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POLYMORPHISM AND THE ANALGESIC ACTIVITY OF N-(3-PYRIDYLMETHYL)-4-HYDROXY-2-OXO-1,2,5,6,7,8-HEXAHYDROQUINOLINE-3-CARBOXAMIDE

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Key words: 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides; polymorphism; X-ray structural analysis; analgesic activity

The advanced study of the analgesic activity of N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide selected by the results of the primary pharmacological screening as the leading structure has revealed a significant change in anesthetic properties in different samples of this compound. Since the substance under study was not soluble in water, and animals received it orally as a thin aqueous suspension, the most likely cause of the effect observed was thought to be the changes in the crystalline structure of N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide occurring in it under the influence of external factors. This assumption has been fully confirmed by the thorough microscopic investigation of high – and low-active samples, as well as more objective data, the methods of powder and single crystal X-ray diffraction analysis. Thus, in particular, it has been found that with all the variety of crystalline and amorphous forms included in the samples studied their qualitative composition appeared to be quite similar. At the same time the quantitative content of some of the phases varies greatly, and obviously, it was the factor determining the size of the analgesic effect.

ПОЛІМОРФІЗМ ТА АНАЛГЕТИЧНА АКТИВНІСТЬ N-(3-ПІРИДИЛМЕТИЛ)-4-ГІДРОКСИ-2-ОКСО-1,2,5,6,7,8-ГЕКСАГІДРОХІНОЛІН-3-КАРБОКСАМІДУ
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Ключові слова: *4-гідрокси-2-оксо-1,2-дигідрохінолін-3-карбоксаміди; поліморфізм; рентгеноструктурний аналіз; аналгетична активність*
Розширене вивчення аналгетичної активності N-(3-піридилметил)-4-гідрокси-2-оксо-1,2,5,6,7,8-гексагідрохінолін-3-карбоксаміду, відібраного за результатами первинного фармакологічного скринінгу як структури-лідера, виявило суттєву зміну знеболюючих властивостей у різних зразків цієї сполуки. Оскільки досліджувана речовина нерозчинна у воді і піддослідним тваринам вводилась перорально у вигляді тонкої водної суспензії, то найбільш ймовірною причиною виявленого ефекту визнали зміни кристалічної будови N-(3-піридилметил)-4-гідрокси-2-оксо-1,2,5,6,7,8-гексагідрохінолін-3-карбоксаміду, які відбуваються у ньому під впливом зовнішніх факторів. Це припущення повністю підтвердилось ретельним мікроскопічним дослідженням високо- та низькоактивного зразків, а також більш об'єктивними даними, одержаними методами порошкового і монокристалічного рентгеноструктурного аналізу. Так, зокрема, встановлено, що при всьому розмаїтті кристалічних та аморфних форм, що входять у досліджувані зразки, їх якісний склад виявився доволі схожим. В той же час кількісний вміст деяких фаз сильно розрізняється, що, очевидно, й послужило визначальним фактором для величини аналгетичного ефекту.

