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Comparison of pharmacophore cinnoline and quinoline systems on the basis of computer calculation and pharmacological screening of their condensed systems

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A series of new pyrimido[5,4-*c*]quinoline derivatives were prepared to compare the pharmacophore systems of cinnoline and quinoline. These compounds were obtained by the cyclocondensation of appropriately substituted 4-amino-3-quinolinecarboxylic acids **3** with acetic anhydride to the respective 2-methyl-1,3-oxazino[5,4-*c*]quinolin-4(3 *H*)-ones **4**. These derivatives reacted with amines and gave *N*-3 substituted 2-methylpyrimido[5,4-*c*]quinolin-4(3 *H*)-ones **6**. 4-Amino-3-quinolinecarboxamide **2** reacted with diethyl carbonate to give 1,2,3,4-tetrahydropyrimido[5,4-*c*]quinolin-2,4-diones **5**. The SAR parameters of the derivatives obtained were analysed with the HyperChem 5.1/ChemPlus 2.0 computer program. The compounds synthesized were screened for their effect on the CNS.

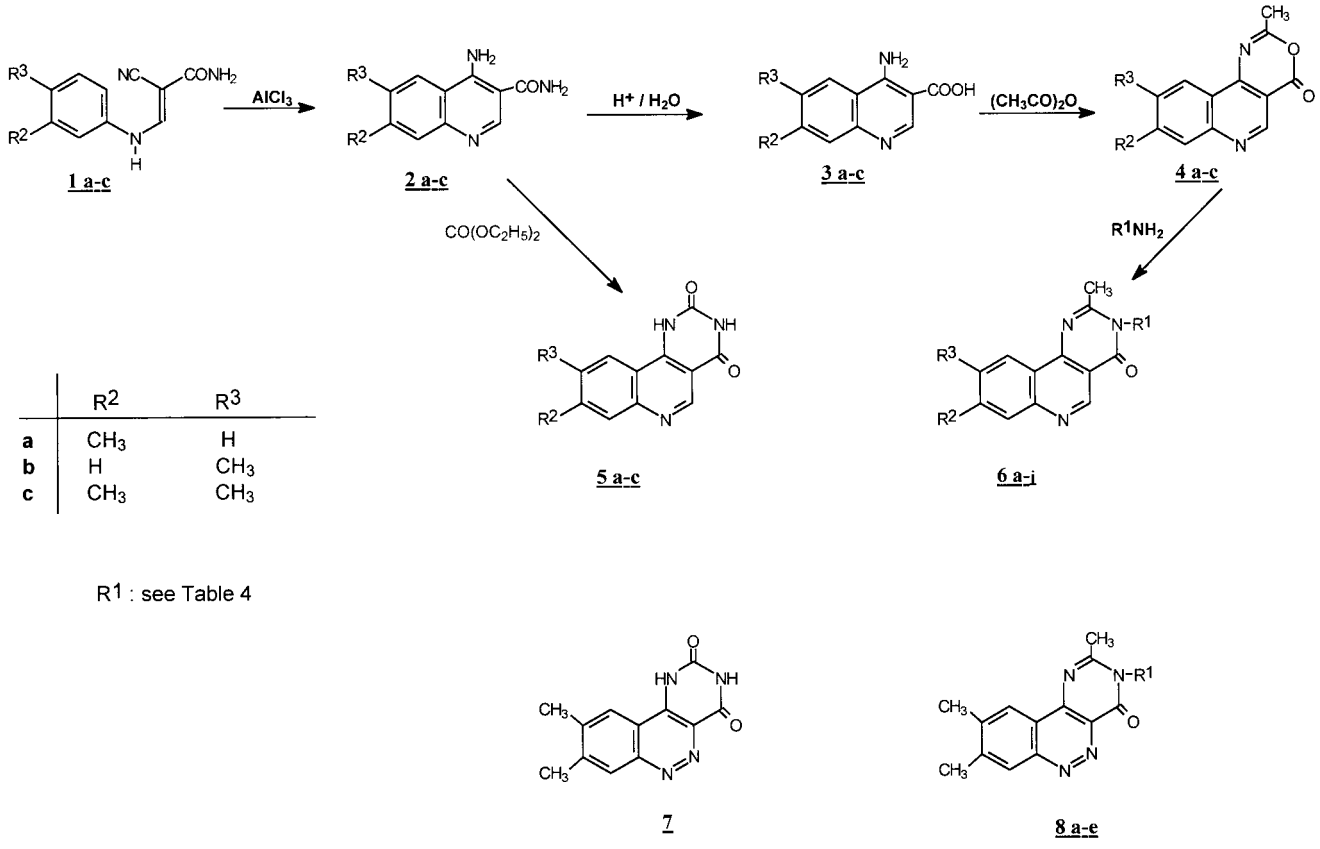
1. Introduction

