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Short communication

Synthesis and pharmacological activity of new carbonyl derivatives of 1-aryl-2-iminoimidazolidine
Part 2. Synthesis and pharmacological activity of 1,6-diaryl-5,7(1*H*)dioxo-2,3-dihydroimidazo[1,2-*a*][1,3,5]triazines

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Abstract

Synthesis and pharmacological activity of 1,6-diaryl-5,7(1*H*)dioxo-2,3-dihydroimidazo-[1,2-*a*][1,3,5]triazines (**C**) are presented. The title compounds were obtained from 1-arylimidazolinurea derivatives in cyclization reaction with difunctional carbonyl reagents—phosgene (method I) or carbonyldiimidazole (CDI) (method II). Their molecular structures were confirmed by the X-ray analysis of 1-phenyl-6-(4-chlorophenyl)-5,7(1*H*)-dioxo-2,3-dihydroimidazo[1,2-*a*][1,3,5]triazine (**C2**) crystals. Compounds **C** exhibited significant depressive action on the central nervous system (CNS) of the laboratory animals, correlated with very low acute toxicity ($LD_{50} > 2000$ mg kg^{-1} i.p.), and showed antinociceptive activity in behavioural models. Reversion of this effect by small dose of naloxone (5 mg kg^{-1}) can suggest opioid-like mechanism of antinociception produced by these and other carbonyl derivatives of 1-aryl-2-iminoimidazolidine. Additionally, an effect on the serotonin neurotransmission pathway was also observed. The receptor mechanism of activity for investigated compounds was confirmed only for the opioid μ receptor in binding affinity assay test. Same tests performed for the serotonin 5-HT_2 and benzodiazepine BZD receptors showed no affinity for tested compounds. The opioid-like and serotonergic activities are similar to these described earlier for chain carbonyl 1-aryl-2-iminoimidazolidine derivatives containing urea moiety, mainly due to similar chemical structure, although compounds **C** are not able to adopt any of the higher energy conformations of urea derivatives. Rigid location of aromatic ring (Ar′) at N6, acting as a spacer blocking any direct access to the carbonyl groups (e.g. through the hydrogen bonding), could be responsible for lack of affinity toward 5-HT_2 expressed in the binding assay test. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: CNS activity; Opioid-like analgesic activity; Molecular structures; 1,6-Diaryl-5,7(1*H*)dioxo-2,3-dihydroimidazo[1,2-*a*][1,3,5]triazines

1. Introduction

Pain is still considered a very complex process involving multiple neurotransmitters and neuromodulators interactions. Some types of pain can be treated now with efficiency (NSAI drugs for inflammatory pain or opioids for acute pain), but the side effect associated with use of these drugs make the search for new approaches inevitable. Search for new biological targets [1] or new more selective drugs showing fewer side effects are the two most important approaches. For all morphine and related analgesics the most severe side effect are euphoria/dysphoria, sedation, depression of respiratory center, depression of vasomotor center with hypotension, nausea, convulsions, tolerance and serious dependence. Commonly, for the pharmacophore models

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of opioid-receptor activity three fragments are placed as the most important ones. Two of them posses hydrophobic character and the third one (basic nitrogen atom) is responsible for strong ionic interaction with the aspartic acid residue in the receptor 'massage' subdomain. So far, all opioid drugs applied have all this features present in their structure but they produce more or less side effects as well.

In our previous paper [2] we presented results on the pharmacological activity of the isomeric carbonyl derivatives of 1-aryl-2-iminoimidazolidine, containing urea moiety, **A** and **B**. In this paper we present the results of similar investigation concerning pharmacological activity of cyclic analogues of the urea derivatives. A main part of these derivatives is the dihydroimidazo-triazine-dione nucleus, which generates the rigid structure **C**. The molecular geometry of the 5,7(1*H*)dioxo-2,3-dihydroimidazo[1,2-*a*][1,3,5]triazine system was not studied so far (CSD ver. 5.22 [3]). All investigated compounds exhibited affinity to the μ opioid receptor although they do not have basic nitrogen atom able to adopt cationic form. Besides, no effect on the respiratory system of the animals (mice) was observed in the behavioural tests, which could be taken as a very promising result.

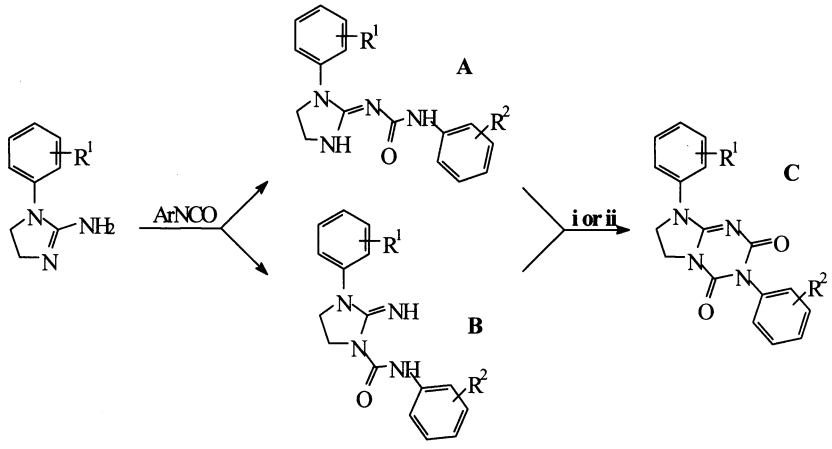
2. Chemical part

The title derivatives of 1,6-diaryl-5,7(1*H*)dioxo-2,3-dihydroimidazo[1,2-*a*][1,3,5]triazine (**C**) were synthesized from the isomeric 1-aryl-2-iminoimidazolidine derivatives containing urea moiety: 1-(1-arylimidazolidin-2-ylidene)-3-arylureas (**A**) or 1-aryl-2-imino-3-arylaminocarbonylimidazolidines (**B**) [2], and difunctional carbonyl reagents such as phosgene (I) and carbonyldiimidazole (CDI) (II) (Scheme 1). Similar results were obtained for both types of urea derivatives used, and for their mixture as well. In Section 6 only reactions with one isomer, **A**, are cited as well as the respective reaction yields are calculated. The physicochemical properties of the synthesized compounds are presented in Table 1.

NMR spectral characteristic of the dihydroimidazo-triazine-diones (**C**) revealed in their ^{1}H-NMR spectra two double-doublet signals of the H2 and H3 hydrogen atoms in the 4.00–4.24 ppm range with the coupling constants of $J \sim 9$ and $J' \sim 7.5$ Hz. Both signals $\Delta\delta$ values were about 0.15, ca. 0.1 ppm lower when compared to the same difference value for the urea derivatives **A**. The ^{13}C-NMR spectra showed almost equal chemical shift values for both carbonyl carbon atoms ($\delta \sim 155$ ppm), about 5 ppm lower when compared to the urea derivatives **A** but almost the same value as for urea derivatives **B** [2]. It can suggest almost same electron distribution on the carbonyl oxygen atoms and similar ability to the hydrogen bond formation. The spectral data of obtained compounds are presented in Table 2.

