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Original article

Antinociceptive activity of new imidazolidine carbonyl derivatives. Part 4. Synthesis and pharmacological activity of 8-aryl-3,4-dioxo-2*H*,8*H*-6,7-dihydroimidazo[2,1-*c*] [1,2,4]triazines

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Abstract

Synthesis and pharmacological activity of 8-aryl-3,4-dioxo-2*H*,8*H*-6,7-dihydroimidazo[2,1-*c*] [1,2,4]triazines (**A**) are presented. The title compounds were obtained from 1-aryl-2-hydrazinoimidazolines (**1**) by cyclization reaction with ethyl oxalate (**2**). They were tested for pharmacological activity in behavioral animal tests (**A1**, **A3**, **A5**, **A6**, **A8**, **A9**). With relatively low acute toxicity (LD_{50} in range from 1100 to over 2000 mg kg^{-1}, intraperitoneally, i.p.), some of them exhibited significant antinociceptive activity as the result of the 'writhing' test indicated. Especially strong antinociception for compound **A8** and significant for **A6** was observed in doses of 12.5–200 mg (0.00625–0.1 LD_{50}) and 37.5–150 mg (0.025–0.1 LD_{50}), respectively. Reversion of the antinociception for **A1** and **A8** produced in the 'writhing' test by 5 mg kg^{-1} dose of naloxon can suggest an opioid-like mechanism of their analgesic activity. Additionally, compound **A9** reduced number of the "head twitch" episodes after 5-hydroxytryptophan (5-HTP) administration with no antinociceptive effect at all and compound **A3** showed significant protection in the pentylentetrazol-induced seizure model. Differences observed in the activity spectrum between **A8** and **A9** derivatives can be explained on the base of difference in the amido-imido tautomeric equilibrium observed between these two compounds.

Keywords: CNS activity; Opioid-like analgesics; Antinociception; Imidazo[2,1-*c*] [1,2,4]triazines; Molecular structure; Amido-imido tautomerism

1. Introduction

The continuous search for new ligands of the opioid receptors with higher activity and lower toxicity and side effects led in recent years to some unexpected results. The most important seem to be recognition of the completely new spatial patterns of the pharmacophoric elements arrangement. The new pharmacophoric groups and new pharmacophores were also proposed [1,2]. The most interesting in the near future could be compounds without basic nitrogen atom as ones showing no typical for other opioids side effects.

The new non-peptide β-turn scaffold agonists [3] or tetrahydroisoquinoline (TIC) derived antagonists [4] with urea and thiourea moieties proved to be powerful ligands toward mu opioid peptide (MOP) or delta opioid peptide (DOP) [5] receptors. The most spectacular discovery was finding the Salvinorin A, a new selective and very potent KOP (kappa opioid peptide receptor [5]) agonist [6]. Salvinorin is a natural diterpenoid with no nitrogen atom present in its structure. It contains oxygen atoms only. It has been found that Salvinorin occupies almost same space within the receptor active domain with U 69,593, also considered a specific KOP agonist. There was yet a different interaction pattern between Salvinorin and U 69,593 [6] recognized, although some similar pharmacophoric groups were present in both structures. Also our results [7–9] of the previous research on the carbo-

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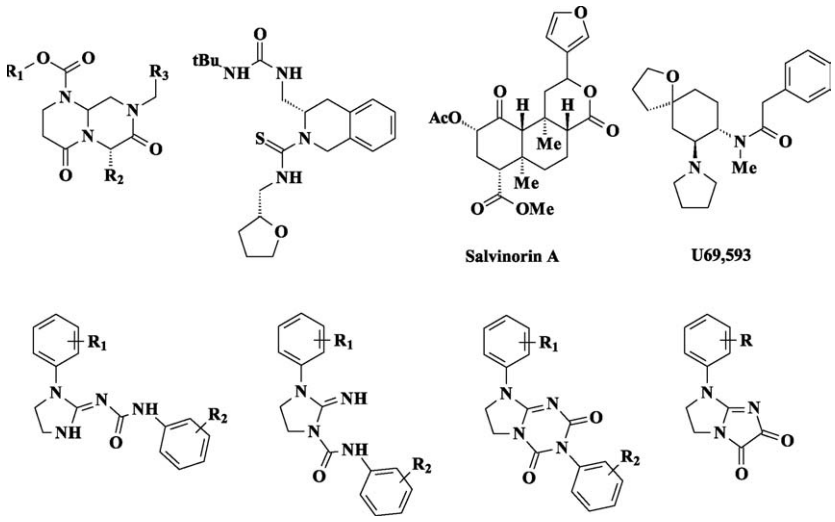
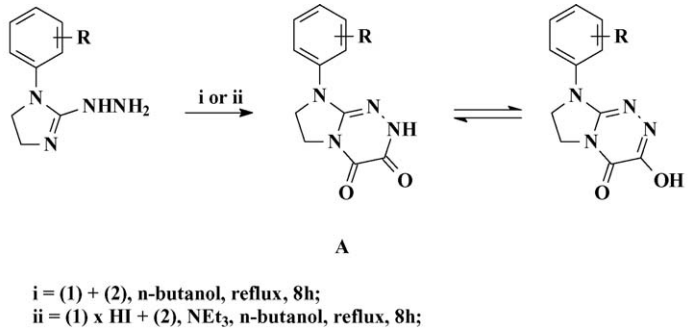
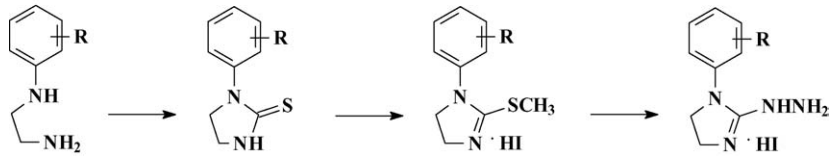