ПОЛИМОРФИЗМ И АНАЛЬГЕТИЧЕСКАЯ АКТИВНОСТЬ N-(3-ПИРИДИЛМЕТИЛ)-4-ГИДРОКСИ-2-ОКСО-1,2,5,6,7,8-ГЕКСАГИДРОХИНОЛИН-3-КАРБОКСАМИДА
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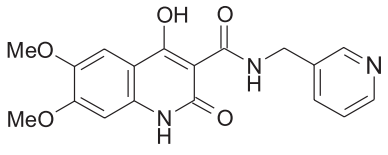
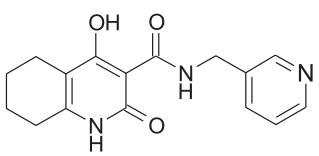
Ключевые слова: *4-гидрокси-2-оксо-1,2-дигидрохинолин-3-карбоксамиды; полиморфизм; рентгеноструктурный анализ; анальгетическая активность*
Расширенное изучение анальгетической активности N-(3-пиридилметил)-4-гидрокси-2-оксо-1,2,5,6,7,8-гексагидрохинолин-3-карбоксамида, отобранного по результатам первичного фармакологического скрининга в качестве структуры-лидера, выявило существенное изменение обезболивающих свойств у разных образцов этого соединения. Поскольку испытуемое вещество не растворимо в воде и подопытным животным вводилось перорально в виде тонкой водной суспензии, то наиболее вероятной причиной обнаруженного эффекта посчитали изменения кристаллического строения N-(3-пиридилметил)-4-гидрокси-2-оксо-1,2,5,6,7,8-гексагидрохинолин-3-карбоксамида, происходящие в нем под воздействием внешних факторов. Это предположение полностью подтвердилось тщательным микроскопическим исследованием высоко- и низкоактивного образцов, а также более объективными данными, полученными методами порошкового и монокристаллического рентгеноструктурного анализа. Так, в частности, установлено, что при всем многообразии кристаллических и аморфных форм, входящих в изучаемые образцы, их качественный состав оказался довольно схожим. В то же время количественное содержание некоторых фаз сильно различается, что, очевидно, и послужило определяющим для величины анальгетического эффекта фактором.

The tendency of many substances to form different crystalline or polymorphic modifications has long attracted the attention of researchers. And if the original interest in such objects was caused solely by curiosity of researchers, but by the accumulation of information about the diversity of the properties of polymorphic substances it gradually became practical. This area of crystallography has found especially bright and productive reflection in medicinal chemistry [1-5]. As it turned out, polymorphism of drugs is capable to change their characteristics radically, and now no serious pharmaceutical manufacturer can ignore the problem [6-9]. For this reason, the issues of obtaining, determining, describing, as well as purity and properties of crystalline forms used in pharmaceutical products do not remain without attention from regulatory authorities. As a result, without such information the registration of a new medicinal substance has now become impossible in many countries. However, it should be recognized that although polymorphism has developed into a separate science, but in many ways it still remains an undiscovered natural phenomenon. Until now, researchers could only notice the formation of one or another polymorphic modification of a substance. Theoretically to predict or calculate this process and, especially, to predetermine the conditions that provide formation of only desired polymorph are not yet possible [10].

We unexpectedly encountered with the problem of polymorphism of biologically active substances when searching for new analgesics among derivatives of 4-hydroxyquinoline-2-ones. In particular, in the integrated optimization studies proposed earlier [11] N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) was involved in the range of the objects studied as a leading compound of N-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (**1**); though it was previously described [12], but, nevertheless, it was of interest as the analogue modified in the benzene moiety of the quinolone nucleus:



1



2

A high analgesic activity of the compound (α-form) found during the primary pharmacological screening immediately caused a keen interest in it as a potential new leading compound (Tab. 1).

However, in further tests serious problems began to appear – the second sample of this compound (β-form) sent to the biological laboratory unexpectedly demonstrated the result approximately two times lower than the first one. And it is despite the fact that they both were the products of the same synthesis. The multiple repetition of the pharmacological experiment under the similar conditions with both samples simultaneously finally confirmed significant differences in their analgesic properties. At first there were even doubts that we dealt namely with N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) in both cases. But NMR spectroscopy and combined gas chromatography mass-spectrometry dispeled these doubts rapidly and confirmed the absolute identity of the first and second samples.

N-(3-Pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) under normal conditions is not soluble in water. It was administered orally to the experimental animals in the form of a fine aqueous suspension stabilized with Tween-80. Since the test substance is ingested in a solid form, one of the most likely factors having such significant impact on its biological properties becomes the crystalline structure [10].