3. Crystallographic part

Triclinic crystals of **C2** are built up of two symmetrically independent molecules (Fig. 1). Bond distances in the 5,7(1*H*)dioxo-2,3-dihydroimidazo[1,2-*a*][1,3,5]triazine system for both molecules are equal within the experimental error. The C8′–N distances within the central guanidine unit indicate different character of each bond with the C8′–N8 being nearly double bond. The



i = dry toluene, N_2, $COCl_2$, NaH, -10°C, 4h,
room temp., 4h, Net_3, reflux, 2h;
ii = DMF, CDI, reflux, 3h;

Scheme 1.

Table 1
Physicochemical properties of 1,6-diaryl-5,7(1*H*)dioxo-2,3-dihydro-imidazo[1,2-*a*][1,3,5]triazines (**C**)

No.	R^1	R^2	R_f	M.p. (°C)	Yield (%)	
					I	**II**
C1	H	H	0.36	250–2	29.5	46.7
C2	H	4-Cl	0.65	258–60	33.4	48.3
C3	H	3-Cl	0.35	293–4	30.6	43.3
C4	H	3,4-Cl_2	0.54	300–3	30.8	40.9
C5	H	2,3-(−CH=)$_4$	0.42	297–8	27.9	44.7
C6	4-CH_3	H	0.49	291–3	34.7	50.2
C7	4-CH_3	4-Cl	0.48	309–10	36.6	49.6
C8	4-CH_3	3-Cl	0.58	260–2	30.9	44.1
C9	4-CH_3	3,4-Cl_2	0.49	301–3	32.0	37.8
C10	4-CH_3	2,3-(−CH=)$_4$	0.42	306–8	28.8	39.9
C11	4-Cl	H	0.38	287–8	36.7	51.1
C12	4-Cl	4-Cl	0.40	299–301	35.9	55.6
C13	4-Cl	3-Cl	0.48	288–90	30.8	46.6
C14	4-Cl	3,4-Cl_2	0.49	298–300	31.3	42.0
C15	4-Cl	2,3-(−CH=)$_4$	0.41	294–6	32.7	38.7
C16	4-CH_3O	H	0.47	247–9	29.7	40.8
C17	4-CH_3O	4-Cl	0.55	257–9	32.2	43.3
C18	4-CH_3O	3-Cl	0.63	269–71	30.1	27.9
C19	4-CH_3O	3,4-Cl_2	0.61	271–3	27.7	39.4
C20	4-CH_3O	2,3-(−CH=)$_4$	0.58	273–5	29.5	41.8

endocyclic torsion-angle values ($\pm 2^\circ$) indicate planarity of the five-membered dihydroimidazole ring while the triazine ring is considerably non-planar; its conformation could be described as a distorted twist-boat. Within the triazine ring two nonequivalent fragments could be distinguished, i.e. N4–C5(O5)–N6 and N6–C7(O7)–N8. They show different electron delocalization, which is indicated by the bond-lengths distribution.

Molecular conformation in the solid state is stabilized by weak C–H···O (dihydro-imidazole/chlorophenyl···carbonyl groups) and C–H···π (phenyl···chloro-chlorophenyl rings) intermolecular contacts.

4. Pharmacological part

4.1. behavioural tests

All tests performed, suggested by Vogel and Vogel [4] are generally accepted as basic in investigation of the central activity by behavioural methods.

Acute toxicity of compounds **C2**, **C3**, **C5** and **C7** was lower than 2000 mg kg^{-1} i.p. The progressive doses of 200, 100, 50 and 25 mg kg^{-1} of compounds tested were used in behavioural experiments. High doses of the investigated substances induced sedation, noticeable hypothermia and decreased responsiveness to external stimuli.

The depressive action on spontaneous locomotor activity in mice was observed after administration of all compounds in dose of 200 mg kg^{-1}. Compounds **C2** and **C3** significantly reduced the motor activity also in dose of 100 mg kg^{-1} (Fig. 2).

All compounds significantly prolonged the time of hexobarbital narcosis. Compounds **C2** and **C3** exhibited more potent effects (significant effects of 100 and 50 mg kg^{-1}) in this test than **C5** and **C7** (Fig. 2).

Body temperature of normothermic mice was significantly depressed by **C2** and **C3** (200 and 100 mg kg^{-1}, respectively, Fig. 3), when **C5** and **C7** induced slighter hypothermia (data not shown).

In the hot-plate test only compound **C7**, given in dose of 200 mg kg^{-1}, significantly prolonged the response to thermal stimuli in mice (data not shown). Strong antinociceptive activity of all compounds was observed in the writhing test in mice (Fig. 4). Their antinociceptive activity in doses used was comparable with effects of morphine administered in doses 0.5–2.0 mg kg^{-1} in the same test (Fig. 4). Naloxone (5 mg kg^{-1}) significantly reversed the antinociceptive effect of **C2**, **C3** and **C7**, and markedly but not significantly attenuated that of **C5** (Fig. 5).

Pentylenetetrazole-induced seizures were not affected by the compounds tested (data not shown).

Amphetamine-induced hyperactivity was not reversed by the **C5** and **C7**, but increased significantly by **C2** and insignificantly by **C3** (Fig. 6).

The head twitch responses after 5-HTP injection were abolished by **C2**, **C5** and **C7**, and significantly attenuated by **C3** (Fig. 7).

It is necessary to underline that the compounds did not exhibited neutrotoxicity. Used in doses of 100 and 200 mg kg^{-1} they did not disturb the motor coordina-

Table 2
NMR data of 1,6-diaryl-5,7(1*H*)dioxo-2,3-dihydroimidazo[1,2-*a*][1,3,5]triazines (**C**)