nyl 1-aryl-2-iminoimidazolidine derivatives can suggest that basic nitrogen atom should not be considered crucial for opioid activity, maybe just important in the recognition stage.

In this paper we would like to present the recent results on the antinociceptive activity investigation of new group of fused imidazole-triazine derivatives bearing α,β-dioxo moiety similar to previously investigated dioxo-imidazo-imidazoles [9]. In the case of compounds investigated the amido-imido tautomerism is possible. It has been found that tautomeric equilibrium was dependent on the substituent present in the N8-aromatic ring. It was also found that this equilibrium was affecting the spectrum of the pharmacological activity, especially visible in case of **A8** and **A9** compounds investigated.

2. Chemical part





The 8-aryl-3,4-dioxo-2*H*,8*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazine derivatives (**A**) were synthesized by condensation of the 1-aryl-2-hydrazinoimidazolines (**1**) and diethyl oxalate (**2**) (Scheme). 1-aryl-2-hydrazinoimidazolines were obtained by earlier described method [10] from respective *N*-arylethylenediamines. The condensation reaction can be carried out starting both from free base (method i) or hydroiodide in the triethylamine presence (method ii). The physicochemical properties of the compounds synthesized are presented in Table 1. NMR spectral characteristic of the imidazo-triazines (**A**) revealed in their ^{1}H NMR spectra signals of the H7 and H6 as two double-doublet at ca. 4.1 and

Table 1
Physicochemical properties of 8-aryl-3,4-dioxo-2*H*,8*H*-6,7-dihydroimidazo[2,1-*c*] [1,2,4]triazines (**A**)

Numbers	R	R_f	M.p. (°C)	Yield (%)	
				(i)	**(ii)**
A1	H	0.4	311–6	62.1	61.4
A2	2-CH_3	0.42	241–3	64.4	60.3
A3	4-CH_3	0.43	330–2	67.3	61.2
A4	2,3-diCH_3	0.44	307–10	63.2	59.8
A5	2-CH_3O	0.36	248–51	60.2	58.5
A6	4-CH_3O	0.38	302–3	68.1	63.4
A7	2-Cl	0.45	307–9	57.6	54.3
A8	3-Cl	0.45	316–8	59.2	57.2
A9	4-Cl	0.46	346–9	72.2	59.6
A10	3,4-diCl	0.45	323–4	62.5	57.9

3.9 ppm with the coupling constants of $J \sim 9$ Hz and $J' \sim 7.5$ Hz or as a broad singlet at ca. 4.0 ppm. Small distance between these two signals can be caused by restriction in conformation of the imidazolidine ring as an effect of the fusion with the triazine and resulting from this is its almost perfect planarity and equalization of both methylene group character. There is also slightly broadened singlet signal in the range 11.42–11.89 ppm. The hydrogen atom with so high acidity could only be an enolic/imidic hydrogen atom. From this signal chemical shift value it can be concluded that the tautomeric equilibrium in solution should be moved towards 3-oxo form rather than 3-hydroxy one. The difference in the substituting group causes also difference in the C3 and C4 character confirmed by the chemical shift values of respective carbon atoms in their ^{13}C NMR spectra (ca. 155 ppm for C4 and ca. 150 ppm for C3). The chemical shift values of C7 and C6 carbon atoms exhibit unequal character as well (ca. 41 ppm for C6 and ca. 48 ppm for C7). The spectral data of the compounds **A** are presented in Table 2.

