 1-aryl-2-imino-3-(4-chlorobenzyl)-aminocarbonylimidazolidines, it was found that the obtained products exist as this first form.

Compound **X** was tested for their pharmacological activity. It exhibited very low acute toxicity: over 2000 mg/kg, *i.p.* (and for it ED_{50} = 2000 mg/kg was accepted for the continuation of the studies). After the highest doses of the investigated substance, sedation was observed.

Compound **XI** significantly decreased body temperature of normothermic mice from 60 to 150 min of observation, at the dose of 200 mg/kg, and from 60 to 120 min after dose of 100 mg/kg (Fig. 1). It also reduced spontaneous locomotor activity (in two used doses: 200 and 100 mg/kg) (Fig. 2) and markedly, but not significantly enhanced the head-twitch responses after L-5-HTP injection (Fig. 3), what can suggest the involvement of serotonin system in its CSN depressive activity.

Antinociceptive activity was tested in the writhing test in mice. Compound **XI** exerted significant antinociceptive activity in the dose of 0.1 ED_{50}, and this activity was reversed by a small dose of naloxone (5 mg/kg) – opioid antagonist (Fig. 4), so we could find out that this analgesia may be the result of interaction with opioid receptors. This compound did not affect amphetamine-induced hyperactivity and did not disturb motor coordination in mice in the tests used (rota-rod and chimney test) (data not presented). The lack of motor-impairing effects is important, because it can change the results of other tests (e.g., motility tests) and affecting reliability of the tests results. The substance also did not protect from clonic seizures, tonic convulsions and death (data not presented).

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