No.	^{1}H-NMR		^{13}C-NMR					
	H2/H3 (2 × dd, *J* [Hz])	Ar/Ar′ (m)	C2	C3	C5	C7	C8a	Ar/Ar′
C1	4.00/4.16 ($J = 9.0$, $J' = 7.6$)	7.10–7.75	40.0	45.9	154.5	154.7	155.4	120.3, 123.3, 125.3, 127.4, 128.5, 129.9, 130.7, 134.7, 135.5, 138.4
C2	4.04/4.19 ($J = 9.0$, $J' = 7.6$)	7.20–7.80	40.0	46.0	154.6	154.9	155.5	120.6, 120.8, 123.7, 128.8, 129.4, 129.7, 129.8, 133.3, 134.4, 136.0
C3	4.06/4.22 ($J = 9.0$, $J' = 7.5$)	7.15–7.75	40.1	45.9	154.6	154.9	155.5	120.9, 126.2, 126.9, 129.0, 129.1, 129.4, 129.6, 130.2
C4	4.06/4.21 ($J = 8.9$, $J' = 7.6$)	7.10–7.80	40.0	45.8	154.6	154.8	155.4	120.9, 122.9, 125.4, 127.3, 130.8, 132.2, 136.2, 137.1, 137.6
C5	4.05/4.18 ($J = 9.0$, $J' = 7.5$)	7.05–7.80	40.0	45.8	154.6	154.8	155.5	118.5, 120.8, 121.8, 122.3, 124.4, 125.4, 125.6, 126.0, 126.7, 127.1, 128.6, 128.8, 129.4, 129.6
C6	4.02/4.19 ($J = 9.1$, $J' = 7.6$)	7.15–7.75	40.1	46.0	154.1	154.7	155.5	119.3, 120.9, 123.4, 126.1, 129.9, 131.2, 133.3, 135.9, 137.1, 138.1
C7	4.05/4.20 ($J = 9.0$, $J' = 7.5$)	7.25–7.70	40.0	46.0	149.0	154.6	155.5	120.8, 129.5, 129.8, 133.2, 134.4, 134.5, 136.1
C8	4.07/4.22 ($J = 8.9$, $J' = 7.5$)	7.15–7.75	40.0	45.9	152.3	155.1	155.7	120.9, 126.2, 126.9, 129.0, 129.1, 129.4, 130.2, 134.8, 136.5
C9	4.11/4.17 ($J = 8.9$, $J' = 7.6$)	7.10–7.80	40.0	46.1	153.4	154.9	155.5	120.2, 122.3, 124.1, 125.5, 126.8, 128.3, 132.7, 137.1, 138.6, 140.1
C10	4.04/4.19 ($J = 9.0$, $J' = 7.5$)	7.05–7.95	40.1	46.0	154.3	154.9	155.3	119.1, 121.8, 122.1, 123.4, 124.7, 126.2, 126.9, 128.1, 129.0, 129.3, 130.7, 131.5
C11	4.03/4.19 ($J = 9.0$, $J' = 7.5$)	7.15–7.75	40.0	45.9	151.7	154.8	155.4	120.4, 122.5, 124.9, 125.0, 127.2, 127.8, 130.1, 135.3, 138.2
C12	4.09/4.23 ($J = 9.0$, $J' = 7.7$)	7.25–7.70	39.9	46.1	153.3	154.9	155.5	120.9, 122.8, 124.1, 126.3, 127.8, 130.2, 131.0, 133.1, 137.9
C13	4.08/4.24 ($J = 9.0$, $J' = 7.6$)	7.15–7.75	39.9	45.0	153.8	155.0	155.5	121.2 123.4, 125.3, 127.8, 128.0, 132.7, 133.1, 136.9, 139.1
C14	4.05/4.20 ($J = 8.9$, $J' = 7.5$)	7.10–7.80	40.0	46.0	154.1	154.8	155.4	121.7, 123.9, 125.9, 127.9, 129.8, 130.1, 131.7, 134.0, 138.7, 140.9
C15	4.05/4.17 ($J = 8.9$, $J' = 7.6$)	7.00–7.95	40.0	45.8	154.0	154.7	155.5	120.3, 120.6, 122.0, 122.2, 123.2, 124.4, 125.6, 126.5, 126.8, 127.9, 128.1, 130.2, 133.2
C16	4.02/4.18 ($J = 8.9$, $J' = 7.5$)	7.05–7.65	39.9	45.9	153.3	154.5	155.3	120.1, 122.7, 127.3, 127.8, 128.3, 129.1, 130.1, 130.9, 135.0, 138.2
C17	4.00/4.19 ($J = 9.0$, $J' = 7.4$)	7.10–7.65	40.0	45.9	154.7	154.9	155.5	120.4, 121.9, 124.2, 126.6, 128.0, 129.2, 132.7, 134.1, 136.0, 137.3
C18	4.02/4.23 ($J = 9.0$, $J' = 7.6$)	7.10–7.70	40.0	46.0	154.9	155.1	155.6	120.3, 123.5, 125.2, 126.9, 127.3, 128.9, 131.9, 132.2, 137.2, 137.9
C19	4.05/4.20 ($J = 9.0$, $J' = 7.6$)	7.05–7.75	40.1	45.9	154.1	154.9	155.5	120.7, 121.2, 123.9, 127.1, 127.9, 130.7, 134.7, 137.3, 137.9, 139.0
C20	4.01/4.19 ($J = 8.9$, $J' = 7.6$)	7.00–7.90	40.0	45.9	154.5	154.8	155.6	118.7, 119.4, 120.7, 121.3, 121.9, 123.2, 124.4, 125.6, 126.1, 126.9, 127.0, 127.2, 130.7

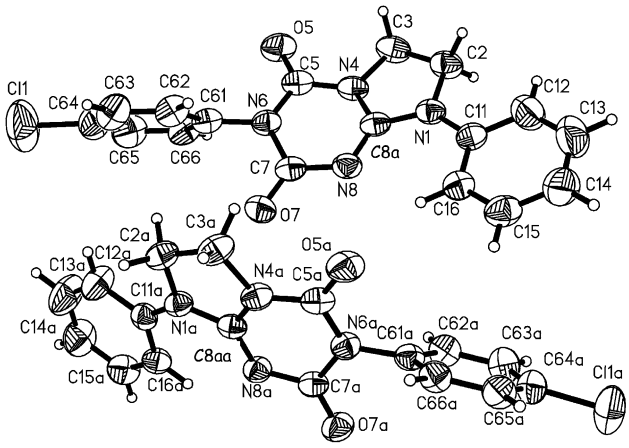


Fig. 1. Atom labelling and conformation of two symmetrically independent molecules of **C2**. Thermal ellipsoids are drawn at 50% probability level and hydrogen atoms are given arbitrary radius. Bond lengths (Å) within the 5,7(1*H*)dioxo-2,3-dihydroimidazo[1,2-*a*][1,3,5]triazine system for the molecule 1 and *molecule a* are:N(1)–C(8′) 1.346(2), *1.342(2)*; N(1)–C(2) 1.469(2), *1.476(2)*; C(2)–C(3) 1.513(3), *1.514(3)*; C(3)–N(4) 1.460(2), *1.459(2)*; N(4)–C(5) 1.361(2), *1.359(2)*; N(4)–C(8′) 1.367(2), *1.366(2)*; C(5)–O(5) 1.215(2), *1.212(2)*; C(5)–N(6) 1.384(2), *1.387(2)*; N(6)–C(7) 1.424(2), *1.423(2)*; C(7)–O(7) 1.213(2), *1.213(2)*; C(7)–N(8) 1.361(2), *1.360(2)*; N(8)–C(8′) 1.302(2), *1.305(2)*.

tion of mice in the rota-rod test. If present, impairment of the motor coordination can express not only neurotoxicity but can also influence the results of the motility tests and reaction to nociceptive stimuli of laboratory animals, affecting reliability of the tests results.