The MS spectra confirmed the molecular weight of the compounds synthesized. The most typical fragmentation pattern was the loss of 24 mu fragment that most probably was C_2H_4. The data are presented in Table 3.

3. Structural and modeling part

The molecular modeling studies were undertaken to investigate the 3D structure and the tautomeric equilibrium of the searched imidazotriazine system. The 3D structures of compound **A8** and **A9** were minimized by ab initio 6-31G* (H–F approximation) method (Fig. 1). The structural data obtained for them indicated planarity in both the heterocyclic dihydroimidazo-triazine system and the chloro-substituted phenyl ring, and a co-planarity of these fragments.

Additionally, the energy minimization and geometry optimization for two tautomeric forms **A8a**, **A9a** and **A8b**, **A9b** were performed using the semi empirical AM1 method [11]. The heat of formation was calculated both for isolated molecule and the molecule in a simulated water environment (the molecule was placed in the center of the box surrounded by 28 water molecules equilibrated at 300 K and 1013 HPa) [12] and the population (p_i) of the tautomers was estimated using a non-degenerate Boltzman distribution (Table 4).

As it can be seen from Table 4 that when a chloro substituent is located at *meta* and *para* position of phenyl ring, the gas phase studies indicate that the 3-oxo form is more stable than 3-hydroxy, with ΔH_f values of 11.61 and 10.68 kJ mol^{-1} for **A8** and **A9**, respectively. The water environment studies show the same relation between oxo and hydroxy forms for *meta*-chloro-substituted phenyl ring in **A8** (ΔH_f = 14.58 kJ mol^{-1}) and predomination of the hydroxy form over oxo for *para*-substituted phenyl ring in **A9** (ΔH_f = 45.41 kJ mol^{-1}).

4. Pharmacological part

4.1. Behavioral tests

Six compounds—**A1**, **A3**, **A5**, **A6**, **A8** and **A9**—were tested for their pharmacological activity. They exhibited weak or very weak acute toxicity (as LD_{50}): over 2000 mg kg^{-1} for **A8** and **A9**, 1800 mg kg^{-1} for **A3**,

Table 2
NMR data of 8-aryl-3,4-dioxo-2*H*,8*H*-6,7-dihydroimidazo[2,1-*c*] [1,2,4]triazines (**A**)

Numbers	^1H NMR	^{13}C NMR							
	H6/H7 (dd, *J* (Hz) or bs)	NH (s)	Ar (m)	C7	C6	C4	C3	C8a	Ar
A1	4.05	11.78	7.01–7.63	49.4	40.8	155.4	152.8	154.3	120.9, 126.6, 129.9, 135.7
A2	3.94/4.09 (J = 9, J' = 7.6)	11.46	7.22–7.42	48.3	40.9	155.0	149.9	154.1	120.7, 127.4, 127.7, 129.5, 132.5, 134.8
A3	4.02	11.75	7.20–7.46	47.1	40.4	155.2	153.1	154.4	120.0, 129.8, 134.2, 135.7
A4	3.91/4.09 (J = 8.9, J' = 7.6)	11.42	7.13–7.24	48.6	41.0	153.0	145.2	154.1	123.9, 126.1, 128.7, 134.6, 137.6, 137.7
A5	4.04/4.19 (J = 9, J' = 7.5)	11.46	7.46–7.94	47.9	40.8	154.9	148.2	154.1	117.5, 120.1, 122.5, 127.1, 131.9, 139.9
A6	4.02	11.70	6.95–7.52	45.8	40.3	154.8	153.0	154.0	114.0, 119.2, 132.8, 143.3
A7	3.95/4.16 (J = 9, J' = 7.6)	11.53	7.33–7.67	48.3	40.9	155.3	150.7	154.2	118.3, 120.7, 122.8, 126.3, 129.9, 137.5
A8	4.05	11.86	7.08–7.85	47.4	41.1	155.9	153.9	154.1	120.1, 124.4, 124.7, 131.5, 136.8
A9	4.02	11.80	7.26–7.51	47.1	40.4	155.2	153.1	154.2	118.5, 130.4, 135.2, 139.8
A10	4.06	11.89	7.44–7.99	47.3	40.5	156.3	153.3	153.9	120.9, 122.9, 136.2, 137.1, 137.7

Table 3
MS data of 8-aryl-3,4-dioxo-2*H*,8*H*-6,7-dihydroimidazo[2,1-*c*] [1,2,4]triazines (**A**)