Based on the above data we considered it appropriate to conduct the study of the phase composition of the high and low active samples of N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**, α- and β-forms, respectively)

Table 1

The analgesic activity of leading compound 1, polymorphic forms of amide 2 and reference drugs on the "acetic acid writhing" model

Compound	Analgesic activity	
	The average number of "writhings"	%
1	18.3±1.0**	78.0
2 (α-form)	11.0±1.3**	86.7
2 (ß-form)	45.5±2.7*	45.1
Metamizole sodium (55 mg/kg)	53.8±1.4**	35.1
Piroxicam (92 mg/kg)	41.6±1.8*	50.0
Diclofenac (5 mg/kg)	40.1±2.3**	51.6
Nabumetone (50 mg/kg)	41.0±3.3*	50.6
Control	83.2±1.3	–

* – differences were significant at $p<0.05$ compared to control;
** – differences were significant at $p<0.01$ compared to control.

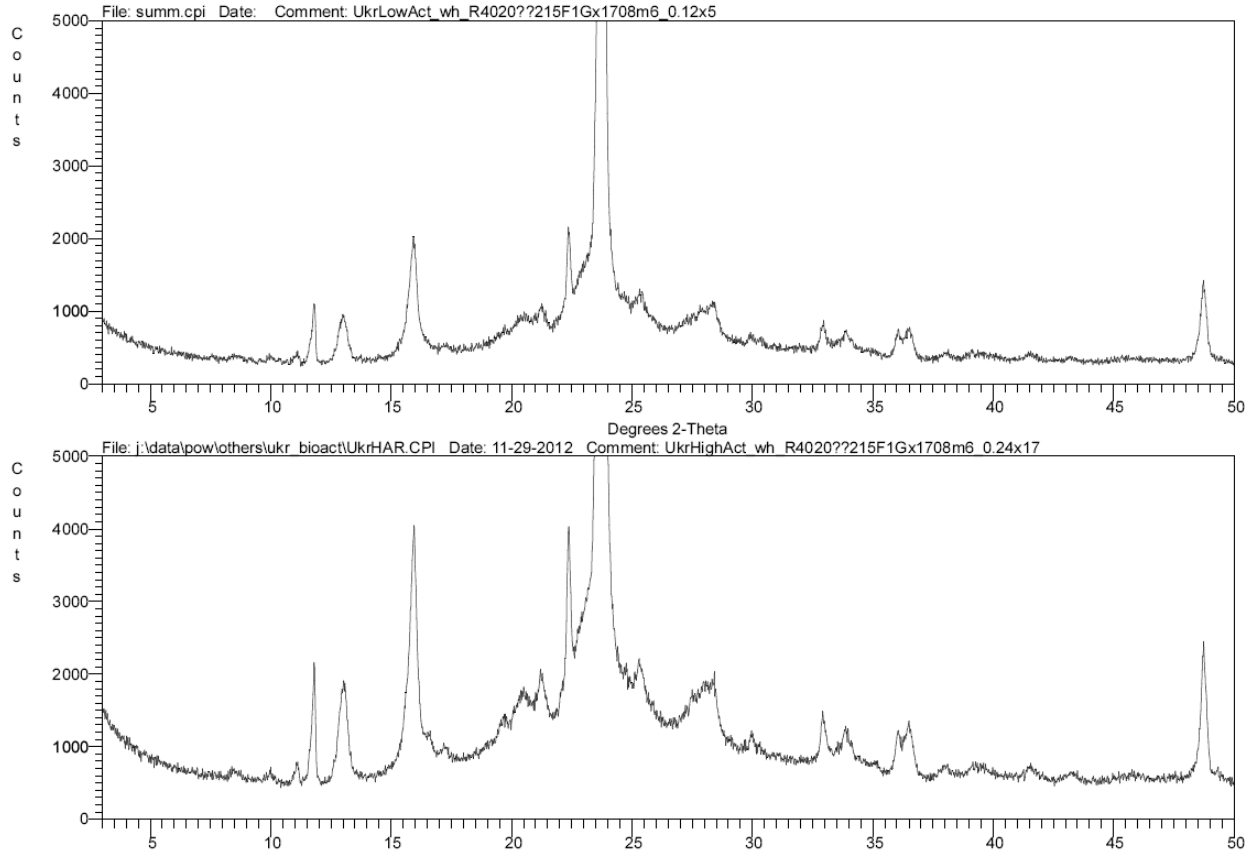


Fig. 1. The X-ray powder diffractogrammes of highly active α-form of amide **2** (bottom) and its low active ß-form (top).

by the methods of X-ray powder and single-crystal X-ray structural analysis.