4.2. Receptors affinity tests

Compound **C2** exhibiting the highest activity in the behavioural tests was subjected to the binding affinity tests. Because of the significant analgesic and depressive action shown in the behavioural tests three types of receptors were investigated: opioid receptor (type μ), serotonin receptor (5-HT_2) and benzodiazepine receptor (BZD). Radioligand displacement method was applied. Selective antagonists for each receptor were used: Naloxone in the presence of sodium ions for opioid μ receptor, Ketanserin for 5-HT_2 receptor and Flunitrazepam for BZD receptor. The affinity was calculated as EC_{50} values and the results are presented in Table 3. The specific binding represented by displacement curves is presented in Figs. 8 and 9.

Compound **C2** exhibited micromolar binding affinity ($EC_{50} = 11$ μmol) only toward opioid μ receptor (Fig. 8). Affinity toward neither serotonin nor benzodiazepin receptors was not confirmed (Fig. 9).

The affinity toward the μ receptor was expressed on the similar level to the chain urea derivatives (**A** and **B** series [2]).

5. Results and discussion

Results of the pharmacological investigation showed that the studied derivatives possessed significant influence on the central nervous system (CNS) of laboratory animals. Central depressive and antinociceptive effects seem to be the most important.

The very significant antinociceptive activity was confirmed both in the 'writhing' test, reversible by a small dose of naloxone, and in the binding assay test. The investigated compounds **C** belong to the group of deprived basic nitrogen atom carbonyl imidazolidine

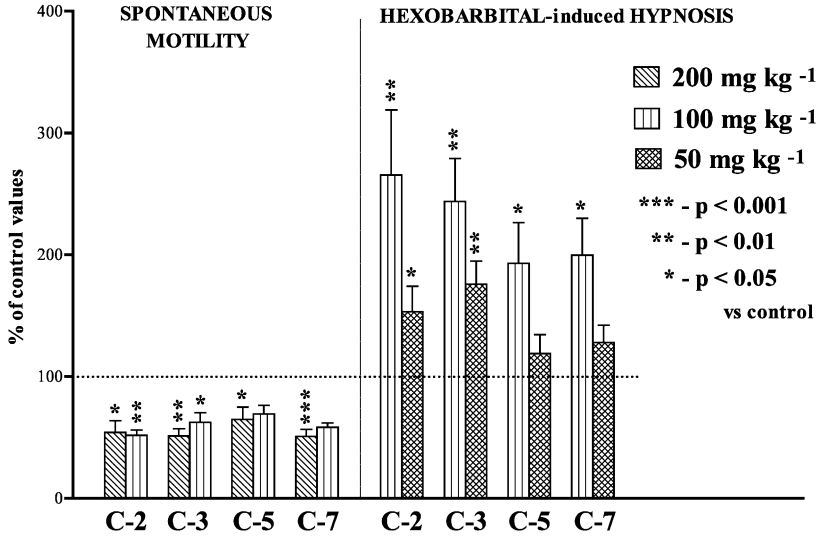


Fig. 2. The influence of the tested compounds on the spontaneous locomotor activity and hexobarbital (65 mg kg^{-1})-induced hypnosis in mice. Note: the mean values of number of movements (226–280), and the time of sleep (14.8–28.6 min) in the control groups are assumed to be 100%. The results are expressed as mean ± SEM for group of 10 mice.

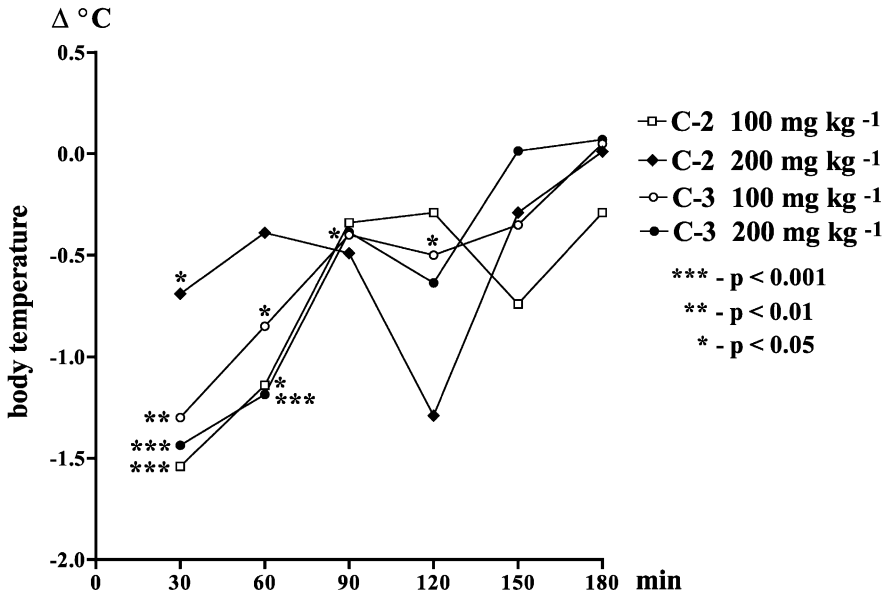


Fig. 3. The influence of tested compounds on the body temperature of mice.

derivatives, exhibiting significant antinociceptive activity connected with the opioid receptors system [2].

Comparison of the solid-state conformation of two molecules of **C2** (Fig. 10) confirms its predicted rigid structure; conformational differences are limited to the phenyl rings orientations which is a result of rotations about the N1–C11 and N6–C61 bonds. Analysis of the distances and angles (Fig. 10) between aromatic rings and the polar C5=O5 group (able to be hydrogen bond acceptor) show that observed and calculated distances Ar–Ar′ (***b***) and Ar′–$O_{carbonyl}$ (***a***) in crystal, and predicted structure **C2′** are almost identical with the distances in conformers **1** (out-stretched) in the chain derivatives of series **A** and **B**, and similar to respective distances in selected ligands of the opioid receptors (Table 4).

The observed depressive effects on the CNS of mice seem not to be connected with dopamine/norepinephrine system (lack of the amphetamine induced hyperactivity antagonization). Exhibited intensification of the post-amphetamine hyperactivity may results from the affinity to the opioid receptors. Similar intensification of the amphetamine hyperactivity was observed after administration of morphine (20 mg kg^{-1}) (Fig. 6). Morphine alone given in the same dose did not affect locomotor activity of naïve mice.