Numbers	Formula m.w. obsd/calcd	MS
A1	$C_{11}H_{10}N_4O_2$, 230.0802/230.0804	M+. 230(100), 202(15), 173(5), 145(6.5), 118(32), 104(7), 91(6), 77(22), 65(4), 51(9), 42(4)
A3	$C_{12}H_{12}N_4O_2$, 244.0961/244.0960	M+. 244(100), 216(10.5), 173(8), 132(12), 131(5), 118(5), 91(12), 65(6), 43(4), 42(3)
A5	$C_{12}H_{12}N_4O_3$, 260.0909/260.0909	M+. 260(100), 229(7.5), 175(5), 174(20), 173(8), 145(4), 134(4.5), 120(4), 118(5), 92(5), 77(6), 43(6), 42(5)
A6	$C_{12}H_{12}N_4O_3$, 260.0911/260.0909	M+. 260(100), 245(17), 217(17), 148(5), 147(4), 133(4), 43(8), 42(4)
A7	$C_{11}H_9ClN_4O_2$, 264.0417/264.0414	M+. 266(33)/264(100), 230(9), 229(68), 179(5), 173(21), 172(6), 159(6), 158(21), 154(8), 152(24), 151(4), 140(5), 138(8), 125(5), 113(4)/111(14), 99(5), 77(5), 75(13), 51(4), 43(7), 42(7)
A8	$C_{11}H_9ClN_4O_2$, 264.0421/264.0414	M+. 266(33/264(100), 238(4)/236(13.5), 179(5), 173(15), 154(7), 152(23), 140(4), 138(5), 113(5)/111(15), 99(4), 75(11), 42(6)
A9	$C_{11}H_9ClN_4O_2$, 264.0414/264.0414	M+. 266(32)/264(100), 238(3.5)/236(11), 154(8), 152(26), 151(5), 140(4), 138(7), 125(5), 113(4)/111(13.5), 99(4), 75(11), 42(5)
A10	$C_{11}H_8Cl_2N_4O_2$, 298.0019/298.0019	M+. 302(11)/300(66)/298(100), 272(8)/270(13), 213(5.5), 207(8), 190(3)/188(20)/186(32), 185(5), 174(7), 173(4), 172(7), 159(4), 147(7), 146(12), 133(4), 124(4), 111(5), 109(9), 75(7), 74(4), 70(5), 43(4), 42(8)

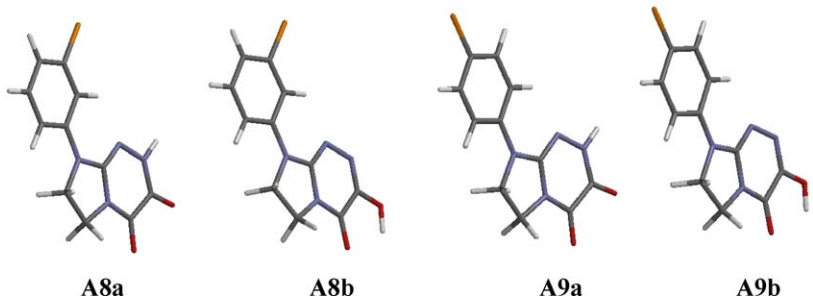


Fig. 1. 3D view of the **A8** and **A9** molecules in both tautomeric forms. Note: energy optimization performed by PC Spartan Pro v. 1.05 [24], ab initio module, RHF approximation, 6-31G (*) basis set.

Table 4
The heat of formation (H_f, AM1-MNDO approximation) and percentage (p_i) for **A8a**, **A8b** and **A9a**, **A9b** tautomeric forms

Tautomer form	H_f (kJ mol^{-1})		p_i (%)	
	Gas	Water	Gas	Water
A8a	150.22	–7642.74	99.0	>>99.0
A8b	161.83	–7628.16	1.0	<<1.0
A9a	149.40	–7611.50	99.0	1.0
A9b	160.08	–7656.91	<<1.0	>>99.0