Previously obtained series of *N*-3 substituted 2-methylpyrimido[5,4-*c*]cinnolin-4(3 *H*)-ones [1] and 1,2,3,4-tetrahydropyrimido[5,4-*c*]cinnolin-2,4-diones [2] showed significant CNS activity. As a continuation of our study, we synthesized the above tricyclic systems with a quinoline core. Our strategy was based on the knowledge of structure-activity relationship and molecular modeling methods. We expected that the synthesis and evaluation of these derivatives would be able to explain the influence of the quinoline core on CNS activity in this group of derivatives. The computer analysis (HyperChem/ChemPlus), as a tool in the drug design process, involved SAR, and hydrophobic, electronic and structural parameters of corresponding pyrimido[5,4-*c*]cinnolines and pyrimido[5,4-*c*]quinolines.

2. Investigations, results and discussion

Phenylaminomethylenecyanoacetamides **1,** the starting material for the cyclocondensation of the quinoline core (Scheme 1) were prepared by condensation of the appropriate aniline, cyanoacetamide and triethyl orthoformate

Scheme 1



	R^2	R^3
a	CH_3	H
b	H	CH_3
c	CH_3	CH_3

Scheme 2

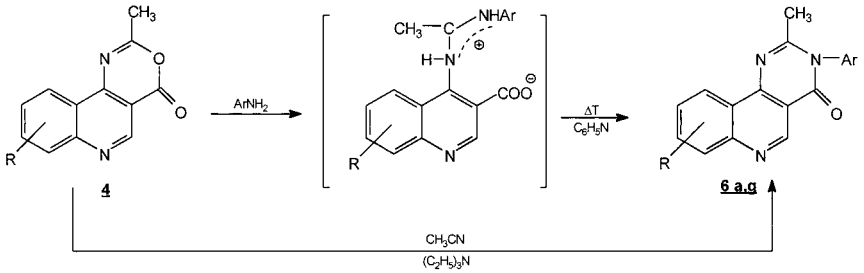


Table 1: Some pharmacological results of pyrimido[5,4-*c*]quinoline derivatives 5a–c, 6a–j