Similarly to the previous observations [2], in behavioural tests, compounds **C** seemed not to affect the respiration process of the mice.

The depressant action, much strongly pronounced by **C2** and **C3** than **C5** and **C7**, seems to be connected with serotonergic neurotransmission. It was expressed in

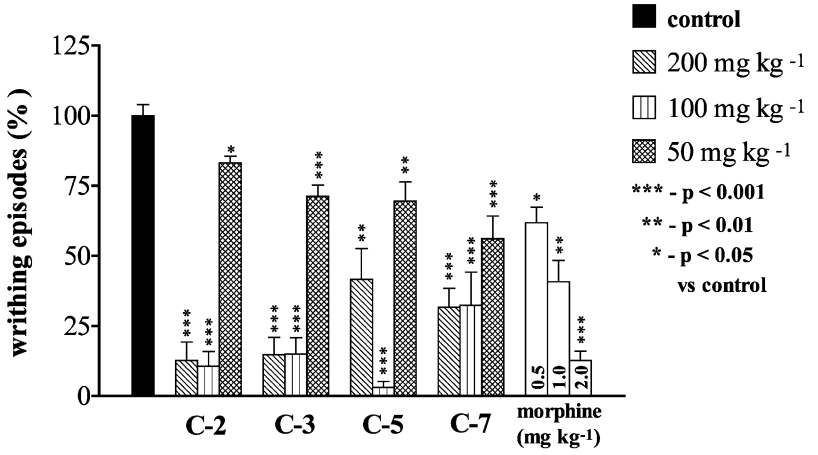


Fig. 4. The antinociceptive effects of the tested compounds and morphine in the writhing test in mice. Note: number of writhing episodes in the control group was 23–35.6 (= 100%). The results are expressed as mean ± SEM for group of 10 mice.

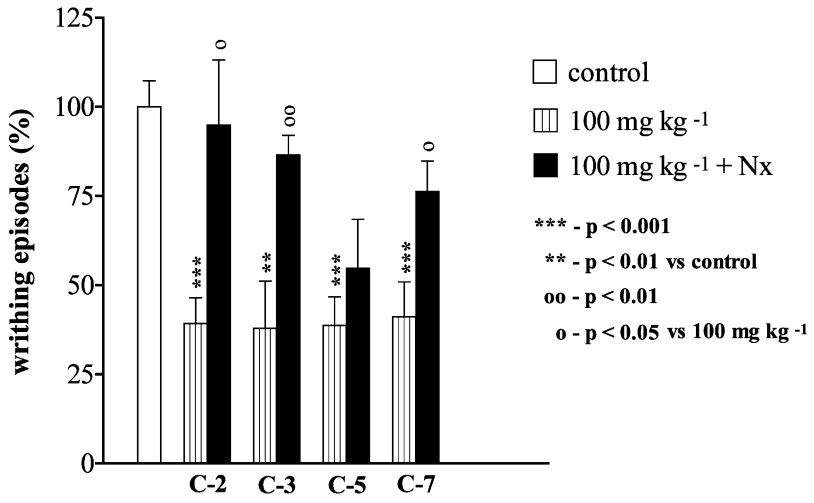


Fig. 5. The influence of naloxone (Nx), 5 mg kg^{-1} s.c., on the antinociceptive activity of 100 mg kg^{-1} of the test tested compounds in the writhing test. Note: number of writhing episodes of control mice was 26.7 (= 100%). The results are expressed as mean ± SEM for group of 10 mice.

decrease of the spontaneous motility, lowering of the body temperature and prolongation of the hexobarbital induced narcosis. Effect on the serotonin neurotransmission (i.e. reducing 'head twitch' responses after 5-HTP administration) was observed for all derivatives.

Interaction with the serotonin neurotransmission was not confirmed in the binding assay test. The lack of affinity in the binding assay test toward serotonin receptor can not yet exclude this possibility, because cyclic imidazo–triazine derivatives (**C**) can easily undergo enzymatic ring opening in the living body, leading to formation of the chain urea derivatives, the group of compounds with already confirmed affinity toward the 5-HT_2 receptor [2]. It seems that the most important structural feature reducing so significantly affinity to serotonin receptor can be the presence of the aromatic ring (Ar′) placed between two carbonyl groups and not allowing any direct access to them, e.g. by formation of the hydrogen bonds.

Electrostatic potential distribution map [5] for compound **C2** shows deep potential wells of almost equal value in the vicinity of both carbonyl O-atoms (Fig. 11).

In conclusion, the continuation of our investigation on the carbonyl derivatives of 1-aryl-2-iminoimidazolidine lead to the observation that a cyclization of these derivatives (containing urea moiety) into the respective fused imidazo–triazine ring system produce compounds of slightly weaker activity but much higher selectivity toward the opioid μ receptor. The respective location of two hydrophobic (aromatic rings) and one H-bond acceptor (carbonyl oxygen atom) sites fit well the proposed earlier [2] non-classical pharmacophore hy-

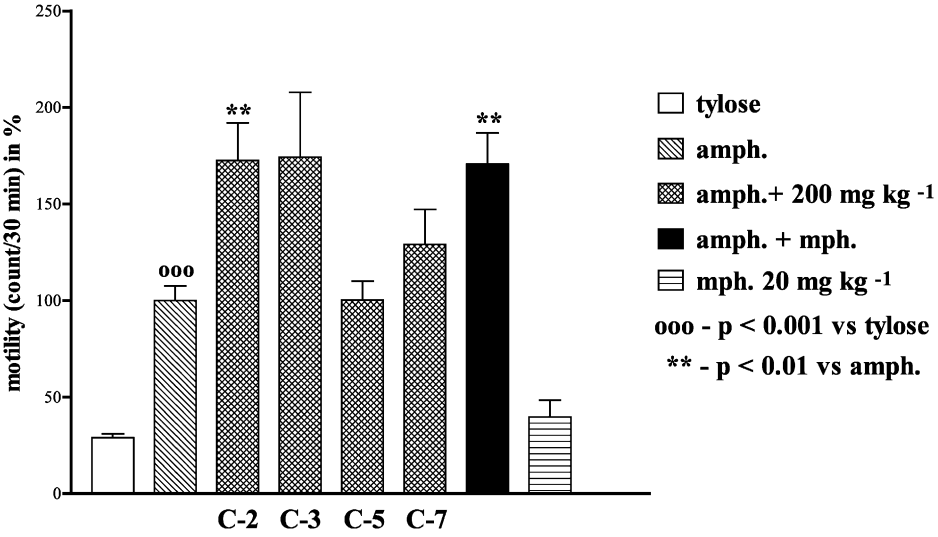


Fig. 6. The influence of the tested compounds and morphine (mph.) (20 mg kg^{-1}) on amphetamine (amph.) (5 mg kg^{-1}) induced hyperactivity in mice. Note: the results are expressed as mean ± SEM of group of 10 mice.