1500 mg kg^{-1} for **A6**, 1400 mg kg^{-1} for **A1** and 1100 mg kg^{-1} for **A5**. The progressive doses, calculated as a part of LD_{50} for compounds tested, were used in behavioral experiments. Spontaneous locomotor activity and also amphetamine-induced hyperactivity in mice were not changed by all investigated compounds given in doses of 0.1 LD_{50} (data not shown). Body temperature of normothermic mice was lowered significantly (within 30 min, **A3**; 60 min, **A8** and **A9** or 90 min, **A6** and **A1**) by all compounds tested except **A5** (Fig. 2). All compounds tested (except **A9**), given in a dose of 0.1 LD_{50} presented significant antinociceptive effect in the writhing test in mice. Compounds **A8** and **A6** exhibited this effect when given in doses as low as 12.5 and 18.75 mg kg^{-1} (0.00625 and 0.0125 LD_{50}, respectively) (Fig. 3). Naloxone (5 mg kg^{-1}) reversed the antinociceptive effects of **A8** and **A1** but not that of **A3**, **A5** and **A6** (Fig. 4). The dose of naloxone

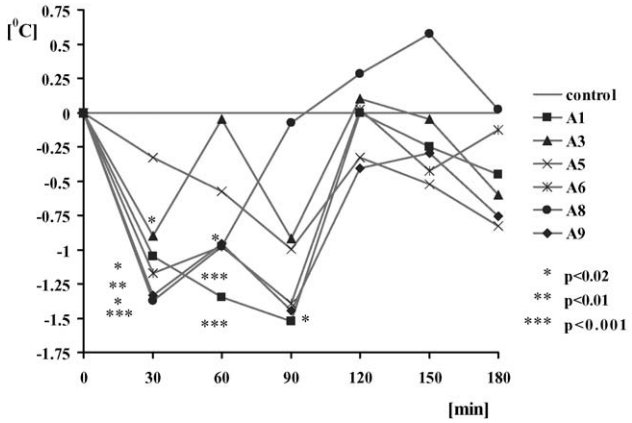


Fig. 2. The influence of the tested compounds on the body temperature of mice. Note: each point represents the mean for a group of 10 mice.

was chosen as acceptable and selective for the opioid (especially MOP) receptors affinity investigation [13–16].

All compounds investigated, with exception of **A3** given in doses 180 and 90 mg kg^{-1}, did not produce any protection in clonic seizures and tonic convulsions evoked by pentetrazole (data not shown). The "head twitch" responses after 5-hydroxytryptophan (5-HTP) administration were significantly decreased only by compound **A9** (Fig. 5). Compounds **A1**, **A3**, **A5**, **A6** and **A9** given in dose of 0.1 LD_{50}, did not

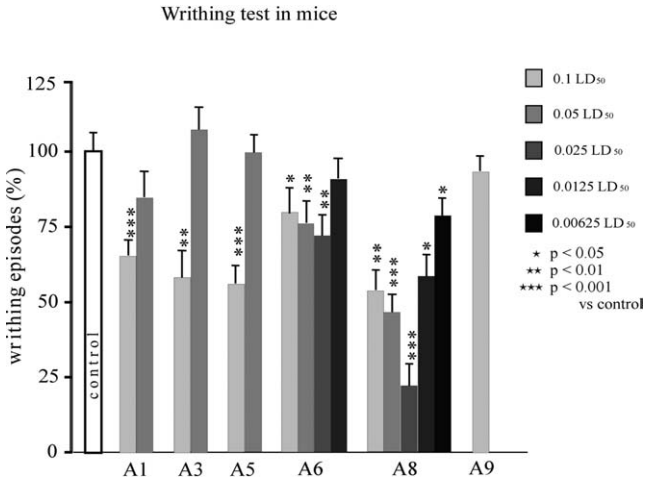


Fig. 3. The antinociceptive effects of the tested compounds, assessed in the 'writhing' test in mice. Note: number of writhing episodes in the control groups was 24–35 (=100%). The results are expressed as mean ± S.E.M. of a group of eight mice.

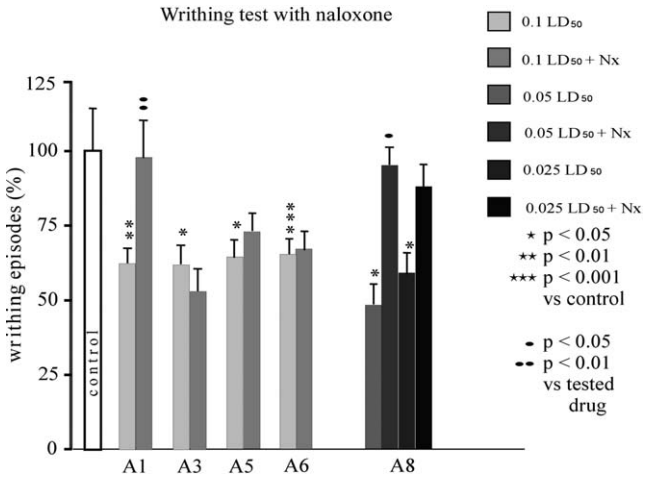


Fig. 4. The influence of naloxone (Nx), 5 mg kg^{-1} s.c. on the antinociceptive activity of the tested compounds in the writhing test. Note: number of writhing episodes of control mice was 22–32 (=100%). The results are expressed as mean ± S.E.M. of a group of eight mice.