Compd.	ALD_{50} (g/kg) i.p.	p.o.	Dose i.p. (Ratio of ALD_{50})	Spontaneous locomotor activity (% of control values) 30 min n = 6–10	Amphetamine hypermotility (% of control values) 30 min n = 6–10	Δt (°C) 1 h	Δt (°C) after m-CPP* 1 h	Four plate test Number of crossings in 1 min. n = 10	Pentetrazole shock n=10 Number of mice with: Clonic seizures	tonic seizures	mortality	Writhing test Number of writhings 30 min n = 10
Control group				100.0	100.0	+0.2	+1.2	5.6 ± 0.6	8	4	4/10	30.3 ± 1.6
5a	>2.0	>2.0	0.1	51.0 ± 5.9[b]	42.0 ± 12.4[a]	−1.0	+0.6	7.11 ± 1.4	8	2	2/10	23.0 ± 2.77
			0.05	24.6 ± 3.1[c]	38.1 ± 4.8[b]							
			0.025	65.0 ± 8.7[a]	39.6 ± 7.9[b]							
			0.0125	67.4 ± 10.3[a]	102.5 ± 21.2							
			0.00625	74.6 ± 9.8								
5b	>2.0	>2.0	0.1	22.4 ± 2.8[c]	37.1 ± 6.6[b]	−0.8	+1.2	5.0 ± 0.7	7	1	0/10	25.0 ± 0.72
			0.05	66.3 ± 6.7[a]	53.0 ± 8.3[a]							
			0.025	46.1 ± 9.6[b]	102.8 ± 15.8							
			0.0125	58.6 ± 6.9[b]								
			0.00625	74.5 ± 7.2								
5c	2.0	>2.0	0.1	34.5 ± 9.3[c]	17.6 ± 4.6[c]	−1.0	−0.8	3.0 ± 0.4[b]	8	0	0/10	18.5 ±1.3[c]
			0.05	56.8 ± 4.6[c]	21.6 ± 4.4[c]							28.5 ± 1.6
			0.025	59.0 ± 10.4[b]	27.5 ± 8.8[a]							
			0.0125	67.0 ± 5.5[b]	23.1 ± 2.6[c]							
			0.00625	80.3 ± 13.0[a]	46.8 ± 12.3[a]							
6a	1.0	>2.0	0.1	29.9 ± 5.5[c]	12.4 ± 2.3[c]	−2.1	−0.5	0.8 ± 0.3[a]	8	0	0/10	15.8 ± 0.8[c]
			0.05	47.4 ± 9.6[c]	29.7 ± 5.6[c]							16.5 ± 1.1[c]
			0.025	91.2 ± 6.1	32.5 ± 3.8[c]							17.0 ± 0.4[c]
			0.0125		35.5 ± 2.2[c]							26.4 ± 1.6
			0.00625		42.6 ± 9.2[b]							
6b	0.5	1.0	0.1	108.9 ± 11.8	20.9 ± 5.7[c]	−0.7	0	4.9 ± 0.4	9	2	2/10	21.3 ± 0.9[c]
			0.05		56.4 ± 14.3							21.3 ± 0.4[c]
			0.025									21.4 ± 0.6[c]
			0.0125									28.8 ± 1.2
6c	2.0	>2.0	0.1	99.2 ± 5.2	38.0 ± 7.0[b]	−1.6	−0.6	9.0 ± 0.5[c]	8	4	3/10	19.6 ± 0.6[c]
			0.05		42.9 ± 9.1[b]			6.7 ± 0.7				21.0 ± 0.8[c]
			0.025		59.7 ± 7.6[a]							22..3 ± 1.5[b]
			0.0125		86.0 ± 6.9							27.1 ± 1.6
6d	1.5	>2.0	0.1	87.1 ± 14.4	56.7 ± 8.3[a]	−1.4	−0.4	5.5 ± 0.5	9	2	5/10	17.4 ± 0.7[c]
			0.05		31.6 ± 4.7[c]							27.0 ± 0.7
			0.025		46.0 ± 7.2[b]							
			0.0125		58.7 ± 10.7[a]							
6e	1.5	>2.0	0.1	119.2 ± 15.9	24.9 ± 4.4[c]	−1.7	−1.4	4.5 ± 0.6	10	1	1/10	21.3 ± 1.2[c]
			0.05		25.4 ± 2.0[c]							23.6 ± 1.2[c]
			0.025		53.0 ± 6.4[a]							28.1 ± 1.6
			0.0125		78.4 ± 10.8							
6f	>2.0	>2.0	0.1	57.2 ± 19.9[a]	150.6 ± 18.0	−0.2	−0.4	8.9 ± 1.4[a]	8	4	2/10	21.4 ± 2.87
			0.05	98.8 ± 14.1				8.4 ± 1.0[a]				
								3.6 ± 0.5				
6g	>2.0	2.0	0.1	15.9 ± 3.1[c]	38.3 ± 9.8[b]	−0.5	−1.3	4.8 ± 0.5	7	4	4/9	23.8 ± 2.36
			0.05	44.0 ± 6.1[c]	39.7 ± 7.7[a]							
			0.025	55.8 ± 5.4[b]	68.6 ± 14.8							
			0.0125	60.2 ± 7.3[b]								
			0.00625	48.3 ± 4.8[c]								
			0.003125	65.1 ± 5.1[b]								
6h	0.5	1.0	0.1	73.8 ± 5.4	165.4 ± 24.6[a]	−1.0	+ 1.3	6.4 ± 0.8	10	1	1/10	26.1 ± 0.46
6j	1.0	>2.0	0.1	77.3 ± 6.3	83.4 ± 16.8	−1.0	+ 0.9	6.1 ± 0.6	7	2	1/9	27.8 ± 3.82