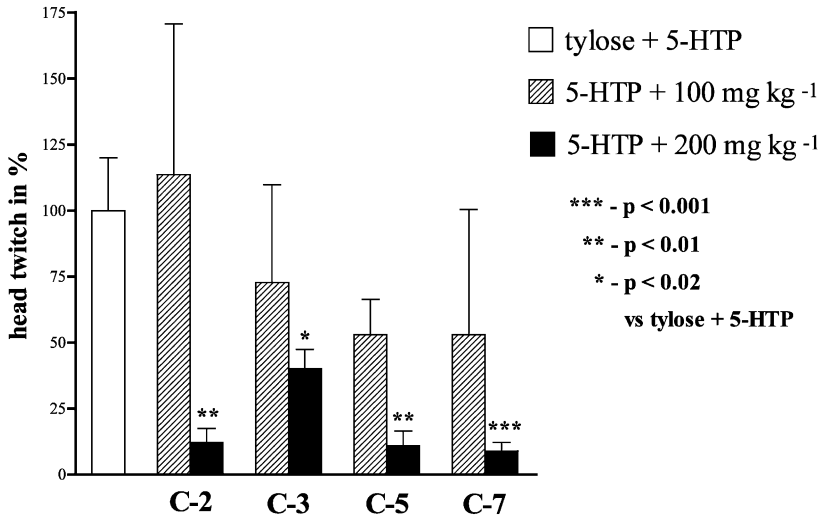


Fig. 7. The influence of the tested compounds on the head twitch responses evoked by 5-HTP (180 mg kg^{-1}). Note: number of 'head twitch' in the control group was 19.6 (= 100%). The results are expressed as mean ± SEM of group of eight mice.

Table 3
EC_{50} values (μmol) in binding tests of compound **C2** on opioid μ, serotonin 5-HT_2 and BZD receptors

	μ	5-HT_2	BZD
Naloxone	0.013	–	–
Morphine	0.20	–	–
Ketanserin	–	0.002	–
Serotonin	–	1.90	–
Clonazepam	–	–	0.001
Diazepam	–	–	0.005
C2	11.0	> 100	> 100

pothesis of the μ receptor activation. It is worth noticing that the compounds **C** did not show any significant influence on the respiration process of mice as well.

Absence of the affinity toward the 5-HT_2 receptor in the binding test and strong effect on the serotonin neurotransmission pathway in the behavioural tests show that the investigated compounds **C** can either easily undergo biochemical triazine ring opening achieving the chain urea derivatives (**A** and **B**) structure [2] or act by different, not receptor dependent mechanism.

In the further investigation, obtained results can lead to the finding of more selective and biochemically stable antinociceptives acting on the opioid receptors.

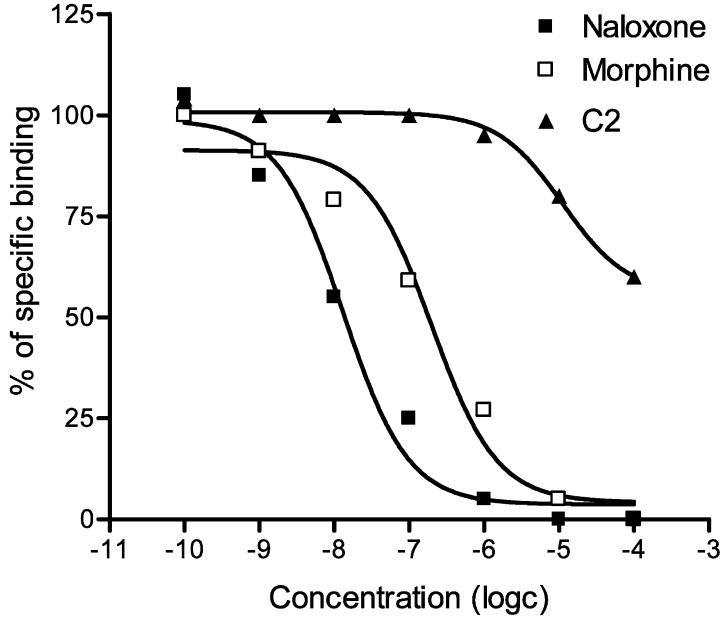


Fig. 8. Displacement of ^{3}H-naloxone from its binding sites by investigated compound **C2** (μ opioid receptor–limbic forebrain).

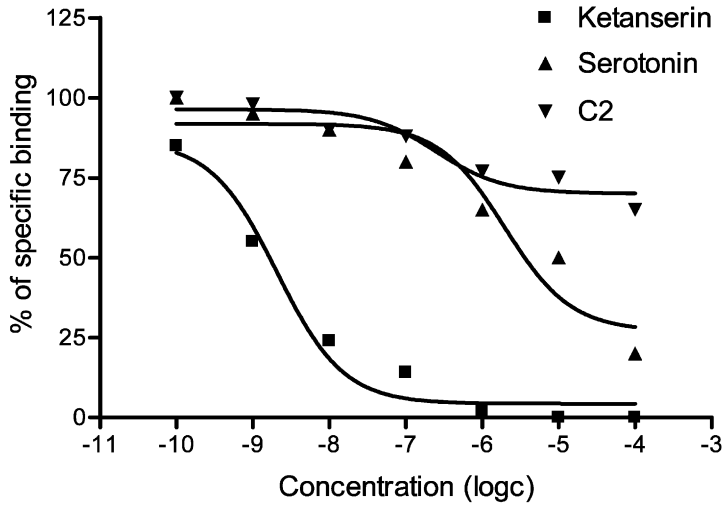


Fig. 9. Displacement of ^{3}H-ketanserin from its binding sites by investigated compound **C2** (5-HT_2 receptor).

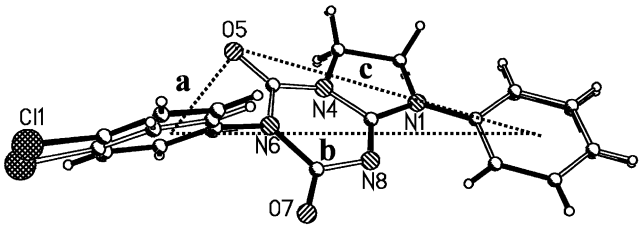


Fig. 10. Fit of two crystallographically independent **C2** molecules and definition of geometric parameters for proposed opioid-activity pharmacophore of series **C**.