impair the motor coordination of mice in the rota-rod and the chimney tests. Only compound **A8**, given in the high dose of 200 mg kg^{-1}, significantly disturbed motor coordination of mice in the rota-rod test. The results of the motility test and reaction to the nociceptive stimuli in mice should be therefore considered significant due to the lack or slight disturbance (**A8**) of motor coordination by compounds tested.

5. Results and discussion

Results of the pharmacological investigation show that some of the series **A** compounds possess significant and structure dependant influence on the central nervous system (CNS) of laboratory animals. The most important seem to be their antinociceptive and serotonergic effects, especially the antinociceptive activity of **A8** compound, whose action in doses used was comparable with effects expressed after mor-

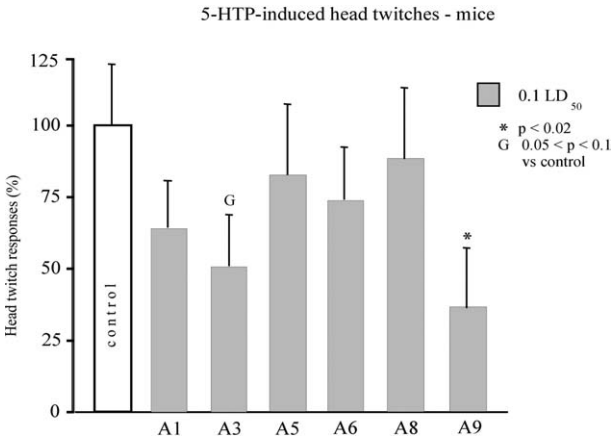


Fig. 5. The influence of the tested compounds on the 'head twitch' responses evoked by 5-HTP (180 mg kg^{-1}). Note: number of 'head twitch' in the control group was 11.5 (=100%). The results are expressed as mean ± S.E.M. of a group of eight mice.

phine administration in doses 0.5–2 mg kg^{-1} in the writhing test in mice [7]. This activity spectrum can be explained by structure similarities to other carbonyl derivatives of 1-aryl-2-iminoimidazolidine, presented in this series of papers [7–9], and underlines the high importance of both hydrophobic and polar (e.g. hydrogen-bond) interactions between ligands and the proteins as well as the spatial location of all pharmacophoric groups.

An analgesic/antinociceptive activity in this group of compounds was expected according to the non-classical MOP pharmacophore [7], although only two of the potential three pharmacophoric points were present, similar to the 1-aryl-5,6(1H)dioxo-2,3-dihydroimidazo[1,2-a]imidazole derivatives [9]. Comparison of the **A8** and 1-phenyl-5,6(1H)dioxo-2,3-dihydroimidazo[1,2-a]imidazole [9]—the most potent compounds from both series—structures (Fig. 6) could lead to the conclusion that except for the presence of the oxo moiety another yet point should be added to the existing MOP receptor pharmacophore. Point which does not exist in the 3-hydroxy tautomer (**b**) of the **A8** but is present in the 3-oxo tautomer (**a**), and also can be found in the 1-phenyl-5,6(1H)dioxo-2,3-dihydroimidazo[1,2-a]imidazole [9]. Comparison of these three structures (Fig. 7) shows that the NH moiety could be a key fragment. In a predominant 3-oxo form, fragments present in the imidazo-

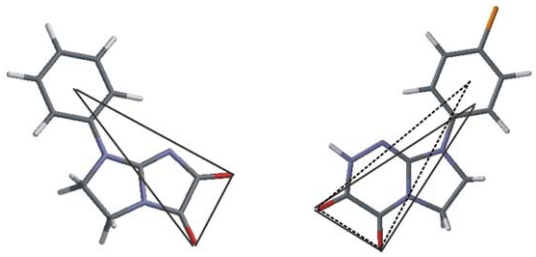


Fig. 6. Comparison of the 1-phenyl-5,6(1H)dioxo-2,3-dihydroimidazo[1,2-a]imidazole and **A9** and the pharmacophores of their serotonergic activity [9]. Note: — pharmacophore of the 5,6-dioxo-2,3-dihydroimidazo[1,2-a]imidazole derivatives; ••• pharmacophore of the **A9a**.