mean values and SEM are given: statistical difference at [a] $p < 0.05$; at [b] $p < 0.01$; at [c] $p < 0.001$ Student "t" test
* *m*-CPP was injected 60 min after compound investigated

[3]. The compounds obtained **1** were cyclized using a method described by Schäfer et al. [4] to the corresponding 4-amino-3-quinolinecarboxamides **2**.

Hydrolysis of the amides **2** with 20% hydrochloric acid solution gave 4-amino-3-quinolinecarboxylic acids **3**.

2-Methyl-1,3-oxazino[5,4-*c*]quinolin-4(3*H*)-ones **4** were prepared by condensation of **3** with acetic anhydride [1]. The ^{1}H NMR of **4** showed a singlet at $\delta = 2.6$ due to a *C*-2 methyl group and an IR band of CO-O at 1730–1750 cm^{-1}. The final step to the *N*-3 substituted 2-methylpyrimido[5,4-*c*]quinolin-4(3*H*)-ones **6** was accomplished by the reaction of 2-methyl-1,3-oxazino[5,4-*c*] quinolin-4(3*H*)-ones **4** with various amines. Similarly, to the 2-methyl-1,3-oxazino[5,4-*c*]cinnolin-4(3*H*)-ones [1], the 2-methyl-1,3-oxazino[5,4-*c*]quinolin-4(3*H*)-ones **4** reacted with aromatic amines to give an intermediate product of the internal amidine salt (Scheme 2). This product was easy to identify on the basis of its ^{1}H NMR spectrum. The spectrum of amidine salt showed two characteristic signals at $\delta = 10.5$ and $\delta = 11.5$ due to NH protons. The 2-methyl-1,3-oxazino[5,4-*c*]quinolin-4(3*H*)-ones **4** were condensed with aromatic amines in the presence of triethyl amine to give the appropriate *N*-3 substituted 2-methylpyrimido-[5,4-*c*]quinolin-4(3*H*)-ones rather than the amidine salt. Their ^{1}H NMR revealed the absence of NH protons in amidine salt.

A different route was employed to prepare 1,2,3,4-tetrahydropyrimido[5,4-*c*]quinolin-2,4-diones **5**. Compounds **5** were obtained by cyclization of amides **2** with diethylcarbonate [2]. The cyclization of **2** was indicated by disappearence of the two signals of the amide in the ^{1}H NMR spectrum and the appearence of a single signal of two NH protons at $\delta = 12.3$. The IR and ^{1}H NMR spectra of the other compounds were assigned in accordance with the proposed stuctures.

CNS effects of the new derivatives of 1,2,3,4-tetrahydropyrimido[5,4-*c*]quinolin-2,4-diones **5** and *N*-3 substituted 2-methylpyrimido[5,4-*c*]quinolin-4(3*H*)-ones **6** were evaluated in mice and in rats with nine behavioural tests.