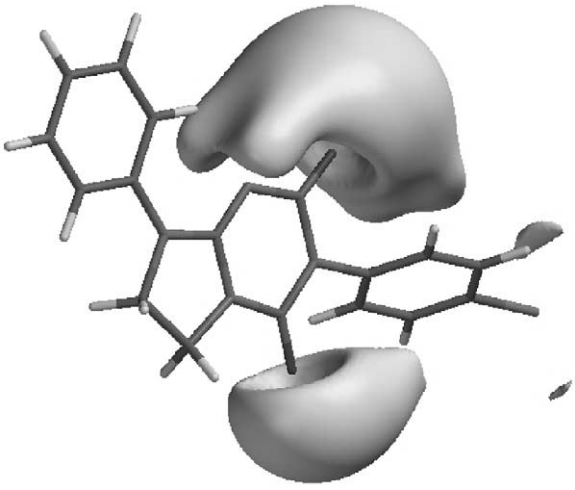


Fig. 11. Electrostatic potential map as an equipotential (−20 kcal mol^{-1}) surface for **C2′**. Note: electrostatic potential map calculated by PC Spartan Pro [4] for the **C2′** local energy minimum by ab initio module, RHF approximation, 3-21G(*) basis set.

6. Experimental

6.1. Chemical analyses

Chemicals were purchased from Merck as 'for synthesis' grade and used without further purification. M.p.s were determined on a Boetius apparatus and are given

Table 4
Observed and calculated distances and angles between aromatic rings and one of the oxo-groups in investigated compounds, and selected ligands of opioid receptors

		Distances (Å)			Angles (°)		
		a	*b*	*c*	*ac*	*ab*	*bc*
C2	Crystal [a]	3.76	8.92	7.19	104.7	51.2	24.1
		3.76	8.95	7.17	105.7	50.5	23.8
	Optimized structure	3.5	8.9	7.1	110	50	20
Bezitramide	Local minimum energy conformer	4.6	10.5	11.4	67	89	24
Fentanyl		4.8	10.4	10.6	77	77	26
Petidine [b]		4.4	5.9	6.9	59	82	39
SNC-80		8.3	8.9	11.8	50	84	46
Naltrexone [b]		5.4	6.4	7.8	82	43	55

Energy optimization performed by PC Spartan Pro [5], ab initio module, RHF approximation, 3-21G(*) basis set.
[a] Values for two symmetrically independent molecules present in the crystal.
[b] Second aromatic ring is replaced by a hydrophobic methyl (Petidine) or cyclopropylmethyl (Naltrexon) moieties.

uncorrected. NMR spectra (^{1}H and ^{13}C) were recorded on Varian Gemini 200 MHz and Brucker 200 MHz spectrometers in d_6-DMSO with TMS as an external standard at 295 K. TLC was performed on commercial Merck SiO_2 60 F_{254} plates with eluent system C_6H_6–ethyl ether (8:1), visualization: UV light $\lambda = 254$ and 355 nm. Elemental analyses were performed on Perkin–Elmer analyzer and were in range of $\pm 0.53\%$ for each analyzed element (C, H, N, Cl)

6.1.1. Synthesis of 1,6-diaryl-5,7(1H)dioxo-2,3-dihydroimidazo[1,2-a][1,3,5]triazines (method **i**) (general procedure)

1-(1-Arylimidazolidine-2-ylidene)-3-arylurea [2] (0.05 mol) was solved in 150 mL of dry $C_6H_5CH_3$ under the atmosphere of dry nitrogen. 0.05 mol (2.4 g of 50% oil suspension) of NaH was added. Mixture was cooled to -10 °C and 0.05 mol (25 g of 20% $C_6H_5CH_3$ solution) of phosgene was slowly added. Mixture was stirred for 4 h in this temperature and for 4 h in room temperature. Then 0.05 mol (5.2 g) of Et_3N was added, mixture was slowly brought to boil and refluxed for 2 h, next left overnight. The precipitation was collected, washed 2 times of 50 mL of water and solved in 25 mL of DMF. Addition of 100 mL of propanol-2 and cooling overnight in the refrigerator started precipitation. White solid was collected and purified by crystallization from DMF–propanol-2 (1:5) mixture.

6.1.2. Synthesis of 1,6-diaryl-5,7(1H)dioxo-2,3-dihydroimidazo[1,2-a][1,3,5]triazines (method **ii**) (general procedure)

1-(1-Arylimidazolidine-2-ylidene)-3-arylurea (0.05 mol) [2] and 0.05 mol (8.6 g) of carbonyldiimidazol (CDI) were solved in 100 mL of DMF and refluxed for 3 h. The solvent was removed by the low-pressure evaporation and the resulting solid was crystallized two times from the DMF–propanol-2 (1:5) mixture.

6.2. X-ray crystal structure analysis

Diffraction data for **C2**, $C_{17}H_{13}ClN_4O_2$, were measured at 295 K on a Nonius CAD4 diffractometer using variable scan speed (ω–2θ scan mode) and graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). A single crystal of dimensions $0.4 \times 0.35 \times 0.28$ mm was used. The crystal is triclinic, space group $P\bar{1}$: a, 9.611(1); b, 10.764(1); c, 15.415(2) Å; α, 93.98(1); β, 100.67(1); γ, 90.59(1)°; V, 1562.9(3) Å^3; Z, 4; d_{calc}, 1.448 g cm^{-3}. Reflection were collected up to $\theta_{max} = 34.9°$; of 9410 measured reflections, 9096 were independent [$R_{(int)} = 0.0137$] and were used in the calculations. Crystal structure was solved by direct methods using SHELXS-93 [6] program and refined by full-matrix least-squares on F^2 using SHELXL-97 [7] program. The non-hydrogen atoms were refined with anisotropic displacement parameters. H-atom positions were located from the geometry, and were given isotropic factors of 1.2 U_{eq} of the bonded C-atoms; the C–H bond 'riding' model was used in the refinement. Final discrepancy factors are $R_1 = 0.0564$, $wR_2 = 0.1252$ for $I > 2\sigma(I)$, and $R_1 = 0.1129$, $wR_2 = 0.1423$ for all data, $S = 1.038$.

6.3. Pharmacological analyses

6.3.1. behavioural tests

6.3.1.1. Materials and methods. The experiments were performed on male Albino Swiss mice (18–30 g). The animals were kept 8–10 to a cage, at room temperature of 20 ± 1 °C, on a 12/12h dark–light cycle. Standard food (Bacutil, Motycz, Poland) and water were available ad libitum. The investigated substances (**C2**, **C3**, **C5**, **C7**) were administered intraperitoneally (i.p.) as suspension in aqueous solution of 0.5% methylcellulose (tylose). The compounds were injected 60 min before the test. The controls received the equivalent volume of the solvent.

The acute toxicity of compounds was assessed in mice according to the method of Litchfield and Wilcoxon [8].