Powder X-ray analysis (Fig. 1) allows to conclude confidently only that the qualitative composition of both samples are quite similar and diverse – in both cases both sharp and wide peaks, which are likely due to the simultaneous presence of several crystalline and amorphous phases, are observed. But the quantitative content of phases (judging by the change in the intensity of the peaks) is very different. It is possible that this factor had a decisive impact on the analgesic effect of the crystalline forms of amide (**2**).

A careful microscopic analysis gave similar results, but at the same time some shiny triclinic crystals suitable for single-crystal X-ray structural analysis were observed in the total powder weight of the active Sample A. This analysis was carried out successfully by us (See Fig. 2 and Tab. 2, 3).

In the independent part of the elementary cell of this crystalline phase of N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) two molecules – **A** and **B** differing in some geometric parameters were found. The cyclohexone fragment in each of these molecules is disordered by two *half-chair* conformations – **A1** and **A2**, **B1** and **B2** (folding parameters [13]: $S = 0.69$, $\Theta = 35.4°$, $\Psi = 29.9°$ in **A1**; $S = 0.81$, $\Theta = 34.3°$, $\Psi = 29.7°$ in **A2**; $S = 0.87$, $\Theta = 32.3°$, $\Psi = 25.1°$ in **B1**; $S = 0.57$, $\Theta = 39.4°$, $\Psi = 28.4°$ in **B2**). Deviation of atoms $C_{(3)}$ and $C_{(4)}$ from the mean-square plane of the rest atoms of the cycle is -0.34 and 0.34 Å in **A1**, 0.40 and -0.40 Å in **A2**, 0.50 and -0.35 Å in **B1** and -0.28 and 0.28 Å in **B2**, respectively. The carbamide fragment of the substituent at atom $C_{(8)}$ is in the plane of the quinolone cycle [the torsional angle is $C_{(7)}$–$C_{(8)}$–$C_{(10)}$–$O_{(3)}$ is -0.3(8)° in **A** and -4.3(8)° in **B**]; it is promoted by formation of intramolecular hydrogen bonds: $O_{(2)}$–H…$O_{(3)}$: (H…O 1.77 Å, O–H…O 149° in **A**, H…O 1.75 Å, O–H…O 150° in **B**) and $N_{(2)}$–H…$O_{(1)}$: (H…O 2.02 Å, N–H…O 135° in **A**, H…O 2.00 Å, N–H…O 135° in **B**). Formation of the given hydrogen bonds leads to electron density redistribution in this fragment of the molecule: bonds of $O_{(1)}$–$C_{(9)}$ 1,259(7) Å in **1A** and 1,286(7) Å in **1B** (mean [14]

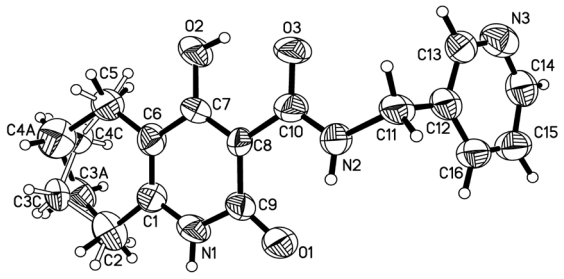


Fig. 2. The structure of N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) with numbering of the atoms.