The approximate LD_{50}(ALD_{50}) of these compounds and positive results of seven tests are given in Table 1. The compounds tested **5a**–**c**, **6a**, **f**, **g** significantly decreased spontaneous locomotor activity in mice. This observation proved the sedative action of these compounds. The most pronounced sedative action was found with **6g**. All compounds at large doses caused catalepsia and ptosis. All compounds produced hypothermia in rats. Compounds **5a**–**c** and **6a**–**e**, **g** significantly inhibited amphetamine – induced hypermotility in mice. None of the compounds altered apomorphine – induced excitation and stereotypic behaviour after apomorphine in rats.

Our results provide substantial evidence for the neuroleptic activity of the tested substances **5a**–**c** and **6a**–**e**, **g**: however these agents do not show a positive response in the apomorphine test. The classic neuroleptics abolish post-apomorphine stereotypy and excitation in animals.

Compound **6c**, **f** exerted anxiolytic activity in the four plate test in mice reflected by an increase of the number of crossings accompanied by electric shocks.

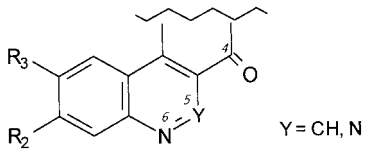
In the pentetrazole test anticonvulsive activity was observed for **5b**, **5c**, **6a**. Compounds **5c**, **6a** completely prevented tonic seizures and mortality of mice in the pentetrazole shock test. Similar, but weak activity was observed in case of compounds **5a**, **6b**, **6e** which reduced tonic seizures and mortality of mice in this test.

The tested compounds **5c**, **6a**–**e** exhibited analgesic activity and reduced writhing episodes in the writhing test in

Table 2: Comparison of SAR properties compounds 5c, 6 a–e and its cinnoline analogs 7, 8 a–e (ChemPlus v. 2.0 calculations)

SAR properties of	5c	7	6a	8a	6b	8b	6c	8c	6d	8d	6e	8e
Surface Area (GRID) of molecule [Å^2]	408.738	406.757	662.540	655.119	639.451	634.434	583.173	583.652	558.890	559.856	619.863	615.844
Volume of molecule [Å^3]	676.217	664.329	1146.417	1133.675	1096.144	1084.283	1023.221	1010.316	981.998	977.939	1074.915	1062.760
LogP of molecule	2.458	2.405	4.046	3.993	3.390	3.338	2.311	2.258	3.338	3.286	2.363	2.310
Polarizability of molecule [Å^3]	26.435	25.726	44.843	44.134	41.963	41.254	39.991	39.282	40.128	39.419	41.826	41.117
Refractivity of molecule [Å^3]	66.092	65.584	114.064	113.556	106.454	105.945	101.168	100.659	101.589	101.080	106.033	105.524
Mass of molecule	241.249	242.237	405.453	406.441	352.479	353.467	352.436	353.424	338.453	339.440	366.453	367.451
Dipole moment [D]	4.82733	6.88546	3.01195	4.68245	3.64796	5.03142	5.09046	6.52612	2.01693	2.46686	4.8057	6.53476

Table 3: The distribution of electronic charge density of some atoms in compounds 5c, 6 a–e and their cinnoline analogues 7, 8a–e (ChemPlus v. 2.0 calculations)



Atoms	5c	7	6a	8a	6b	8b	6c	8c	6d	8d	6e	8e
C-5	0.035	–	0.012	–	0.013	–	0.014	–	−0.039	–	0.013	–
N-5	–	0.090	–	0.069	–	0.070	–	0.070	–	0.013	–	0.069
N-6	−0.094	–	−0.080	–	−0.081	–	−0.083	–	−0.073	–	−0.081	–
N-6	–	−0.028	–	−0.016	–	−0.017	–	−0.019	–	−0.014	–	−0.018

mice. Compounds **6a**, **6b** and **6c** showed activity in this test even at doses of 0.025 ALD_{50}.