The activity of the compounds was assessed in the following tests:

- locomotor activity was measured in photoresistor actometer for single mouse for 30 min as: (a) spontaneous activity; (b) amphetamine-induced hyperactivity—mice received subcutaneously (s.c.) 5 mg kg^{-1} of amphetamine 30 min before the test;
- nociceptive reactions were studied in: (a) the 'hot plate' test (56 °C) described by Eddy and Leimbach [9]—the test was carried out 60 min before and 30, 60, 90 and 120 min after the compounds administration; (b) the acetic acid (0.6%)-induced 'writhing' test [10]—the number of writhing episodes was measured for 10 min, starting 5 min after acid solution i.p. administration;
- hexobarbital (65 mg kg^{-1})-induced narcosis was recorded as the time elapsing between the loss and recovery of the righting reflex;
- motor coordination was evaluated in the rota rod test [11];
- body temperature in normothermic mice was measured in the *rectum* by means of thermistor thermometer;
- pentylenetetrazole (110 mg kg^{-1}, s.c.)-induced convulsions were evaluated as the number of mice with clonic seizures, tonic convulsions and dead animals;
- 'head twitch' responses after 5-hydroxytryptophan (5-HTP), according to Corne et al. [12]. Mice received 5-HTP (180 mg kg^{-1}, i.p.) and number of head twitches was recorded in six 2-min intervals (4–6, 14–16, 24–26, 34–36, 44–46, 54–56 min);

– influence of naloxone (5 mg kg^{-1}, s.c.) on antinociceptive effect of tested compounds was assessed in the writhing test.

The compounds were injected in doses 200, 100, 50 and 25 mg kg^{-1}, respectively (equivalent to 0.1, 0.05, 0.025 and 0.0125 LD_{50}). Statistics: the obtained data were calculated by Student's t-test and χ^2-tests with Yates correction (pentylenetetrazole-induced seizures).

6.3.2. Binding assays

The affinity of compound **C2** toward opioid μ, serotonin 5-HT_2 and benzodiazepine BDZ receptor was investigated by radioligand displacement method. The radioligands [^{3}H]-Flunitrazepam (specific activity 81 Ci $mmol^{-1}$), [^{3}H]-Ketanserin (specific activity 63.7 Ci $mmol^{-1}$) and [^{3}H]-naloxone (specific activity 52 Ci $mmol^{-1}$) were purchased from Amersham. In all experiments the samples were counted for radioactivity in a Beckman LS 3801 scintillation counter. The specific binding was defined as the difference between total and unspecific binding. EC_{50} was estimated using GraphPad Prism computer program.

6.3.2.1. Displacement of ^{3}H-naloxone (52 Ci $mmol^{-1}$) by investigated compound. Tissues (cerebral cortex) from individual naive animals were processed separately. They were homogenized at 0 °C in 20 volumes of 50 mmol L^{-1} Tris–HCl buffer, pH 7.6. The homogenate was centrifuged at 0 °C and 25 000 × g for 30 min, and the pellet was rehomogenized and incubated in a shaking water bath at 37 °C for 45 min (preincubation), and then recentrifuged at 0 °C and 25 000 × g for 30 min. The pellet (fraction P_1+P_2) was stored at −20 °C not longer than 48 h. For incubation it was reconstituted in 20 volumes the incubation buffer pH 7.6. The incubation mixture contained 100 mmol L^{-1} NaCl, final volume 550 μL was consisted of 450 μL membrane suspension, 50 μL of ^{3}H-naloxone solution (2.5 nmol) and 50 μL of buffer containing seven concentrations (1 nmol–100 μmol) of investigated compound **C2**. For measuring unspecific binding, naloxone in a final concentration of 10 μmol was present. The incubation was carried out in duplicates, in a shaking water bath, at 30 °C for 30 min. Addition of the radioligand initiated the incubation, which was terminated by rapid filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 mL portions of ice-cold incubation buffer and were placed in plastic scintillation minivials. Scintillation fluid (3 mL) was added, and the samples were counted for radioactivity.

6.3.2.2. Displacement of ^{3}H-ketanserin (63.7 Ci $mmol^{-1}$) by investigated compound. Tissues (structures) from individual naive animals were processed separately. They were homogenized at 0 °C in 20 volumes of 50 mmol L^{-1} Tris–HCl buffer, pH 7.6. The homogenate was centrifuged at 0 °C and 25 000 × g for 15 min, and then the pellet was rehomogenized and incubated in a shaking water bath at 37 °C during 15 min. (preincubation), and then recentrifuged at 0 °C and 25 000 × g for 30 min. After decanting the supernatant, the pellet (fraction P_1+P_2) was stored at −20 °C not longer than 48 h. For incubation it was reconstituted in 20 volumes the incubation buffer pH 7.6. The incubation mixture (final volume 550 μL) was consisted of 450 μL membrane suspension, 50 μL of ^{3}H-ketanserin solution (0.6 nmol) and 50 μL of buffer containing seven concentrations (1 nmol–100 μmol) of investigated compound **C2**. For measuring unspecific binding, cold ketanserin in a final concentration of 10 μmol was present. The incubation was carried out in duplicates, in a shaking water bath, at 37 °C for 20 min. Addition of the radioligand initiated the incubation, which was terminated by rapid filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 mL portions of ice-cold incubation buffer and were placed in plastic scintillation minivials. Scintillation fluid (3 mL) was added, and the samples were counted for radioactivity.

6.3.2.3. Displacement of ^{3}H-flunitrazepam (81 Ci $mmol^{-1}$) by investigated compound. Tissues from individual naive animals were processed separately. They were homogenized at 0 °C in 20 volumes of 50 mmol L^{-1} Tris–HCl buffer, pH 7.6. The homogenate was centrifuged at 0 °C and 1000 × g for 15 min, and then recentrifuged at 0 °C and 25 000 × g for 30 min. After decanting the supernatant, the pellet (fraction P_2) was stored at −20 °C not longer than 48 h. For incubation it was reconstituted in 20 volumes the incubation buffer pH 7.6. The incubation mixture (final volume 550 μL) was consisted of 450 μL membrane suspension, 50 μL of ^{3}H-flunitrazepam solution (2 nmol) and 50 μL of buffer containing seven concentrations (1nmol–100 μmol) of investigated compound **C2**. For measuring unspecific binding, cold diazepam in a final concentration of 10 μM was present. The incubation was carried out in duplicates, in a shaking water bath, at 0 °C for 60 min. Addition of the radioligand initiated the incubation, which was terminated by rapid filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 mL portions of ice-cold incubation buffer and were placed in plastic scintillation minivials. Scintillation fluid (3 mL) was added, and the samples were counted for radioactivity.

7. Supplementary material

The lists of atomic coordinates, displacement parameters and complete geometry have been deposited at

the Cambridge Crystallographic Data Centre, CCDC No. 180329. Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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