Table 2

Interatomic distances (*l*) in the structure of triclinic modification of amide **2**

Bond	*l*, Å	Bond	*l*, Å	Bond	*l*, Å
O(1A)-C(9A)	1.259(7)	C(6A)-C(7A)	1.419(6)	C(1B)-C(6B)	1.334(7)
O(2A)-C(7A)	1.331(6)	C(7A)-C(8A)	1.390(8)	C(1B)-C(2B)	1.521(6)
O(3A)-C(10A)	1.246(6)	C(8A)-C(9A)	1.432(6)	C(2B)-C(3D)	1.540(1)
N(1A)-C(1A)	1.362(7)	C(8A)-C(10A)	1.489(7)	C(2B)-C(3B)	1.540(1)
N(1A)-C(9A)	1.381(6)	C(11A)-C(12A)	1.517(7)	C(3B)-C(4B)	1.540(1)
N(2A)-C(10A)	1.322(7)	C(12A)-C(16A)	1.360(7)	C(4B)-C(5B)	1.540(1)
N(2A)-C(11A)	1.473(5)	C(12A)-C(13A)	1.395(7)	C(3D)-C(4D)	1.540(1)
N(3A)-C(14A)	1.311(6)	C(14A)-C(15A)	1.403(7)	C(4D)-C(5B)	1.540(1)
N(3A)-C(13A)	1.334(6)	C(15A)-C(16A)	1.365(7)	C(5B)-C(6B)	1.511(7)
C(1A)-C(6A)	1.344(7)	O(1B)-C(9B)	1.286(7)	C(6B)-C(7B)	1.420(6)
C(1A)-C(2A)	1.517(6)	O(2B)-C(7B)	1.321(5)	C(7B)-C(8B)	1.413(7)
C(2A)-C(3A)	1.540(1)	O(3B)-C(10B)	1.251(6)	C(8B)-C(9B)	1.403(7)
C(2A)-C(3C)	1.540(1)	N(1B)-C(1B)	1.368(7)	C(8B)-C(10B)	1.497(7)
C(3A)-C(4A)	1.540(1)	N(1B)-C(9B)	1.398(6)	C(11B)-C(12B)	1.504(7)
C(4A)-C(5A)	1.540(1)	N(2B)-C(10B)	1.330(7)	C(12B)-C(16B)	1.362(7)
C(3C)-C(4C)	1.540(1)	N(2B)-C(11B)	1.499(6)	C(12B)-C(13B)	1.383(6)
C(4C)-C(5A)	1.540(1)	N(3B)-C(14B)	1.320(7)	C(14B)-C(15B)	1.360(7)
C(5A)-C(6A)	1.507(8)	N(3B)-C(13B)	1.359(7)	C(15B)-C(16B)	1.358(7)

1.210 Å), $O_{(3)}$–$C_{(10)}$ 1.246(6) Å in **1A** and 1.251(6) Å in **1B** (1.210 Å), and $C_{(7)}$–$C_{(8)}$ 1.390(8) Å in **1A** and 1.413(7) Å in **1B** (1.326 Å) are extended, and bonds of $O_{(2)}$–$C_{(7)}$ 1.331(6) Å in **1A** and 1.321(5) Å in **1B** (1.362 Å), and $C_{(8)}$–$C_{(9)}$ 1.438(6) Å in **1A** and 1.403(7) Å in **1B** (1.455 Å) are shortened compared to their mean values.

3-Picolyl substituent is in the antiperiplanar position in relation to $C_{(8)}$–$C_{(10)}$ bond [the torsional angle is $C_{(11)}$–$N_{(2)}$–$C_{(10)}$–$C_{(8)}$ is 173.4(5)° in **A** and 169.6(5)° in **B**], and its aromatic cycle is in *–sc*-conformation in relation to $C_{(10)}$–$N_{(2)}$ bond and noticeably turn to $N_{(2)}$–$C_{(11)}$ bond [torsional angles are $C_{(10)}$–$N_{(2)}$–$C_{(11)}$–$C_{(12)}$ are -83.7(6)° in **A** and -78.2(7)° in **B**; $N_{(2)}$–$C_{(11)}$–$C_{(12)}$–$C_{(16)}$ -68.6(7)° in **A** and -69.7(7)° in **B**].