None of compounds studied decreased the immobility time of mice in the behavioural despair test, which is regarded as the most universal model for evaluation of antidepressant action.

Compounds **5c**, **6a**–**g** prevented m-CPP – induced hyperthermia in rats. Compounds **5c** and **6e, 6g** completely abolished m-CPP – induced hyperthermia.

Thus, these results indicate that compounds **5c**, **6a**–**g** exhibit antiserotonin activity.

It is therefore concluded that:

- these substances exhibit many features of classic neuroleptic agents, and show analgesic and antiserotonic activity,
- **6g** has the highest sedative activity,
- **5** and **6a** display additional anticonvulsive activity,
- compound **6c** displays anxiolytic action.

Biological activity may be a function of steric and electronic factors. Pharmacological results of the selected 1,2,3,4-tetrahydropyrimido[5,4-*c*]quinolin-2,4-dione (**5c**), *N*-3 substituted 2-methylpyrimido[5,4-*c*]quinolin-4(3 *H*)-ones **6a**–**e** and their pyrimido[5,4-*c*]cinnoline analogues **7**, **8a**–**e** [1, 2] were then examined in terms of SAR properties, using the ChemPlus 2.0 (Hypercube) program [5]. The results are shown in Table 2.

Structure-activity relationships of selected pyrimido[5,4-*c*] quinoline derivatives in comparison with pyrimido[5,4-*c*] cinnoline analogues showed higher activity for quinoline, especially in tests of temperature and locomotor activity. Moreover, only the pyrimido[5,4-*c*]quinoline derivatives showed analgesic activity. Analysis of SAR data (GRID, volume of molecule, logP, polarizability, refractivity, dipole moment) for the pyrimido[5,4-*c*]cinnoline and the pyrimido[5,4-*c*]quinoline analogues (Table 2) did not give a clear solution, as most values were similar. The structures analysed differed in values of dipole moment, these being higher for the pyrimido[5,4-*c*]cinnoline analogues. Significant differences of the electron densities at positions 5 (nitrogen in cinnoline or carbon atom in quinoline molecule) and 6 (nitrogen atom) were also found. The electronegativity of the *N*-6 atom was higher for the pyrimido[5,4-*c*]quinoline system derivatives (Table 3). The three-dimensional arrangement of atoms also determined molecular properties and biological activity. Minimized pyrimido[5,4-*c*]cinnoline and pyrimido[5,4-*c*]quinoline analogues showed the same shape, and similar bond lengths and bond angles. The structures were overlayed with quite low RMS error. Also pyrimido[5,4-*c*]cinnolines like pyrimido[5,4-*c*]quinolines could exist as flat, with the exeption of the *N*-3 substituent, whose arrangement depended on the conformation of the molecule. It seems probable that *N*-3 substituents do not determine the activity of the derivatives studied, because the 1,2,3,4-tetrahydropyrimido[5,4-*c*]quinolin-2,4-diones **5** were more active, than *N*-3 substituted 2-methylpyrimido[5,4-*c*]quinolin-4(3 *H*)-ones **6**.

We suggest that differences in activity for the derivatives examined may be associated with another pathway of biotransformation of structures with a quinoline core. The other hypothesis concerns the large difference in the value of the negative charge on position *N*-6. The electrostatic potentials as the energy of interaction of a point positive charge with the nuclei and electrons of a molecule clearly showed those areas particulary rich or poor in electrons. This factor may be essential for the binding of these compounds with appropriate receptors.

3. Experimental

3.1. Apparatus

Melting points were determined on an Electrothermal apparatus in open capillaries and have not been corrected. ^{1}H NMR (Varian Mercury 300 MHz) and IR spectra (Mattson Infinity Series FTIR) were consistent with the assigned structures. ^{1}H NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Carbon, hydrogen, and nitrogen elemental analyses were performed using a Perkin Elmer series II. CHNS/O Analyzer 2400 and were within 0.4% of theoretical values.