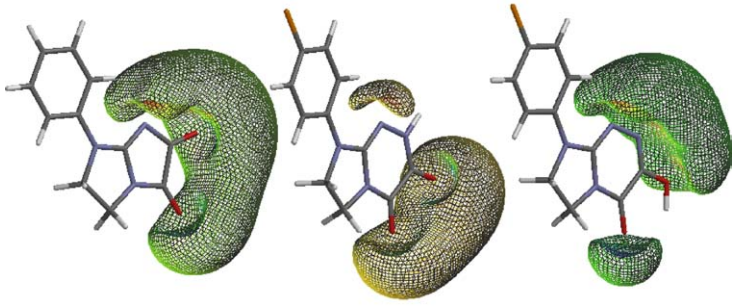


Fig. 7. Distribution of the equipotential surfaces (–20 kcal mol^{-1}) for 1-phenyl-5,6(1H)dioxo-2,3-dihydroimidazo[1,2-a]imidazole (**D1**, [9]), **A9a** and **A9b**. Note: energy optimization performed by PC Spartan Pro v. 1.05 [24], ab initio module, RHF approximation, 6-31G (*) basis set.

imidazole derivatives [9] (hydrophobic aromatic ring and α,β-dioxo moiety) can be also found. Imidazo-imidazole derivatives exhibited antinociceptive, MOP receptor mediated activity [9], so probably some of the investigated **A** series compounds do (e.g. **A1** and **A8**). Imidazo-imidazole derivatives exhibited also but in much stronger manner the serotonergic activity, which is not present at all in the group of imidazo-triazine derivatives showing an antinociception. Only **A9** compound shows serotonergic effect while it is deprived of the antinociceptive action. According to the molecular modeling results, there could be only one structural difference between **A8** and **A9** (applicable probably also for the whole series)—**A9** compound exists predominantly in the 3-hydroxy form (Table 4). Therefore, the exchange of the 3-oxo group by 3-hydroxy one seems to be the main factor affecting selectivity of the action. Exclusion of the one oxo group (3-oxo namely) from the α,β-dioxo moiety reduced antinociceptive activity of the **A9** and its probable affinity for the opioid receptors. In the same time it triggered on the affinity for the serotonin receptor (e.g. 5-HT_2). From this point of view both pharmacophoric models (opioid and serotonergic activity) presented earlier [7,9] need some adjustments.

It could be concluded that aromatic ring (hydrophobic/pi–pi interactions) and the carbonyl group (hydrogen bond acceptor) play in the selected **A** series compounds opioid action pharmacophore more important role than the third fragment (hydrophobic interaction). It is possible that there is also the fourth point in this pharmacophore having a hydrogen acceptor bond character (nitrogen atoms—N7 in imidazo-imidazole derivatives or N1 in imidazo-triazine derivatives). It is also possible that NH group can make an additional pharmacophoric point as a HD center, replacing the hydrophobic one. The lack of the antinociceptive activity observed for **A9** derivative underline the high importance of the 3-oxo group. It seems to be much more important than 4-oxo group, because its change into the 3-hydroxy one also causes drastic change in the activity. In contrary 4-oxo group seems to be much more important for the serotonergic activity. Interaction with the oxygen atom as a HA center should be accompanied rather with similar type interaction (HA) with the ring nitrogen atom (N7 of the imidazole ring or N2 of the triazine ring) than with the adjacent oxygen atom in position 3. This interaction (as three-centered hydrogen bond) would be formed much easily with 3,4-dioxo tautomers but no serotonergic activity is observed for other than **A9** compounds from the **A** series. From two available nitrogen atoms interaction with N2, one seems to be more important for the serotonergic activity. In the imidic tautomeric form it is freely available as HA center. In the amidic tautomeric form it changes its character rather to the HD center because the access to the nitrogen atom would be possible only from the perpendicular directions (from above or below) (Fig. 8).

6. Experimental protocols

6.1. Chemical analysis

Chemicals were purchased from Merck as 'synthesis grade' and used without further purification. Melting points (m.p.) were determined on a Boetius apparatus and are given uncorrected. NMR spectra (^1H and ^{13}C) were recorded on a Brucker 200 MHz spectrometer in D_6-DMSO with TMS as an external standard at 295 K. EI-MS spectra were recorded at 70 eV (direct insertion probe, ion source temperature 160 °C) on the VG ZabSpec spectrometer (Manchester, UK). TLC was performed on commercial Merck SiO_2 60 F_{254} plates with toluene/ethyl acetate/methanol (1:3:0.5) eluent system and visualization in UV light λ = 254 and 355 nm.