In the crystal of molecule **A** and **B** owing to several intramolecular hydrogen bonds of C–H…π stacking-dimers **A-A** and **B-B** are formed by the "head-to-tail" type (the distance between π-systems is 3.8 Å):

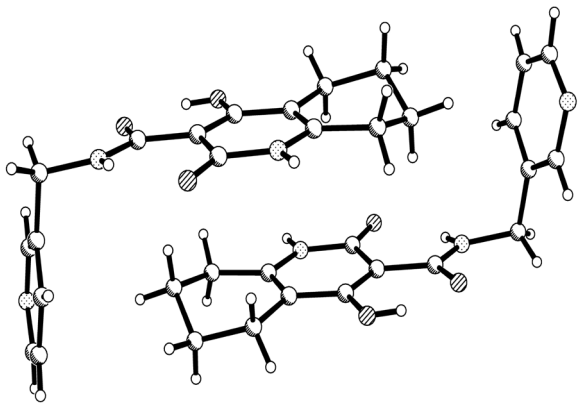


Fig. 3. A stacking-dimer of molecules of amide **2** in the crystal of triclinic modification.

In dimer **A-A**:
C(3a)-H(3ab)…C(8a)' (π) (- *x*, - *y*, 1 - *z*) H…C (π) 2.83 Å, C-H...C (π) 157°;
C(3a)-H(3ab)…C(10a)' (π) (- *x*, - *y*, 1 - *z*) H…C (π) 2.59 Å, C-H...C (π) 166°;
C(4c)-H(4ca)…C(9a)' (π) (- *x*, - *y*, 1 - *z*) H…C (π) 2.82 Å, C-H...C (π) 146°;
C(4c)-H(4ca)…C(10a)' (π) (- *x*, - *y*, 1 - *z*) H…C (π) 2.87 Å, C-H...C (π) 161°.
In dimer **B-B**:
C(3d)-H(3db)…C(10b)' (π) (- *x*, 1 - *y*, 2 - *z*) H…C (π) 2.75 Å, C-H...C (π) 176°;
C(4b)-H(4bb)…C(10b)' (π) (- *x*, 1 - *y*, 2 - *z*) H…C (π) 2.78 Å, C-H...C (π) 167°.

Dimers are connected by intermolecular hydrogen bonds: N(1a)-H…N(3b)' (- *x*, 1 - *y*, 1 - *z*) H…N 2.07 Å, N-H…N 170° and N(1b)-H…N(3a)' (- *x*, - *y*, 2 - *z*) H…N 2.05 Å, N-H…N 165°.

In the low active β-sample of N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) due to a relatively much lower content the triclinic crystalline phase is not visually detected, and it may become a cause of the biological activity decrease. This conclusion is not final, of course, since any polymorphic modification of amide **2** has not been obtained and studied in the pure form. The external factors caused the changes of the phase composition of the second sample while its sending are not clear yet. Nevertheless, based on the available data it is definitely arguable that N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) is highly prone to polymorphism. And the main thing is that it is not likely reasonable