Computer analysis was performed with version 5.1 of the HyperChem/ ChemPlus 2.0 package running on a Pentium III (450 MHz) microprocessor and 128 MB RAM.

3.2. Chemistry

*3.2.1. Synthesis of phenylaminomethylenecyanoacetamides **1a–c** [**3**]*

Mixtures of 10 mmol of the corresponding aniline, 10 mmol of cyanoacetamide, 10 mmol of triethyl orthoformate and 50 ml C_2H_5OH were refluxed for 3 h. The mixture was cooled, and the solid filtered off, washed with C_2H_5OH and dried.

1a: Yield: 62.3% (dioxane). M.p. 178–180 °C. $C_{11}H_{11}N_3O$ (201.2)
1b: Yield: 76.4% (dioxane). M.p. 234–236 °C. $C_{11}H_{11}N_3O$ (201.2)
1c: Yield: 84.1% (dioxane). M.p. 221–222 °C. $C_{12}H_{13}N_3O$ (215.2)

*3.2.2. Synthesis of 4-amino-3-quinolinecarboxamides **2 a–c***

Compound **2** was synthesized from **1** as described [4].

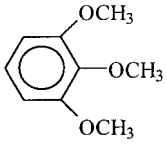
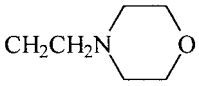
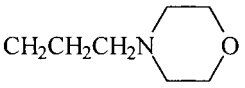
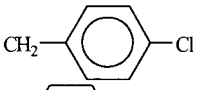
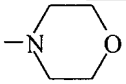
2a: Yield: 67.3% (DMF). M.p. > 300 °C. $C_{11}H_{11}N_3O$ (201.2)
2b: Yield: 62.9% (DMF). M.p. > 300 °C. $C_{11}H_{11}N_3O$ (201.2)
2c: Yield: 69.9% (DMF). M.p. > 300 °C. $C_{12}H_{13}N_3O$ (215.2)

*3.2.3. Synthesis of 4-amino-3-quinolinecarboxylic acids **3a–c***

Mixtures of 10 mmol of **2** and 75 ml of 20% HCl were refluxed for 6 h. After cooling, the solid was filtered off and made alkaline with 10% ammonia.

3a: Yield: 86.0% (DMF). M.p. 289–291 °C. $C_{11}H_{10}N_2O_2$ (202.2)
3b: Yield: 95.6% (DMF). M.p. 286–288 °C. $C_{11}H_{10}N_2O_2$ (202.2)
3c: Yield: 93.2% (DMF). M.p. 276–277 °C. $C_{12}H_{12}N_2O_2$ (216.2)

Table 4: Physical and analytical data of compounds 6a–j

Compd.	R^1	R^2	R^3	Molecular formula (weight)	M.p. (°C)	Yield (%)
6a		CH_3	CH_3	$C_{23}H_{23}N_3O_4$ (405.4)	296–298	78.2
6b	$(CH_2)_3N(C_2H_5)_2$	CH_3	CH_3	$C_{21}H_{28}N_4O$ (352.5)	82–84	63.4
6c		CH_3	CH_3	$C_{20}H_{24}N_4O_2$ (352.4)	186–188	68.3
6d	$(CH_2)_2N(C_2H_5)_2$	CH_3	CH_3	$C_{20}H_{26}N_4O$ (338.4)	142–144	70.7
6e		CH_3	CH_3	$C_{21}H_{26}N_4O_2$ (366.4)	148–150	62.6
6f	CH_2CH_2OH	CH_3	CH_3	$C_{16}H_{17}N_3O_2$ (383.3)	287–289	58.6
6g		CH_3	CH_3	$C_{21}H_{18}N_3OCl$ (363.8)	248–250	65.2
6h		H	CH_3	$C_{17}H_{18}N_4O_2$ (3310.3)	192–194	72.3
6j	$CH(CH_3)_2$	H	CH_3	$C_{16}H_{17}N_3O$ (267.3)	256–258	69.7