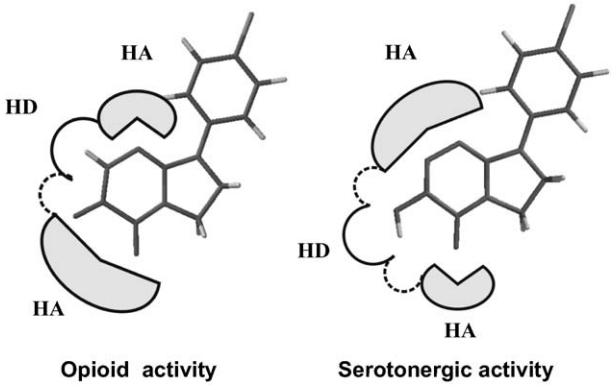


Fig. 8. Possible hydrogen bond acceptor (**HA**) and donor (**HD**) interaction sites for opioid and serotonergic activity of **A9**.

Elemental analyses were performed on a Perkin–Elmer analyzer and were in range of ±0.5% for each element analyzed (C, H, N, Cl).

6.1.1. Synthesis of 8-aryl-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c] [1,2,4]triazines (method i) (general procedure)

Free base of 1-aryl-2-hydrazinoimidazoline [10] (0.05 mol) was solved in 80 ml of *n*-butanol. The 0.05 mol (7.3 g) of diethyl oxalate was added and the mixture was heated under reflux for 8 h. During that time, precipitation of the solid started. The mixture was refrigerated overnight and the precipitation yielded was collected and purified by crystallization from DMF/methanol (1:2) mixture.

6.1.2. Synthesis of 8-aryl-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c] [1,2,4]triazines (method ii) (general procedure)

Diethyl oxalate (7.3 g, 0.05 mol) was added to the suspension of appropriate 1-aryl-2-hydrazinoimidazoline hydroiodide [10] (0.05 mol) in 80 ml of *n*-butanol. The mixture was stirred and triethylamine (5 ml) was added. The reaction was carried out under reflux for 8 h. During that time, precipitation of solid started. The crude product obtained after cooling was collected, washed off with cold methanol and finally purified by recrystallization from DMF/methanol (1:2) mixture.

6.2. Molecular modeling and theoretical calculations

The heat of formation for two possible tautomeric forms of investigated imidazotriazine derivative was calculated at the restricted Hartree–Fock level (RHF) using AM1 semiempirical SCF-MO method implemented in the program package HyperChem rel. 4.5 [17]. The structures were fully optimized (bond lengths, bond angles and torsion angles) without any constraint to a gradient norm of <0.1.

6.3. Pharmacological analysis

6.3.1. Behavioral tests

6.3.1.1. Materials and methods. The experiments were performed on male Albino Swiss mice (17–30 g). The animals were kept in 8–10 to a cage, at room temperature of 20 ± 1 °C, on a 12/12 h dark–light cycle. Standard food (Bacutil, Motycz, Poland) and water were available ad libitum. The investigated compounds were administered intraperitoneally (i.p.) in volume of 10 ml kg^{-1} as suspensions in aqueous solution of 0.5% methylcellulose (tylose). The compounds were injected 60 min before the test. The controls received an equivalent volume of the solvent.

All tests performed, suggested by Vogel and Vogel [18] are generally accepted as basic in investigation of the central activity by behavioral methods.

The acute toxicity of the compounds was assessed in mice according to Litchfield and Wilcoxon [19].

The activity of the compounds was assessed in the locomotor activity tests (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.

Body temperature in normothermic mice was measured in the rectum by means of thermistor thermometer.

Nociceptive reactions were studied in the acetic acid (0.6%) induced 'writhing test' [20]—the number of writhing episodes was measured for 10 min starting 5 min after the i.p. administration of the acid solution. Influence of naloxone (5 mg kg^{-1}, s.c.) on antinociceptive effect of tested compounds was assessed in the same test.

Pentylenetetrazole (110 mg kg^{-1}, s.c.)-induced convulsions were evaluated as the number of mice clonic seizures, tonic convulsions and dead animals.

'Head twitch' responses after 5-HTP administration was assessed according to Corne et al. [21]. 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).

The compounds were injected in doses equivalent to 0.2, 0.1, 0.05, 0.025, 0.0125 and 0.00625 LD_{50}.

Motor coordination was evaluated in the rota-rod [22] and the chimney [23] tests.

Statistics: The obtained data were calculated by Student's *t*-test and χ^2-test with Yates correction (pentylenetetrazole-induced seizures).

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