Table 3

Valence Angles (ω) in the structure of triclinic modification of amide **2**

Angle	ω, deg.	Angle	ω, deg.
C(1A)-N(1A)-C(9A)	126.2(5)	C(10A)-N(2A)-C(11A)	123.4(5)
C(14A)-N(3A)-C(13A)	116.7(5)	C(6A)-C(1A)-N(1A)	118.8(5)
C(6A)-C(1A)-C(2A)	124.6(6)	N(1A)-C(1A)-C(2A)	116.5(5)
C(1A)-C(2A)-C(3A)	112.7(7)	C(1A)-C(2A)-C(3C)	110.5(6)
C(2A)-C(3A)-C(4A)	112(1)	C(3A)-C(4A)-C(5A)	111.7(8)
C(2A)-C(3C)-C(4C)	109.9(8)	C(5A)-C(4C)-C(3C)	109.0(7)
C(6A)-C(5A)-C(4A)	114.6(7)	C(6A)-C(5A)-C(4C)	112.5(6)
C(1A)-C(6A)-C(7A)	119.4(6)	C(1A)-C(6A)-C(5A)	121.8(5)
C(7A)-C(6A)-C(5A)	118.8(5)	O(2A)-C(7A)-C(8A)	121.8(5)
O(2A)-C(7A)-C(6A)	117.1(6)	C(8A)-C(7A)-C(6A)	121.1(5)
C(7A)-C(8A)-C(9A)	119.5(5)	C(7A)-C(8A)-C(10A)	119.1(5)
C(9A)-C(8A)-C(10A)	121.2(5)	O(1A)-C(9A)-N(1A)	118.7(5)
O(1A)-C(9A)-C(8A)	126.4(5)	N(1A)-C(9A)-C(8A)	114.9(6)
O(3A)-C(10A)-N(2A)	120.9(5)	O(3A)-C(10A)-C(8A)	120.3(6)
N(2A)-C(10A)-C(8A)	118.9(5)	N(2A)-C(11A)-C(12A)	109.5(4)
C(16A)-C(12A)-C(13A)	116.4(6)	C(16A)-C(12A)-C(11A)	122.8(5)
C(13A)-C(12A)-C(11A)	120.8(5)	N(3A)-C(13A)-C(12A)	124.6(5)
N(3A)-C(14A)-C(15A)	123.4(6)	C(16A)-C(15A)-C(14A)	117.5(6)
C(12A)-C(16A)-C(15A)	121.0(6)	C(1B)-N(1B)-C(9B)	123.5(5)
C(10B)-N(2B)-C(11B)	121.8(5)	C(14B)-N(3B)-C(13B)	115.0(5)
C(6B)-C(1B)-N(1B)	122.5(5)	C(6B)-C(1B)-C(2B)	124.2(6)
N(1B)-C(1B)-C(2B)	113.2(5)	C(1B)-C(2B)-C(3D)	111.5(6)
C(1B)-C(2B)-C(3B)	108.7(7)	C(2B)-C(3B)-C(4B)	107.0(8)
C(3B)-C(4B)-C(5B)	109.8(7)	C(2B)-C(3D)-C(4D)	119(1)
C(3D)-C(4D)-C(5B)	110.1(8)	C(6B)-C(5B)-C(4D)	117.0(7)
C(6B)-C(5B)-C(4B)	109.5(6)	C(1B)-C(6B)-C(7B)	117.3(6)
C(1B)-C(6B)-C(5B)	123.3(5)	C(7B)-C(6B)-C(5B)	119.2(5)
O(2B)-C(7B)-C(8B)	121.5(5)	O(2B)-C(7B)-C(6B)	117.9(6)
C(8B)-C(7B)-C(6B)	120.5(5)	C(9B)-C(8B)-C(7B)	121.1(5)
C(9B)-C(8B)-C(10B)	121.0(6)	C(7B)-C(8B)-C(10B)	117.9(5)
O(1B)-C(9B)-N(1B)	117.8(5)	O(1B)-C(9B)-C(8B)	127.1(5)
N(1B)-C(9B)-C(8B)	115.0(6)	O(3B)-C(10B)-N(2B)	120.9(5)
O(3B)-C(10B)-C(8B)	120.6(6)	N(2B)-C(10B)-C(8B)	118.4(5)
N(2B)-C(11B)-C(12B)	110.0(4)	C(16B)-C(12B)-C(13B)	117.1(6)
C(16B)-C(12B)-C(11B)	123.0(5)	C(13B)-C(12B)-C(11B)	119.9(6)
N(3B)-C(13B)-C(12B)	124.1(6)	N(3B)-C(14B)-C(15B)	125.0(6)
C(16B)-C(15B)-C(14B)	118.5(6)	C(15B)-C(16B)-C(12B)	120.3(6)

to study it further as a potential pain-killer at least since the conditions, which would allow obtaining highly active polymorphic modifications of this substance in regard to pharmacology and providing their stability while storing, will not be found.