3.2.4. Synthesis of 2-methyl-1,3-oxazino[5,4-c]quinolin-4(3H)ones ***4a–c***

Compound **4** was synthesized from **3** as described in [1].
4a: Yield: 73.5% (CH_3CN). M.p. 238–241 °C. $C_{13}H_{10}N_2O_2$ (226.2)
4b: Yield: 69.2% (CH_3CN). M.p. 237–238 °C. $C_{13}H_{10}N_2O_2$ (226.2)
4c: Yield: 71.3% (CH_3CN) M.p. 232–234 °C. $C_{14}H_{12}N_2O_2$ (240.2)

3.2.5. 1,2,3,4-tetrahydropyrimido[5,4-c]quinolin-2,4-diones ***5a–c***

Compound **5** was synthesized from **2** and diethylcarbonate as described earlier [2].
5a: Yield: 84.2% (DMF). M.p. > 300 °C. $C_{12}H_9N_3O_2$ (227.2)
5b: Yield: 82.6% (DMF). M.p. > 300 °C. $C_{12}H_9N_3O_2$ (227.2)
5c: Yield: 68.5% (DMF). M.p. > 300 °C. $C_{13}H_{11}N_3O_2$ (241.2)

3.2.6. 2-Methylpyrimido[5,4-c]quinolin-4(3H)-ones ***6a–j***

Compound **6** was synthesized from **4** as described earlier [1].
Physical and analytical data for **6** are shown in Table 4.

3.3. Computational calculations

The calculations were performed using the semiempirical PM3 method supplied by HyperChem Release 5.1 (Hypercube, Inc.) code. The Polak-Ribiere algorithm was employed for geometry optimization. The convergence criteria were adapted to an energy gradient of 0.01 kcal mol^{-1} $Å^{-1}$ for all compounds. Partial atom charges were determined using single-point AM1 calculations.

3.4. Pharmacology

Swiss white mice (18–27 g) of either sex and male Wistar rats (180–290 g), fed on standard diet, were used. The compounds investigated were administered i.p. or (in the writhing test) p.o. as a suspension in a 1% aqueous solution of methylcellulose. Drugs: D-amphetamine sulfate (Sigma), apomorphine hydrochloride (Sandoz), pentetrazole (Cardiazolum, Poland), *m*-chlorphenylpiperazine (*m*-CPP, Institute of Pharmacology, Polish Academy of Sciences). The approximate LD_{50} (ALD_{50}) values were determined according to the Deichmann and Le Blanck method [6]. The pharmacological screening included the following tests: spontaneous- and amphetamine-induced locomotor activity (in photoresistant cages) in mice, apomorphine-induced stereotypy in rats [7], pentetrazole shock in mice, four plate test in mice [8], rectal body temperature in rats, *m*-CPP-induced hyperthermia in rats [9], behavioural despair test in mice [10], writhing test in mice [11]. The compounds tested were administered 60 min before examination, except for the behavioural despair test, in which the compounds were injected once a day for 14 days. Amphetamine (5 mg/kg s.c.), apomorphine (2.5 mg/kg s.c.), pentetrazole (85 mg/kg s.c.), *m*-CPP (10 mg/kg i.p.), and 3% acetic acid solution (0.1 ml i.p. in the writhing test) were given 60 min after the study compounds. The effect of the compounds was examined for 30 min in the case of the spontaneous- and amphetamine-induced locomotor activity tests, the pentetrazole test and the writhing test, for 60 s after a 15 s exploration period in the four plate test, for 6 min in the behavioural despair test (60 min after the last dose of study compound), for 1 h at 15 min intervals in the apomorphine-stereotypy test, and for 3 h at 30 min intervals in the rectal body temperature test and in the m-CPP-induced hyperthermia test.

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