Experimental Part

N-(3-Pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) was synthesized by the known method [12].

X-Ray Structural Analysis. Crystals of amide **2** are triclinic (ethanol), $C_{16}H_{17}N_3O_3$, at 20°C a = 7.865(2), b = 12.077(2), c = 16.106(2) Å, α = 80.06(1)°, β = 85.31(1)°, γ = 71.97(2)°, V = 1432.1(4) Å^3, M_r = 299.33, Z = 4, space group P, $d_{calc.}$ = 1.388 g/cm^3, μ(MoK$_\alpha$) = 0.098 mm^{-1}, F(000) = 632. The unit cell parameters and intensities of 10071 reflections (5046 independent with R_{int} = 0.117) were measured on an Xcalibur-3 diffractometer (MoK$_\alpha$ radiation, CCD-detector, graphite monochromator, ω-scanning, $2\theta_{max}$ = 50°).

The structure was decoded by the direct method using the SHELXTL programme package [15]. When refining the structure restrictions were imposed on the bond lengths in disordered cycles (Csp^3-Csp^3 1.54 Å). The positions of the hydrogen atoms were found from the electron density difference map and refined using

the rider model with U_{iso} = n U_{eq} of a nonhydrogen atom bound with the hydrogen atom (n = 1.5 for hydroxyl group and n = 1.2 for the other hydrogen atoms). The structure was refined in relation to F^2 by the method of least squares anisotropically for nonhydrogen atoms to wR_2 = 0.207 for 4833 reflections (R_1 = 0.083 for 1450 reflections with $F>4\sigma(F)$, S = 0.839). The complete crystallographic data about the structure of triclinic modification of amide **2** were deposited at the Cambridge Crystallographic Data Centre – deposit No. CCDC 1044949. The interatomic distances and angles are shown in Tab. 2 and 3, respectively.

The powder X-ray analysis of α- and β-forms of amide **2** was performed using a Siemens D500 diffractometer according to Bragg-Brentano scheme in the range of angles $2°\leq 2\theta \leq 60°$ (radiation – CuKα, graphite monochromator on the secondary beam, scanning step – 0.02°, accumulation time – 20 s at each point, a horizontal divergence of the primary beam – 1°, the receiving slit – 0.1°).

The biological studies presented in this paper were conducted in full compliance with the provisions of the European Convention on protection of vertebrates used for experimental and other scientific purposes and the Ukrainian Law No. 3447-IV "On protection of animals from severe treatment" (2006).

Analgesic properties of α- and β-forms of N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2**) were studied in white nonlinear male mice weighing 18-23 g (10 animals for each test substance) using the "acetic acid writhing" standard model [16]. The nociceptive effect was reproduced by the intraperitoneal injection of 0.6% acetic acid solution in the amount of 0.1 ml per 10 g of the animal's body weight in an hour after introduction of the test sample. The animals were observed for 20 min, the number of "writhings" was counted. The analgesic effect was assessed by the ability of compounds to reduce the number of "writhings" in the groups under study compared to the control group and expressed in percentage (Tab. 1). Testing was carried out in comparison with the leading structure – N-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (**1**) [11], as well as with the known non-opioid analgesics: Metamizole sodium (Darnitsa, Ukraine), Piroxicam (Jenapharm, Germany), Diclofenac (KRK, Slovenia) and Nabumetone (SmithKline Beecham, Germany). All test compounds were administered orally in the dose of 20 mg/kg in the form of a thin aqueous suspension stabilized with Tween-80. Medicines were used similarly or as aqueous solutions in the doses corresponding to their ED_{50} for this experimental model [17]. The control group received an equivalent amount of water with Tween-80. The results of biological tests were processed by statistics using Student's t-criterion.

Conclusions

1. An increased tendency to form different crystal modifications has been revealed in N-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide being of interest as a potential analgesic.

2. Another experimental evidence that polymorphism is a very important property of any biologically active substances that can significantly change its characteristics and therefore requires close attention and thorough study has been obtained.

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